

## Food supply in honeybee colonies improved kiwifruit (*Actinidia deliciosa* Liang & Ferguson) (Actinidiaceae: Theales) pollination services

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### Suplemento alimenticio en colonias de abejas para la mejora del servicio de polinización de kiwi (*Actinidia deliciosa* Liang & Ferguson) (Actinidiaceae: Theales)

**RESUMEN.** El modelo actual de agricultura determina una disminución en los hábitats seminaturales lo que conduce a una mala nutrición de las colonias de abejas, las cuales generalmente necesitan ser suplementadas con alimentos. Las abejas se utilizan para transferir polen entre plantas de kiwi (*Actinidia deliciosa* Liang & Ferguson masculina y femenina, aumentando así la calidad de la fruta y el rendimiento de los cultivos. El objetivo principal fue determinar el efecto de la estimulación de las colonias de *Apis mellifera* L. con suministros alimentarios estándar sobre la recolección de polen de kiwi. Los tratamientos (n = 5 colmenas cada uno) se realizaron en un huerto de kiwis en Mar del Plata, Argentina: Grupo J/A: suministrado con jarabe de azúcar (2:1) + suplemento proteico líquido ("Api-promotor®"); Grupo J/P: suministrado con jarabe de azúcar (2:1) + suplemento de proteínas sólidas ("patty"); Grupo J: suministrado con jarabe de azúcar (2:1); Grupo C: control, no suministrado. Las colonias abastecidas con J, J/P y J/A recolectaron más polen de kiwifruit que el tratamiento control, incluso bajo la presencia de otras especies florales en áreas cercanas. Aunque las abejas recolectaron la mayor parte del polen de otras especies de plantas de hábitats seminaturales, los tratamientos bajo estimulación artificial (J/P, J/A y J) pueden mejorar significativamente el servicio de polinización de kiwis que realizan las abejas.

**PALABRAS CLAVE.** *Apis mellifera*. Estimulación. Jarabe de azúcar. Polen. Suplemento proteico.

**ABSTRACT.** The current agriculture model determines a decrease in semi-natural habitats leading to poor nutrition for honeybee colonies, which usually need to be food supplemented. Honeybees are used to transfer pollen between male and female kiwifruit (*Actinidia deliciosa*

Liang & Ferguson) plants, increasing fruit quality and crop yield. Our main goal was to determine the effect of stimulating *Apis mellifera* L. colonies with standard food supplies on the collection of kiwifruit pollen. However, honey bees can also forage other flowering species in the crop site's surrounding areas. We selected kiwifruit as a model to analyze the effects of food supply on pollen collection of the target crop. The following experimental treatments (n = 5 hives each) were conducted in a kiwifruit orchard in Mar del Plata, Argentina: Group J/A: supplied with sugar syrup (2:1 + liquid protein supplements ("Api-promotor®"); Group J/P: supplied with sugar syrup (2:1 + solid protein supplements ("patty"); Group J: supplied with sugar syrup (2:1); Group C: control, not supplied. Colonies supplied with J, J/P and J/A collected more kiwifruit pollen than the other two treatments, even under other flowering species in areas nearby. Although honeybees collected most pollen from other plant species of semi-natural habitats, J/P, J, and J/A treatments can significantly improve the honeybees' kiwifruit pollination service.

**KEYWORDS.** *Apis mellifera*. Pollen. Protein supplemental. Stimulation. Sugar syrup.

## INTRODUCTION

In nature, plants supply bees with nectar and pollen to satisfy their nutritional requirements. Nowadays, the current industrial agriculture model leads to a nutritional deficit for pollinators and honeybees mainly because of the homogenization of the landscape related to monoculture proliferation and biodiversity loss (IPBES, 2016). Within this context, the supplementation diet is a technique that can compensate (to a certain extent) both nectar and pollen deficits for honeybee colonies, allowing their proper development (Standifer et al., 1977; Brodschneider & Crailsheim, 2010). Commonly, the food supply for honeybees contains sugars (sugar syrup, high-fructose corn syrup) and proteins (soybean flour, brewer's yeast, and dairy substitutes), which partially complement their natural food requirements (Araujo Freitas & Echazarreta, 2001; Barragán et al., 2015; Manning, 2016).

The simultaneous flowering of wildflowers near the crop could affect the pollinator movements between the semi-natural habitats and the target crop, negatively affecting the pollination service sought (Muñoz Rodríguez et al., 2005). Some studies support this hypothesis showing that *Apis mellifera* L. (Hymenoptera: Apidae) frequently preferred wild pollen-nectariferous plant species settled nearby crops (Andrada, 2003; Andrada et al., 2004; Joseph et al., 2019). Additionally, a reduced amount of stored pollen becomes the main factor stimulating the pollen foraging behavior; the stimuli seem to have three main components based on the number of young larvae (the amount of brood pheromone in the colony modulates the numbers of pollen foragers), amount of stored pollen, and proportion of occupied space (e.g., Dreller et al., 1999).

Kiwifruit (*Actinidia deliciosa* Liang & Ferguson) (Actinidiaceae: Theales) is a functionally dioecious species, presenting separate male and female individuals. Although both male and female flowers

produce pollen collected by bees, the female pollen is infertile, and none of these flowers produce nectar (Delaplane & Mayer, 2000). Moreover, the main strategies in kiwifruit pollination service involve the presence of *A. mellifera* hives (Goodwin et al., 2013). However, the efficiency of honeybees in pollen removal from kiwifruit flowers depends on its availability (amount of bloomed kiwifruit flowers), pollen size, and pollen scent (due to sexual dimorphism between sexes in kiwifruit) (Goodwin, 1986b, Pernal & Currie, 2002). However, according to Pernal & Currie (2001), honeybees are insensitive to pollen quality and generalist, visiting many types of flowers. Also, pollen offered by kiwifruit as a reward has been pointed out as a weak attractant for honeybees compared to the presence of nectariferous-flowering species in the surroundings, despite honeybees help to increase kiwifruit yields and fruit quality (Costa et al., 1993; Free, 1993; Howpage et al., 2001). In particular, colonies supplied with sugar syrup increased the kiwifruit pollen collection up to 100% (Goodwin, 1986a). Thus, such food stimulation is recommended when using honeybees to improve the pollination service for kiwifruit (Gardi et al., 2003; Gameda et al., 2018).

Within this scenario, our goal was to determine the effect of providing *A. mellifera* colonies with standard food supplies, such as sugar syrup plus two alternatives of protein supplements, on the collection of kiwifruit pollen. To test our goal, we collected pollen from the different flowering species in the crop site's surrounding areas during the flowering period of kiwifruit. Specifically, we aimed to analyze if there is a better combination of food supplements to honeybees that markedly increase pollen collection of kiwifruit, independently of the availability of other flowering species in the surroundings.

## MATERIAL AND METHODS

### Study site

Kiwifruit was selected as a model to analyze the effects of food supply on pollen collection of the target crop. The southeastern area of Buenos Aires province has adequate climatic and edaphic conditions for kiwifruit production. The experiment was carried out in a commercial orchard of *A. deliciosa* located beside kilometer 10 of 226 Provincial Road, in General Pueyrredón district, Buenos Aires province, Argentina. In recent years, commercial *A. deliciosa* growing areas have expanded rapidly through General Pueyrredón, and further expansion in the near future is also expected. Field experiments were conducted on a half-hectare with 5-year old kiwifruit plants (cv. Howard), with a 1:10 male-female ratio, from November 20<sup>th</sup> to December 1<sup>st</sup>, 2010.

### Characteristics of the hives

A total of 20 colonies of *A. mellifera* bees placed in Langstroth hives (4 treatments, N = 5 each) were used for the experiment. All the hives were equally standardized: 5 frames covered by open and closed brood, 4 frames with reserves, 8-9 frames covered by bees, honey super  $\frac{3}{4}$  with 9 frames, ventilation grille, inner cover, Doolittle internal feeder, and plastic pollen traps ("Apipolen" type). Queens in all colonies were sisters of the same age. The traps were placed between the brood chamber and honey super, while the entrances were closed to avoid foragers entering the hive. So, the time with pollen deprivation has been standardized equally among treatments.

### Treatments

Hives were placed in the orchard on November 21<sup>th</sup>, when kiwifruit flowering reached 10%, as Gardi et al. (2003) recommended. During the following days, flowering reached its maximum values (70-100% of opened flowers). Samples were collected once a day (one sample *per* colony) during the entire flowering period (five days). Each pollen sample represents the pollen loads collected for the colony from 9 AM to 1 PM. All colonies could reach the same flowers according to the foragers' range (*e.g.*, Dyer, 1996). Colony stimulation started 15 days before moving the colonies to the orchard, with a single supplementation for all colonies of 0.5 liters of sugar syrup (2:1). Once hives were placed alongside the kiwifruit orchard, 1.5 liters of syrup was supplied every 48 h during the whole trial (except for the control treatment). The colonies were divided into four groups of five hives each: **Group J/A**: energy stimulation with sugar syrup (2:1) and addition of liquid protein supplements ("Api-promotor®"); **Group J/P**: energy stimulation with sugar syrup (2:1) and supply of solid protein supplements ("patty"); **Group J**: energy stimulation with sugar syrup (2:1); **Group C**: control, no stimulation during the trial (without any artificial

stimulation). The treatment "only protein supplement" and "only water" were not used as treatments because protein supplement or water alone is not usually provided to hives by beekeepers.

Protein supplementation involved two alternatives according to the treatments. 1) Group J/A: a liquid supplementation diluted in sugar syrup, constituted by "Api-promotor®", rich in amino acids and proteins, provided within the hive's feeder every time sugar syrup was provided (10.5 ml *per* 1.5 liters of sugar syrup). 2) Group J/P: a solid supplementation (the "patty") made of soybean flour, fish flour, dry natural pollen, vitamins, and minerals (Apilab bee food mass®). The patty was placed only once on the top frames (one patty of 200 g *per* hive) two days before hives' transportation. This supplement was processed by bees slowly during the whole trial and contained high-row protein concentration (5.2% M/M).

### Sampling

Pollen collection by honeybees began around 9 AM and decreased in the afternoon (Goodwin, 1986b). Such a decline in pollen collection might be due to pollinators' continuous pollen removal during the day (Goodwin, 1995). Thus, once hives were settled within the orchard, a standard stimulus among treatment was induced through pollen deficit within the hives by trapping corbicular loads of pollen in the hive's entrance from 9 AM to 13 PM (Webster et al., 1985; Delaplane & Mayer, 2000). Pollen samples were collected once a day at the same time (1 PM), obtaining one sample *per* colony during five consecutive days, so, at the end of the trial, there were five samples *per* colony. Afterwards, the obtained samples of pollen were weighted (total pollen loads; *i.e.*, the total number of pollen pellets), and those samples exceeding 15 g were homogenized, and 15 g of pollen pellets were sub-sampled (Goodwin et al., 1994). Then, these sub-samples were used to separate and count pollen pellet according to their color (pollen type). The counted unity within the sample was each pollen pellet, classified by color according to Pantone 747 XR table to standardize color assignment. The number of pollen pellets of the different species was estimated in those samples heavier than 15 g, extrapolating counted pellets in 15 g to the total weight of each sample. The nearby flowering plants (possible food resources) to the *A. deliciosa* orchard were recorded as the available flora for honeybees. The pollen type for each plant species was identified using Wodehouse (1935) technique through a Nikon E600 microscope.

### Statistical Analysis

Statistical analyses were performed using R software (version 3.4.1, 2017). Even after applying several transformations, the data did not meet the conditions of normality and homoscedasticity, so it was decided to perform the analysis using a non-parametric test.

Kruskal-Wallis test was used to determine the effect of the treatments on (a) total number of pollen pellets collected *per day/hive*, (b) the proportion of kiwifruit pollen collected *per day/hive*, and (c) the proportions of pollen collected in the other plant species (available for honeybees) *per day/hive*. Differences among treatments were assessed using Wilcoxon test ( $p < 0.05$ ).

## RESULTS

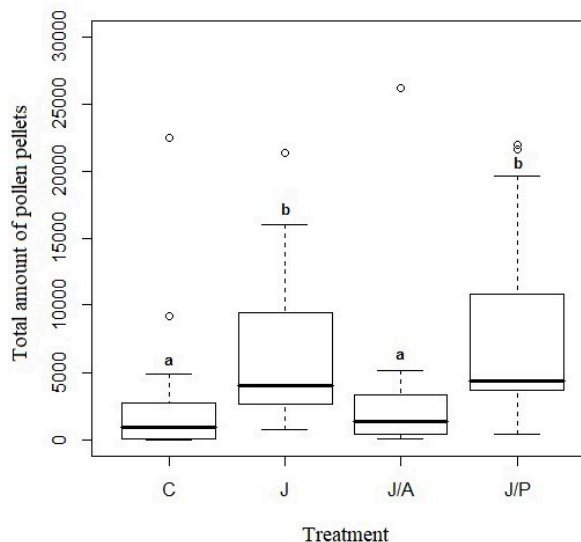
The number of pollen pellets collected by honeybees showed significant differences among the treatments (Kruskal-Wallis, Chi-square = 26.56,  $df = 3$ ,  $P = 7.29 \times 10^{-6}$ ) (Fig. 1). Treatments J/P and J did not show differences between them, but these treatments registered higher values than treatments C and J/A in the number of total pollen collected.

The proportion of the kiwifruit pollen collected by honeybees showed significant differences among the treatments (Kruskal-Wallis, Chi-square = 11.91,  $df = 3$ ,  $P = 0.007$ ) (Fig. 2). Treatments J/P, J/A, and J did not show differences in the number of kiwifruit pollen collected related to the total number of pollen pellets; these treatments registered higher and significantly different values than the control treatment (Fig. 2).

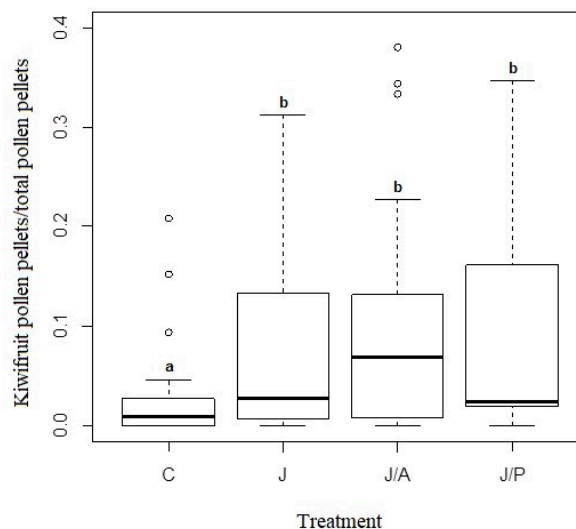
The palynological analysis of the corbicular pollen showed the presence of the following species: *Brassica campestris* L., *Rubus* sp., *Taraxacum officinale* (L.) Weber ex F.H. Wigg, *Raphanus sativus* L., *Pinus* sp., *A. deliciosa*, and one from an unidentified genus. *Brassica campestris* and *Rubus* sp. together gather 89.45% of the collected pollen during trials. The former was equally collected independently of the experimental treatment (Kruskal-Wallis, Chi-square = 1.04,  $df = 3$ ,  $P = 0.79$ ). Meanwhile, for *Rubus* sp. there were differences (Kruskal-Wallis, Chi-square = 20.27,  $df = 3$ ,  $P = 0.0001$ ). Treatments J/P and J did not show differences between them, but these treatments registered higher values (Wilcoxon rank test,  $P = 0.00228$  and  $P = 0.00035$  respectively) than treatments C and J/A, respect the amount of total pollen collected of this species. The collected pollen of the remaining species represents a lower proportion (<11%) of the total collected pollen.

## DISCUSSION

Other studies found that supplemental feeding increased foraging on targeted crops (Barker, 1971; Goodwin, 1986a; Goodwin et al., 1991; Gameda et al., 2018), while others did not (Free, 1964, 1967; Lois et al., 2020). These differences may be due to a complex interaction of different factors within the agricultural systems (e.g., climate, the nutritional quality of nectar and pollen, crop intensity, and semi-natural patches) (Goodwin, 1997; Benedek, 2003; Colwell et al., 2017; Garibaldi et al., 2020). In kiwifruit, our results showed that different combinations of food supplementation in honeybee colonies increased the collection of kiwifruit



**Fig. 1. Total amount of pollen pellets collected by colonies under different treatments: C (control, no food supply); J (syrup); J/A (syrup + api-promotor, liquid protein); J/P (syrup + patty, solid protein).** Different letters indicate statistical differences between treatments (Wilcoxon rank sum test,  $p < 0.05$ ).



**Fig. 2. Proportion of kiwifruit (*Actinidia deliciosa*) pollen pellets collected by colonies under different treatments: C (control, no food supply); J (syrup); J/A (syrup + api-promotor, liquid protein); J/P (syrup + patty, solid protein).** Different letters indicate statistical differences among treatments (Wilcoxon rank sum test,  $p < 0.05$ ).

pollen. Also, our results support what Goodwin (1986a) obtained in New Zealand, where sugar syrup was proposed as an efficient stimulus for honeybees to collect pollen from kiwifruit. Additionally, Goodwin et al. (1994) claimed that patty alone did not increase kiwifruit

pollen collection. Thus, it would be better to maintain the sugar stimulus when a protein supplement is added to fulfill hives' food requirements and maximize kiwifruit pollen collection. Although some beekeepers induced pollen deficit through pollen traps to increase foraging (Webster et al., 1985; Delaplane & Mayer, 2000) by augmenting the number of foraging bees (e.g., Fewel & Winston, 1992; Dreller et al., 1999), there is no empirical evidence supporting that such practice induces a better pollination service for *A. deliciosa*. Likely, the flowering crop length, pollination biology, and the flower density in the surrounding semi-natural patches are also variables to evaluate and manage (Garibaldi et al., 2020). As a stimulus to pollen foraging, pollen deprivation was an equally standardized variable among treatments, so the differences observed were independent of this factor. On the other hand, it is crucial to keep colonies without nutritional deficit, which improves their resistance and tolerance against viruses, *Nosema* infections (DeGrandi-Hoffman et al., 2016), and *Varroa* infestations (Annoscia et al., 2017). Therefore, providing protein and syrup together as a nutritional strategy (J/P, J/A treatments) increased the kiwifruit pollen's collection and also allowed protein supplementation.

*Brassica campestris* and *Rubus* sp. are pollen-nectariferous species (Andrada, 2003) and were highly visited by honeybees during the trial (89.5% of the total collected pollen). The flowering period of these two species overlapped with the flowering of *A. deliciosa*. *Brassica campestris* was evenly foraged independently of the treatment applied on hives. On the other hand, the pollen collection of *Rubus* sp. was greater under J/P and J treatments than under the other treatments. These patterns showed that honeybees were looking for additional nectar and/or pollen sources, independently of food supply. Hence, the forage of pollen did not change evenly among floral resources, so the pollination service for kiwifruit production through honeybees should be assessed according to the conditions of a given crop, considering the foraging dynamics in the nearby semi-natural habitats.

Future studies should focus on the dynamics in pollen foraging by honeybees for a particular crop, considering the relevance of studying the landscape complexity when delivering the pollination service for *A. deliciosa*. The semi-natural areas in the surroundings not only provide pollen and nectar resources for *A. mellifera*, but also sustain populations of native pollinators that could be involved in kiwifruit pollination. For example, *Bombus* and *Xylocopa* species might have a much more accurate pollen release mechanism through buzzing for this crop (De Luca & Vallejo-Marin, 2013). Thus, *A. mellifera* could be interacting with other pollinators during the flowering of *A. deliciosa* through a potential synergistic effect, which has not been studied yet.

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