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Improved Pollination Efficiency and Reduced Honey Bee Colony Decline in Greenhouses by Allowing Access to the Outside During Part of the Day

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Abstract

Although honey bees are efficient pollinators of many crops cultivated in greenhouses, it is difficult to maintain colony strength and consequently pollination efficiency. Many bees die under greenhouse conditions and the colonies rapidly weaken. We examined the effect of adaptations to the hive entrance that allowed control of whether and when bees had access to the outside environment to see how it would affect pollination efficiency and colony condition in greenhouses with flowering cucumber (*Cucumis sativus*) plants in comparison with colonies that remained constantly inside the greenhouse. We recorded the type and period of visitation to the cucumber flowers, numbers of honey bees entering and leaving the two-entrance hives and the effect of this type of management on the quantity of brood and food. Five frame Langstroth "nucleus" colonies were equipped with two 30 square centimeter entrances and two 3.0 cm diameter circular openings. Allowing the bees to make visits outside the greenhouse in early morning with redirection of bees into the greenhouse at 8:30 a.m. did not reduce visitation to cucumber flowers in the greenhouse. Maintaining colonies in the greenhouse reduced brood area and food stores. These losses were significantly reduced in colonies that had access to the outside during the early morning. Another advantage of alternating access to the inside and the outside of the greenhouse was that there was less possibility of interactions between bees and people working on the crop; also, pesticide applications could be made without directly affecting foraging bees.

Introduction

Closed agricultural cultivation systems (greenhouses) were developed in order to optimize vegetable production and to obtain high quality products (Muijzenberg, 1980). Greenhouses allow the control of various climatic variables; they reduce the vulnerability of crops to insect pests through the use of screens or other types of barriers; also, irrigation and fertilization can be applied with greatly increased efficiency (Van Straten et al., 2010). However, more than 40% of the crops produced in greenhouses require pollinating agents (Guerra-Sanz, 2008), principally bees (Klein et al., 2007), which do not easily adapt to these closed environments (Nye, 1962; Abrol, 2012).

Bees are commonly used for greenhouse pollination of various crops, including tomatoes and bell peppers, which are normally pollinated by bumble bees, mainly *Bombus terrestris* (L.) (Free, 1993; Velthuis & van Doorn, 2006). Stingless bees, such as *Tetragonisca angustula* (Latreille) and *Nannotrigona* spp., have also been found to be efficient pollinators of tomatoes (Del Sarto et al., 2005), peppers (Santos et al., 2008), strawberries (Malagodi-Braga & Kleinert, 2004) and cucumbers (Nicodemo e al., 2013). These stingless bees are considered effective and useful pollinators in greenhouses because they are generalist foragers, have a tendency for flower fidelity, do not attack agricultural workers who tend the crop, rarely abandon their nests and store food (Slaa et al., 2006).

Though *Bombus* spp. bees have been widely used in various countries for greenhouse pollination (Velthuis & van Doorn, 2006), they are not globally distributed and are considered a difficult to control exotic pest in some regions where they have been introduced (Dafni et al., 2010). Stingless bees, Meliponini, have a more restricted distribution, and when they are introduced into a greenhouse environment, they need to adapt to foraging in a restricted area; also, they do not invariably visit the flowers that need pollination when under confinement (Bomfim et al., 2014). Given the current knowledge about honey bee management and the facility in finding beekeepers who have colonies available for pollination services practically throughout the world, honey bees have the potential to become an optimal solution for pollinating crops in greenhouses, if techniques can be improved to adapt these bees to foraging in closed environments (Katayama, 1987; Guerra-Sanz, 2008).

Honey bees are the most widely used insects for pollination services of agricultural crops grown in open fields, since they have numerous foragers per colony that visit extensive areas, superior to many other species of bees (McGregor, 1976; Southwick & Southwick, 1992; Beekman & Ratnieks, 2000; Garibaldi et al., 2013; Bartomeus et al., 2014). In addition, they forage under a broad ambient temperature range (Abou-Shaara, 2014) and have efficient communication (Winston 1987), which favors the collection of large amounts of food resources necessary for the development of their colonies (Couto & Couto, 2006; Brodschneider & Crailsheim, 2010). However, their use on greenhouse crops is often limited because colonies in greenhouses normally decline rapidly (Sabara & Winston, 2003).

Given the advantages of using honey bees for pollination services and the problems that are still unresolved concerning adapting these bees for working in closed environments, we investigated whether allowing bees to alternatively forage inside and outside the greenhouse would allow efficient pollination, while maintaining colony integrity.

Material and Methods

Greenhouse and crop details

The work was carried out in Ribeirão Preto, São Paulo State, Brazil (21°10'02" S and 47°51'53" W), at altitude 546 m. Cucumbers (*Cucumis sativus* L.) cultivar Aodai were planted from seed in trays containing planting substrate. After 20 days, the seedlings were transplanted to the three greenhouses, each with dimensions of 13 meters long x 8 meters wide, by 1.8 m tall, covered with low density polyethylene film with UV blocking and anti-aphid screening on the sides (totally enclosed).

Cucumber seedlings (n = 144) were planted in each of the three greenhouses with a spacing of 0.5 x 1.0 m. Fertilizer was added to the irrigation water based on soil analysis and recommendations in IAC Technical bulletin 100 (Raij et al., 1997). Pest control was carried out using registered products for cucumber cultivation (Mapa, 2018) at night, when there were no more bees working outside the hives.

Preparation and development of the colonies

One week before they were introduced into the greenhouses, three colonies housed in five-frame standard Langstroth hives were equalized in terms of brood and food comb areas. A comparison of the development of the colonies kept in the greenhouses was evaluated by mapping all the combs on the day the hives were introduced and again one day after they were removed from the greenhouses (Al-Tikrity et al., 1971).

Hive and outside temperature within the greenhouses were measured with two thermometers. One was placed in the greenhouse at 1.4 meters height and a second on the hive floor of the colonies in the treatment named 24h-normal, as described below.

Hive placement and management

Colonies of Africanized honey bees (*Apis mellifera* L.) were submitted to three different managements of hives. Each greenhouse was supplied with a water source accessible to the bees. In the first, a five-frame hive was placed in a hive-sized cutout in the side screen of the greenhouse, at a height of 1.4 meters. In this way, the foraging bees could be directed to either inside or outside the greenhouse.

These five-frame hives had two 30 cm² rectangular entrances, on opposite ends. At 2.5 cm above each of these two entrances, an additional opening 3.0 cm in diameter was made. A fine mesh metal screen funnel directed to the inside of the hive was placed in this opening, through which foragers bees could enter the hive but not leave it. The opening at the point of this screen funnel was about 0.5 cm in diameter (Fig 1). In order to direct the bees to forage outside of the greenhouse, the lower entrance was blocked with a metal plate. Those bees that were still foraging in the greenhouse could enter the hive via the circular upper entrance, which was always kept open. When the objective was to direct the bees to forage inside the greenhouse, the lower hive entrance that was directed towards the outside was blocked with a metal plate and the entrance to the inside was opened (Fig 1).

Since the screen funnel point would be crushed and blocked by one of the comb frames, one deep frame was replaced with a shallow frame to allow space for the funnel. The hive then had four standard deep frames and one shallow frame. This management was called two-entrance hive.

A second greenhouse was pollinated by bee colonies that went through an adaptation period aiming to induce the bees to reorient in the greenhouse. The hives were kept in a dark room at 30 ºC and 70% relative humidity for 72 hours before being introduced into the greenhouse. During this confinement period the hive cover was replaced with an 8-mesh screen and the single hive entrance closed. Water and sucrose syrup (1:1 w/v) were supplied through this screen *ad libitum*. On the day that the hive was introduced into the

greenhouse, the screen top was removed and replaced with a normal wooden cover and the normal entrance was opened. The hive was placed 1.4 m above the greenhouse floor. This management was named 24 h-dark. The third greenhouse was given a hive that was introduced directly from the apiary and the management of this hive was entitled 24 h-normal.

The colonies were placed inside the greenhouses after the cucumber plants had begun flowering. Each of the three greenhouses received one colony, which remained there for 10 days. This process was repeated three times, so that each greenhouse received three colonies over 30 days. The hives with inside and outside entrances were manipulated at different times during each of the three 10-day periods. During the first 10-day period, the bees could visit the cucumber flowers from 6-12:30; during the second 10-day period, they had access to the flowers inside the greenhouse from 6-10:30. During the third 10-day period, the bees were directed towards the outside until 8:30. After 8:30, the bees were redirected to forage inside the greenhouse, until 12:30, when they were again allowed access only to the outside. We chose to carry out the bee visitation tests in the morning, as this is the period in which pollen grains are more viable compared to the afternoon period (Nicodemo et al., 2012).

Flight activity

Visual counts of the number of bees entering and leaving the hive during five-minute intervals were made throughout the period of flight activity of the bees. A random blocks design (blocks = days) was used, with four repetitions per colony in partitions divided in time, with five treatments (colonies managed in different ways) in the partitions.

Visits to the cucumber flowers

The frequency of bee visits was estimated during all the day by counting bees during 10 minutes each hour, from the first visit until bees stopped visiting, observed by walking through each greenhouse. A random blocks design (blocks = days) was used, with four repetitions per colony in partitions divided in time, with five treatments (colonies managed in different ways) in the partitions.

Cucumber pollination

To determine fruit production with and without bee visits, 25 pistillate flowers were selected before anthesis and some were covered with fine mesh nylon bags during part of the day, allowing bee visitation only during one of four periods: 6:00-8:30, 6:10:30, 6:00-12:30, 6:00-18:00 (n = 5 for each period). The other five remained covered, preventing visitation. This was repeated during five days for each of the bee access regimes. The percentage fruit set and mean fruit weight were measured later.

Statistical analyses

The data were submitted to analysis of variance. Multiple comparisons were made using the Tukey test, at a significance level of 5%, using SAS software (SAS Institute, 2003).

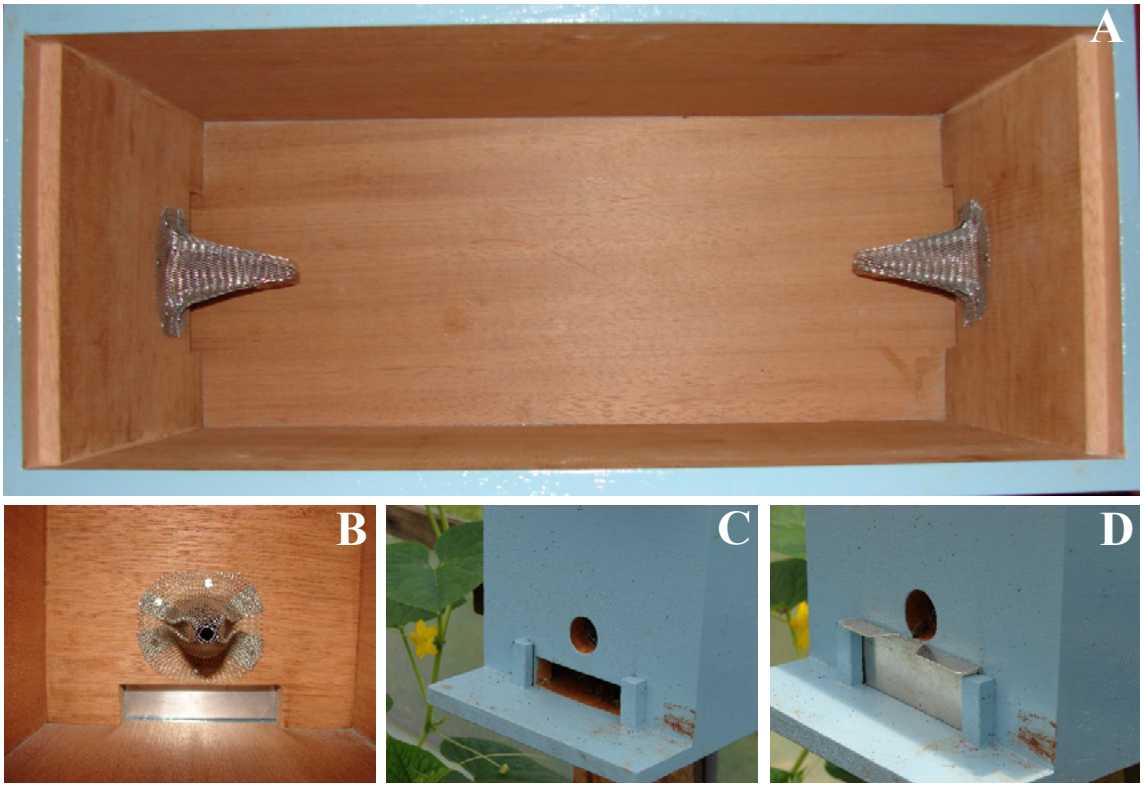

Fig 1. (A) View from above of the two-entrance hive without cover and frames; (B) internal view of the usual entrance which is closed and an upper entrance with a fine mesh metal screen funnel, external view of the hive installed in the greenhouse with the usual entrance (C) open and (D) closed.

Results

The bees quickly adapted to the two-entrance hives, including landing of foragers on the screens of the circular entrances, even when the normal entrance was available. The bees were not able to overcome the screen cones and were successfully directed to the inside or the outside of the greenhouses.

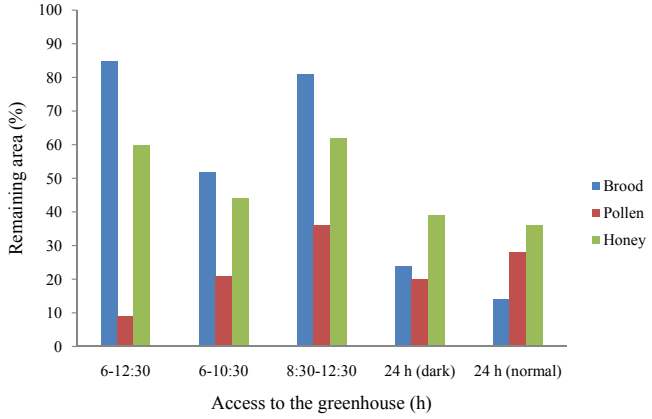

Fig 2. Remaining comb area (%) of brood, pollen and honey in colonies introduced into greenhouses in which the bees had access to the outside or not during a period of 10 days.

All of the colonies used in the greenhouses had a reduction in the comb areas occupied by bee brood, pollen and honey (Fig 2). This reduction was less accentuated in the two-entrance colonies, which were able to forage to the outside during part of the day. The reduction in the brood area among the colonies that were completely confined to the greenhouses was over 70%, indicating that these colonies would have a drastic population decline in the following weeks if they were to remain in the greenhouse.

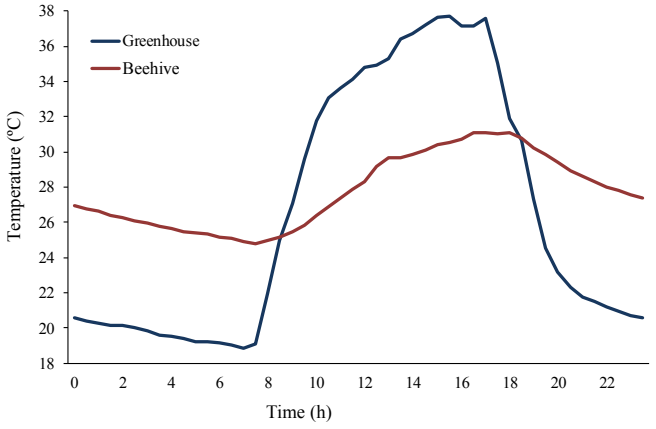

Fig 3. Mean temperature during the day within the greenhouse and within the hive (temperature probes on the floor of the hive and outside the hive at about 1.4 meters height). Temperatures were recorded daily during 10 days.

Bees were sometimes found to be lost within the greenhouses, independent of the management schedule; they kept hitting against the side screening or the plastic covering and did not return the colonies. The reduction in pollen in the comb was less in the colonies that had access to the outside earlier in the morning, with about 36% remaining after 10 days in the greenhouse. The greatest reduction (91%) in comb area with pollen was observed for the two-entrance hive that had the least access to outside the greenhouse. When comb area with honey was compared, the best performance was among bees with access to the outside, with 44–62% remaining. Among the colonies completely confined in the greenhouse, less than 40% of the comb area with honey remained.

Inside hive temperatures ranged from 24.8 to 31.1 ºC, compared to an ambient temperature of 18.8–37.7 ºC (Fig 3), demonstrating that these bees thermoregulated efficiently.

The flow of bees entering and leaving the hives was greatest in the colonies that could forage to the outside early in the morning (Fig 4). Besides beginning to work about an hour earlier than the other colonies when they had access to the cucumber plants, they also visited the plants more intensively.

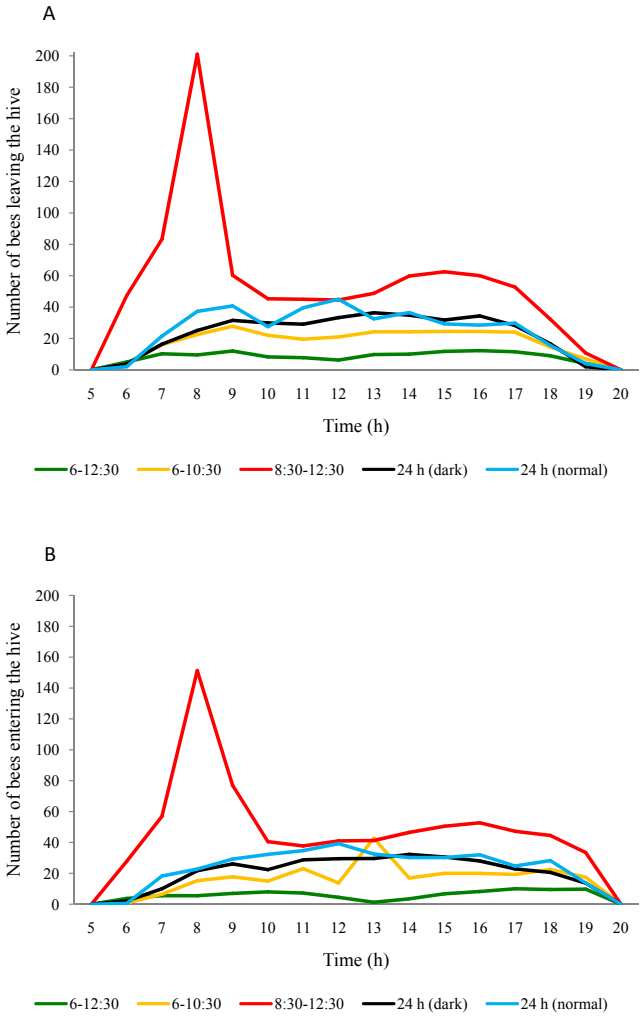

Fig 4. Number of bees leaving (A) and entering (B) the hives introduced into greenhouses in which the bees had access to the outside or not during a period of 10 days.

The number of bees that left the colonies to forage outside the greenhouse early in the morning, between 6 and 7:00 was 13.8 times greater than the mean number of bees that could only fly into the greenhouse.

After the bees stopped collecting nectar and pollen from the cucumber flowers at about 15:00 (Fig 5), the bees kept confined in the greenhouse no longer had access to outside food sources; however, they continued to fly in and out of the entrance. The number of bees returning to the hives after 16:00 was 25.2% of the total number of bees returning during the whole day.

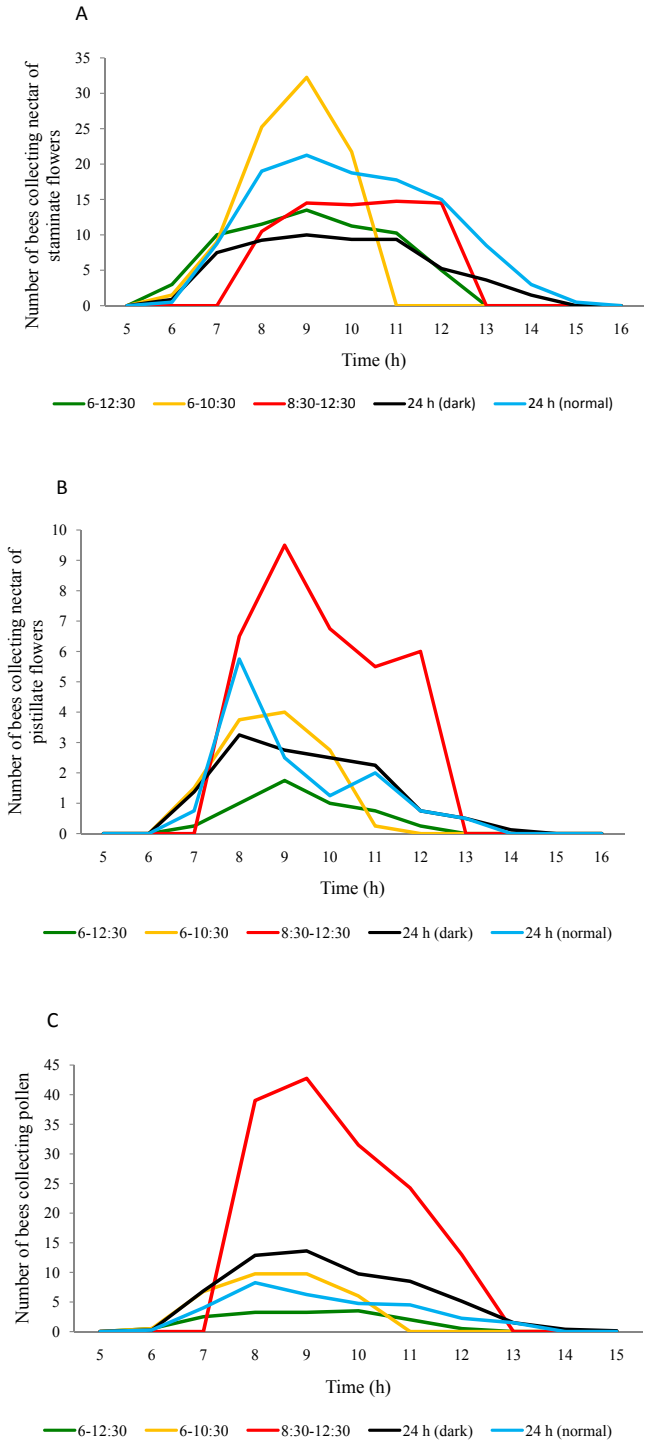

Fig 5. Mean number of nectar collecting bees visiting staminate (A) and pistilate (B) and pollen collecting bees visiting staminate (C) cucumber flowers in the greenhouse. Bees had access to the outside or not during a period of 10 days.

The number of bees flying out of the two-entrance colonies increased with the period of time available to forage outside the greenhouse. The total number of bees flying out of the hives among bees that could fly outside early in the morning and after 12:30 was 570% and 209% superior to the number of bees that left the colonies in hives that only had access to the outside after 12:30 and 10:30, respectively. These bees could collect food, resins and water from outside the greenhouse. When the main entrance was closed, the bees returned through the screen cone entrance within 30 minutes, both for bees coming from inside and outside the greenhouse.

Bees visited the flowers after initiation of the anthesis period, which began about 6:00 (Nicodemo et al., 2012), with a gradual increase in the frequency of visits until 9:00 in all of the greenhouses. From then on, the number of bees on the flowers declined. Comparing the different management schedules, the best performance was made by bees that had access to the outside early in the morning, especially when comparing pollen foraging on the cucumber flowers (Fig 5C), which is fundamental to assure pollination.

The bees from the colonies that were confined for three days in the dark before being introduced into the greenhouse made fewer visits to the cucumber flowers. Some of the workers of these colonies were occupied removing bees that had died during the time that the colonies had been kept closed in the dark. All of the greenhouses had both bees that visited flowers and bees that incessantly flew against the screening, trying to get out. Flowers that were not visited by bees did not produce cucumbers.

The visitation by the bees until 10:30 allowed the obtaining of good fruit set rates (Table 1). Allowing bees to continue foraging until 12:30 did not increase fruit formation. In fact, less fruit was formed when the flowers were visited by the bees during the entire of anthesis period. Fruit weight was greatest when the flowers were visited up to 12:30 (Table 2); the heaviest fruits were obtained when the bees could visit from 8:30 to 12:30 and from anthesis until 10:30.

Table 1. Cucumber fruit set (%) after four pre-established daily periods of visitation by bees that were maintained in hives that allowed access to the outside of the greenhouse during part of the day or not. Half of the 24h hives were maintained in a dark room for 72 h before placing them in the greenhouse (= dark).

Period of bee access to the greenhouse	**Period of availability of flowers to visitation of bees**			
	6:00 – 8:30	6:00 – 10:30	6:00 – 12:30	6:00 – 18:00
6:00–12:30	90	90	90	80
6:00–10:30	90	100	90	90
8:30–12:30	–	90	100	90
24h (dark)	80	100	80	80
24h (normal)	80	90	80	70

Table 2. Average weight of cucumber fruits (g) in four pre-established daily periods of visitation of bees that were installed in hives that allowed access to the outside of the greenhouse during part of the day or not.

Period of bee activity inside the greenhouse	Period of availability of flowers to visitation of bees			
	6:00–8:30	6:00–10:30	6:00–12:30	6:00–18:00
6:00–12:30	272.8 aB*	370.1 aA	261.6 bB	274.9 bB
6:00–10:30	270.8 aB	375.2 aA	371.4 aA	375.9 aA
8:30–12:30	–	375.2 aA	372.8 aA	373.1 aA
24h (dark)	265.4 aB	363.1 aA	243.5 bB	244.1 cB
24h (normal)	261.9 aB	357.8 aA	244.0 bB	247.2 cB

*Means followed by the same letter (upper case in the row and lowercase in the column) were not significantly different based on the Tukey test.

Allowing access to flowers until 8:30 was not sufficient to give satisfactory pollination; the fruits that came from flowers open to visitation until 10:30 were heavier, indicating that bees should be allowed to visit the flowers until this time. Allowing bees to visit flowers during the whole time of anthesis did not result in heavier fruit. Fruit was lighter when the bees visited the flowers in the greenhouse more than five hours per day.

Discussion

Besides helping overcome the reductions in brood, honey and pollen stores in the brood combs that occur in colonies confined to the greenhouse, the use of two-entrance hives could facilitate crop management, since the work can be done when the bees are foraging outside, without concern with getting stung. Pesticides can also be applied without needing to remove the hives from the greenhouse if the bees are directed to leave the crop and the hive is covered to avoid direct contamination.

Inadequate diet, especially when it involves protein, impedes the full development of the hypopharyngeal glands of the bees. The nurse bees then cannot adequately feed the larvae (Dustmann & Ohe, 1988; Crailshem et al., 1992). Greenhouses can have cultivated areas from less than 100 m² to over 1,000 m²; however, even in the larger greenhouses, the nutritional necessities of the bees will rarely be met, meaning that the bees will have deficiencies (Naug, 2009).

Using bees for pollination in greenhouses generally results in thermal stress for the colonies because greenhouses are normally hotter than the outside environment during the day (Cermeño, 1979; Sganzerla, 1995). Consequently, the colonies will need to put extra effort into temperature regulation in order safeguard their brood. The higher temperatures are beneficial for some crops, such as peppers (Sganzerla, 1995). However, for the bees the microclimate and the available food resources are normally not ideal. Since bees are essential for production of many crops because of their role in pollination, the use of a two-entrance hive could help reduce stress in comparison with the normal option of maintaining the bees inside the greenhouse 24 hours per day.

Bees that are allocated to the work of cooling the colony could otherwise participate in the collecting and processing of pollen and nectar if the temperature inside the hive was less of an issue. If the bees do not have access to the outside, a water supply can be offered within the hive for the bees to use for evaporative cooling (Couto & Couto, 2006).

An important factor to consider about the performance of the bees within greenhouses is the type of material used to cover these structures. The greenhouses that we used, similar to most used throughout the world, are covered with polyethylene plastic, which blocks ultraviolet light (Diaz & Fereres, 2007), which is important for bee navigation (Edrich et al., 1979; Guerra-Sanz, 2008). The efficiency of the bees in the greenhouse can be negatively affected by a lack of or low intensity of ultraviolet light (Dag & Eisikowitch, 1995).

The bees that had access to the outside early in the morning began to forage earlier than the bees in the other flight control regimes. The first hours of the day are especially important for bees that collect pollen (Free, 1993), an essential diet component for brood production (Herbert Jr. et al., 1977; Brodschneider & Crailsheim, 2010). In nature, the bees mainly collect pollen early in the day, because that is when most flowers open and become available (McGregor, 1976; Couto & Couto, 2006; Reyes-Carrillo et al., 2007).

Cucumbers plants of the Aodai cultivar are monoecious and highly dependent on animal pollinators, especially honey bees (McGregor, 1976; Klein et al., 2007). Other investigations have shown that honey bees are also efficient pollinators of cucumbers grown in greenhouses (Nogueira-Couto & Calmona, 1993; Nicodemo et al., 2013). Although the effectiveness of honey bees as pollinating agents of cucumber was already known, our work brings contributions regarding the management of hives into greenhouses, in order to facilitate the maintenance of colonies and pest management of crops when hives are introduced to the greenhouses for pollination services.

In our study, all of the management schemes helped produce cucumbers with an average weight above 350 g when flower visitation occurred up until 10:30. When bees were able to visit the flowers more than five hours per day, the

mean weight of the fruit was less than 300 g. Excess visitation can induce the bees to remove pollen grains that have been deposited on the stigmas, whether intentional or not, as was found in a field experiment involving pollination of pumpkin (*Cucurbita maxima*) in an area with 1,200 m² of plantings and 30 colonies of honey bees (Nicodemo et al., 2009).

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Authors' Contribution

RHNC collaborated with definition of the pollination methods. DDJ collaborated with the definition of methods for evaluating the performance of beehives. EBM was responsible for defining the statistical design. DN was responsible for designing the beehive with two entrances and collecting the data. All authors participated in the analysis of the results and in article writing.

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