

## *Varroa destructor*: ITS INTERACTION WITH *Apis mellifera* COLONY STRENGTH IN TWO LOCALITIES OF SAN LUIS POTOSÍ, MEXICO

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### ABSTRACT

The mite *Varroa destructor* is one of the greatest threats to the apiculture sector worldwide. Generation of knowledge on its behavior and of the factors that favor its development under different environmental conditions, as well as the level of influence of these factors, is essential to the development of strategies for integral management of the mite. With the objective of analyzing the interaction between the variables of *Apis mellifera* colony strength and the percentage of *V. destructor* infestation, a group of 40 beehives were monitored during two flowering seasons, spring and summer-fall, in San Luis Potosí, Mexico. The variables were analyzed using the Pearson correlation analysis as well as an analysis of variance with repeated measures in time ( $p \leq 0.05$ ). The level of *V. destructor* infestation was significantly related to the quantity of honey (+0.58) and pollen (+0.62) reserves and negatively correlated with hygienic behavior (-0.65). The percentage of *V. destructor* infestation in spring was not significantly different from that in summer-fall, while the quantity of food (honey and pollen) reserves in spring were statistically superior as of the second half of the season. Hygienic behavior had statistical differences only at the beginning of the flowering seasons; it was superior in summer-fall. However, this difference was not maintained over time.

**Keywords:** level of infestation, hygienic behavior, flowering season.

### INTRODUCTION

In Mexico, apiculture is one of the animal production activities that generates the most foreign currency for the country, reaching 65 million dollars in 2020 (SIAP, 2020). This activity benefits the rural sector, especially in marginalized areas where livestock production is not extensive (Medina-Flores *et al.*, 2014). San Luis Potosí is in a privileged position in Mexico where three different apicultural regions converge. This endows the region with great potential to produce honeys from different flowers. However, like the rest of the country and other regions of the world, bees face numerous challenges, among them is the presence of the ectoparasite *V. destructor*,

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considered by many researchers to be one of the most significant sanitary problems for *Apis mellifera* colonies worldwide (McMenamin & Genersch, 2015). At the national level, its frequency is more than 80%, and an annual loss of more than 33% of the bee colonies on the central high plateau and in the northern regions of the country is associated with the presence of the mite (Medina-Flores *et al.*, 2018). Its impact on management and profitability of apiculture is due to the major alterations that it causes in bee behavior, malformations, reduction in life expectancy and in honey production. However, the most catastrophic effect of the mite is its function as a biological vector of different RNA viruses and as a suppressor of bee immune response (Beaurepaire *et al.*, 2020).

Growth of the population of *V. destructor* depends on the balance between the rate of reproduction and the rate of mortality of the individuals inside the beehive at a given moment. However, they are affected by *A. mellifera*'s different mechanisms of resistance and tolerance, such as reduction in population growth, reduction of the post-capping period, and low fertility, fecundity and reproductive success of the founding females, grooming, hygienic behavior, cell size, self-medication, social apoptosis, attractiveness, and low susceptibility of the swarm play an important role in regulating the mite population (Strauss *et al.*, 2015).

Hygienic behavior is the ability of the workers to detect sick or parasitized larvae, discriminate between normal and abnormal larvae, and remove the cap and the sick larvae or the parasite. Even when this removal does not guarantee death of the mite, it can interrupt its reproductive cycle and decrease female fertility (Mondet *et al.*, 2020). Also, environmental conditions such as variations in temperature and relative humidity, beekeeper management practices, availability of pollen and flow of nectar, long brooding periods, and number of drone larvae can induce a drastic increase in the size of the mite population (Le Conte & Navajas, 2008).

To design strategies that tend to control and reduce the occurrence of this parasitosis in *A. mellifera* populations, it is necessary to quantify it, identify the factors associated with colony strength, apiary management and the different environments in which apiculture is practiced that contribute to the presence and development of *V. destructor*. The strength of a colony is understood to be the result of the interaction among the demography of the colony, energy sources, and the temporal and spatial pattern of availability of environmental resources in the landscape (EFSA-AHAW Panel, 2016). Therefore, the objective of this study was to analyze the role of the different factors associated with the strength of a colony that affect the *A. mellifera* – *V. destructor* interaction during two flowering seasons in two localities of San Luis Potosí, México.

## MATERIALS AND METHODS

### Monitoring scheme

In 2020, a group of 40 *A. mellifera* beehives in the High Plateau and Central regions of the state of San Luis Potosí were monitored during two flowering seasons. Spring

(April-May) monitoring was carried out in the locality of El Mezquite, municipality of Villa de Arista at the coordinates 22° 40' 46.10" N and 100° 55' 4.85" W, altitude 1621 m. Climate is dry and warm with an average temperature of 22.8 °C, low of 13.5 °C and high of 32.1 °C. Total precipitation during the sampling period was 13.8 mm. Here, the mesquite (*Prosopis laevigata*) is the species of greatest importance for apiculture of the season. Later, at the beginning of the summer-fall season (August-October) the same group of beehives were transported to the locality of El Mezquital, municipality of Villa de Arriaga, at the coordinates 22° 7' 45.61" N and 101° 16' 33.62" W, altitude 2160 m; climate is semiarid temperate with mean temperature of 16.5 °C, low 8.7 °C and high 24.4 °C. Total precipitation during the period was 76.4 mm. This season is characterized by a broader diversity of floral resources, although *Bidens odorata* is the species recognized traditionally as the most important of the season. The data on low and high temperatures during the sampling periods were requested from the Coordinación General del Servicio Meteorológico Nacional, CONAGUA, for the weather stations closest to the location of the apiaries (24098 and 32127).

The sampling period was 45 days; according to the knowledge and experience of local migratory beekeepers, this is the average resident time of a beehive in a site during one harvest. Sampling was carried out every two weeks during each flowering season. The monitored beehives had a Jumbo-type brood chamber (46.5 cm long, 38 cm wide and 29.5 cm high) with capacity for 10 frames and no honey super. For each beehive, we used one commercial freely fertilized Italian queen in her first year of production. Before monitoring, the queens were introduced to orphan beehives in wooden cages for their recognition. On the third day after introduction, the candy stopper (mixture of powdered sugar and honey) that obstructed the exit from the cage was perforated with a wooden toothpick to stimulate the worker bees to eat the candy and conclude the release of the queen. Finally, one week later, a routine inspection of the hive was carried out to verify that the queen had initiated oviposition. The hives had homogeneous conditions in terms of honey and pollen reserves, brood, and adult bee population, as well as statistically similar levels of *V. destructor* infestation. The parameters in the brood chamber of all the hives were estimated using the method described below.

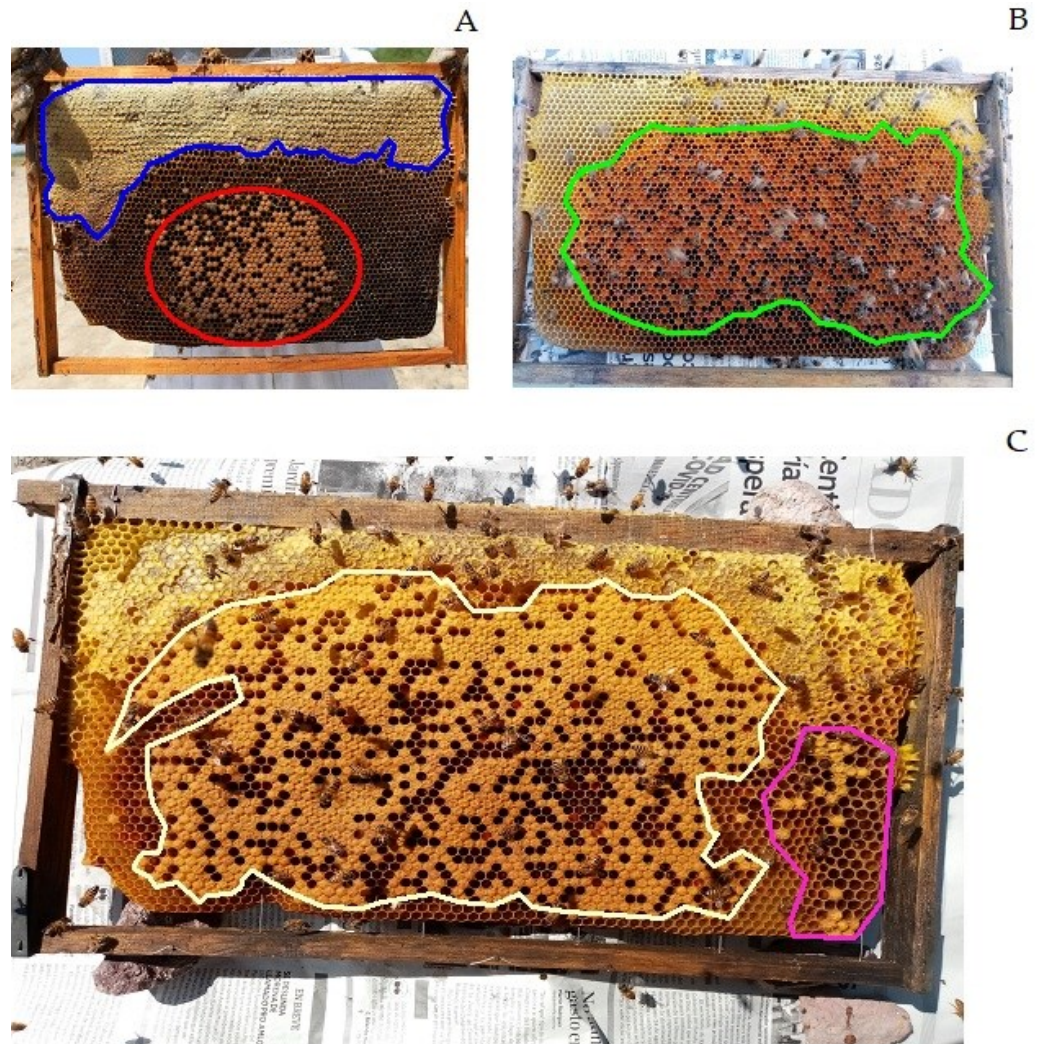
**Brood solidness (BS):** This pattern was determined by placing a grid delimiting 100 cells over a section of capped brood. Empty cells were then subtracted to estimate the percentage of brood solidity. The procedure was repeated on different patches of brood to derive a mean of at least ten observations.

**Adult bee population (ABP):** The adult bee population was determined by the difference in weight in kilograms between the complete hive during the night and the sum of the weight of its elements without adult bees.

**Hygienic behavior (HB):** The percentage was estimated using the technique of perforating 50 capped brood cells using an entomological pin #00. The area was delimited with colored pins, and 24 h later it was inspected again to estimate the percentage of removed brood.

*V. destructor* infestation percentage (VDIP) in adult bees: This was determined by the jar method described by De Jong *et al.* (1982).

Worker brood (WBR), drone brood (DBR), capped honey (CH), and pollen stored in the brood chamber (POL): Each of these was determined as the area in square centimeters occupied by each type of input by analyzing photographs of all the frames in the brood chamber (Figure 1) using the ImageJ tool of the National Institute of Health or by counting individual cells.



**Figure 1.** Variables evaluated in the brood chamber. A: capped honey outlined in blue; worker brood outlined in red; B: stored pollen marked in green; C: worker brood inside white line; cells of the drone brood outlined in pink are larger and projected outward.

### Statistical analysis

Data were analyzed with the SAS tool OnDemand for Academics: Studio. Initially, a bivariate analysis was performed with Pearson correlation ( $p \leq 0.05$ ) to establish the degree of association between the study variables. An analysis of variance with repeated measures ( $p \leq 0.05$ ) was then applied considering two treatments: spring (Villa de Arista site) and summer-fall (Villa de Arriaga site). For each treatment, the 40 hives were considered repetitions. This analysis was applied to only the variables that had significant correlations. To satisfy the assumption of normality, the data on capped honey and worker brood were transformed to square root, the quantity of pollen to logarithm, and the percentage of *V. destructor* infestation to arcsine of the square root. The results for these variables were presented as means expressed in transformed units.

### RESULTS AND DISCUSSION

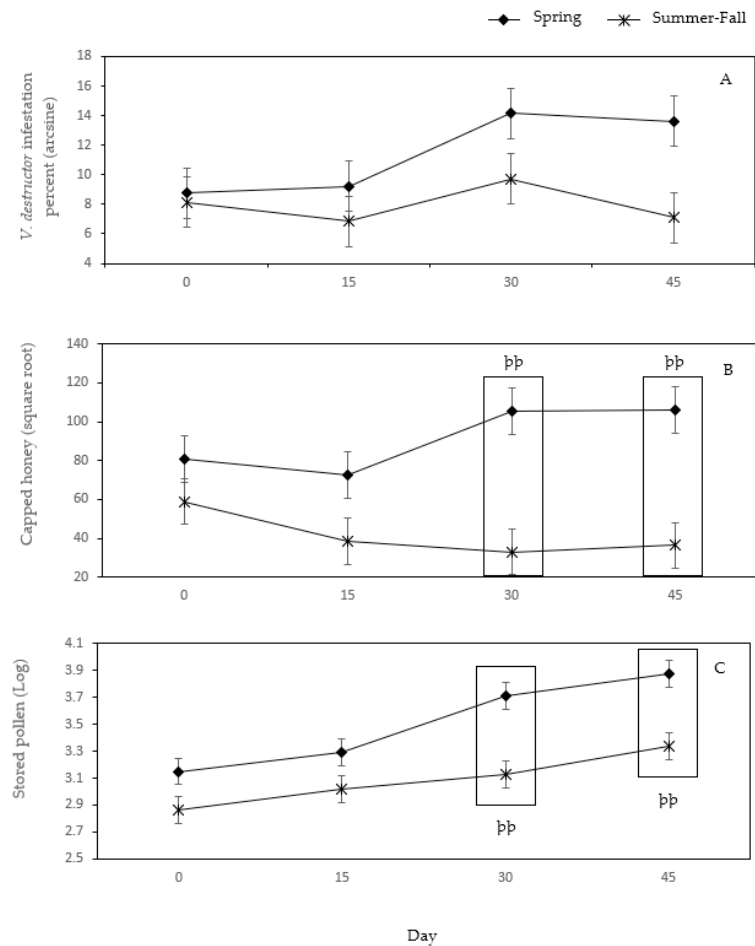
The results of this study enable analysis of the complex relationship between *A. mellifera* and *V. destructor*. The correlation analysis shows that only the variables honey (+0.58) and pollen (+0.62) reserves, as well as hygienic behavior (-0.65), correlated significantly with the level of mite infestation (Table 1).

**Table 1.** Pearson correlation between variables of colony strength and percentage of *V. destructor* infestation.

|                    | CH <sup>†</sup>     | POL                | WBR   | DBR   | BS                 | HB                  | ABP   |
|--------------------|---------------------|--------------------|-------|-------|--------------------|---------------------|-------|
| POL <sup>‡</sup>   | 0.65 <sup>bb</sup>  |                    |       |       |                    |                     |       |
| WBR <sup>§</sup>   | -0.15               | 0.24               |       |       |                    |                     |       |
| DBR <sup>¶</sup>   | 0.29                | 0.10               | 0.27  |       |                    |                     |       |
| BS <sup>**</sup>   | -0.22               | -0.02              | -0.02 | -0.14 |                    |                     |       |
| HB <sup>††</sup>   | -0.66 <sup>bb</sup> | -0.54              | -0.34 | -0.20 | 0.17               |                     |       |
| ABP <sup>‡‡</sup>  | -0.56 <sup>bb</sup> | -0.00              | 0.36  | -0.20 | 0.40 <sup>bb</sup> | 0.27                |       |
| VDIP <sup>§§</sup> | 0.58 <sup>bb</sup>  | 0.62 <sup>bb</sup> | 0.33  | 0.24  | 0.07               | -0.65 <sup>bb</sup> | -0.16 |

<sup>†</sup>CH: capped honey; <sup>‡</sup>POL: stored pollen; <sup>§</sup>WBR: worker brood; <sup>¶</sup>DBR: drone brood; <sup>\*\*</sup>BS: brood solidness; <sup>††</sup>HB: hygienic behavior; <sup>‡‡</sup>ABP: adult bee population; <sup>§§</sup>VDIP: *V. destructor* infestation percent. <sup>bb</sup>Significant correlation at  $p \leq 0.05$ .

Regarding the level of *V. destructor* infestation, no significant differences were found between seasons during the 45 days of monitoring (Figure 2a), likely because the differences in climatic conditions of the two flowering seasons were not so accentuated or significant. As Szabo & Walker (1996) mention, the populational development of the mites may be more favored by the continuity that exists in the availability of *A. mellifera* broods in hot, humid climate regions and not in temperate climates, as is the case of this study. Medina-Flores *et al.* (2014) and Tapia-González *et al.* (2019) coincide with this hypothesis since they did not find significant differences in *V. destructor*



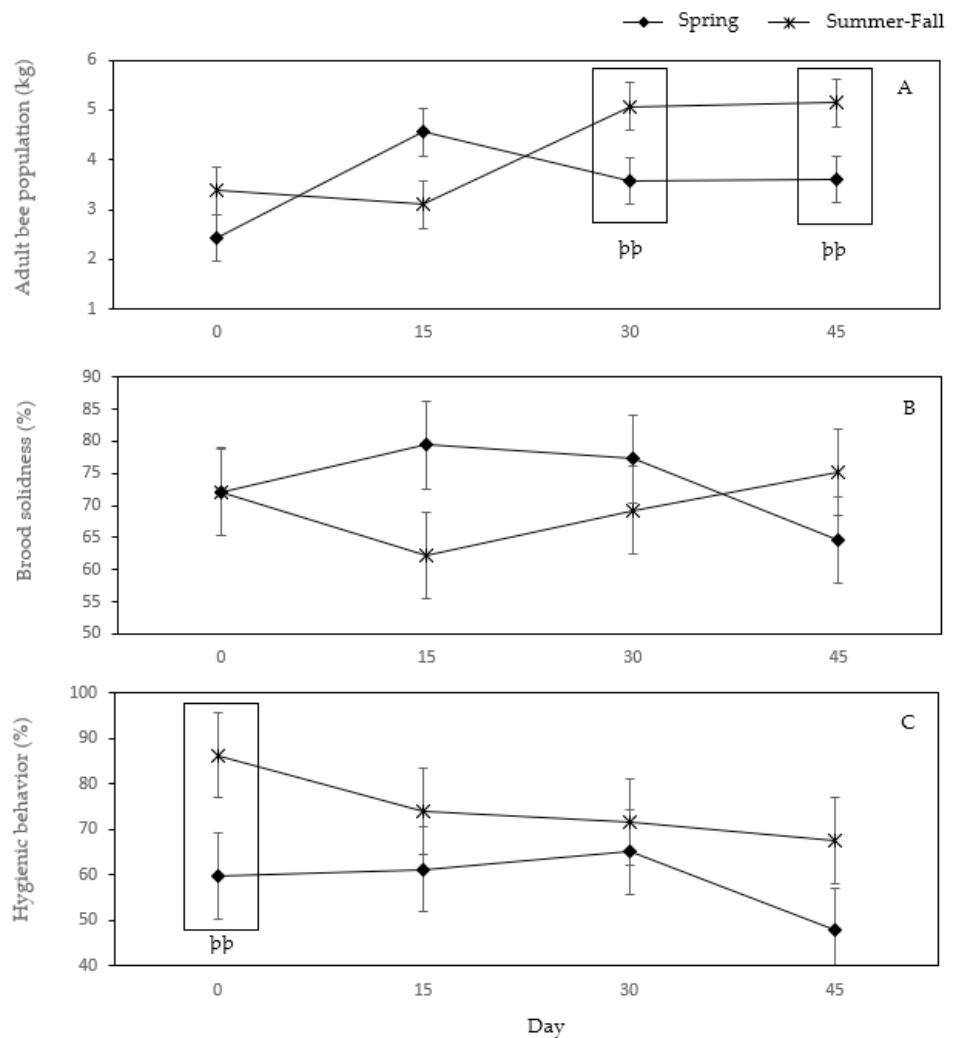
**Figure 2.** Behavior of honey and pollen reserves and level of *V. destructor* infestation during two flowering seasons (spring and summer-fall) in San Luis Potosí, Mexico. The values of the variables are presented in transformed units. <sup>p̂p̂</sup>Significant difference at  $p \leq 0.05$ .

infestation levels in conditions of temperate semiarid and hot climates in the states of Zacatecas and Jalisco (Mexico), respectively.

Regarding hygienic behavior (HB), a significant negative correlation ( $r = -0.65$ ) was found with level of *V. destructor* infestation (Table 1). This coincides with results obtained by Masaquiza-Moposita *et al.* (2017), who found a negative correlation ( $r = -0.54$ ) between HB and the number of mites parasitizing adult bees. In contrast, Strauss *et al.* (2015) found no correlation between the rate of brood removal and *V. destructor* infestation levels in colonies of *A. m. scutellata*, but this correlation did exist with European *A. mellifera* hybrids. In this regard, Medina-Flores *et al.* (2014) mention that there are many inconsistencies among the results found in the literature on the degree of influence hygienic behavior can have on the level of *V. destructor* infestation, even when these studies have assessed this interaction in bee colonies selected for

their expression of HB and fertilized artificially over generations. These authors attribute this variation in the results to a strong environmental influence that exists in the expression of hygienic behavior, as well as to methodological differences in the experiments.

When analyzing the effect of interaction of the flowering season in each sampling period, we observed that hygienic behavior had statistical difference only at the beginning of the experiment; it was higher in summer-fall, with a mean of 86.3% compared with 59.8% in spring. However, this difference was not maintained during the rest of the experiment (Figure 3c). Likewise, Medina-Flores *et al.* (2014) found similar percentages of HB in spring and fall, despite the important variation in the expression of hygienic behavior during the entire experiment.



**Figure 3.** Behavior of the adult bee population, brood solidness, and hygienic behavior during two flowering seasons (spring and summer-fall) in two communities of San Luis Potosí, Mexico. The variables are presented in their original units. <sup>bb</sup>Significant difference at  $p \leq 0.05$ .

In this sense, it is important to consider that HB plays an important role in the natural resistance of *A. mellifera* populations to *V. destructor*. Also, although the trait is genetically determined (Locke, 2016), environmental factors, as with the beekeeper's management techniques, can strongly affect its expression. Uzunov *et al.* (2014) studied 21 apiaries in different parts of Europe and 16 genotypes of different European subspecies of honeybees from 2009 to 2012 and concluded that the expression of HB was highly affected by harvest season and location of the apiaries, factors that interact to generate unique combinations of nectar and pollen availability and translate into widely diverse conditions of colony strength.

It is not possible to speculate that the HB response is directly influenced by the strength of the population since there was no correlation with ABP. Moreover, the population had statistical differences as of the second half of monitoring; ABP was higher in summer-fall with 5.07 kg on day 30 and 5.14 kg on day 45, against 3.58 and 3.61 kg in spring, respectively (Figure 3a). In the same sense, a negative correlation was found between HB and the amount of capped honey (CH) in the brood chamber ( $r = -0.66$ ). The amount of capped honey was statistically different between seasons as of day 30 of monitoring (Figure 2b); there was more in spring than in summer. This difference in the hive's honey storage capacity is possibly associated with a decrease in the foraging activity of worker bees derived from higher precipitation during the summer and thus fewer sunny days during the season. It has been shown that the presence of rains in the foraging areas decreases traffic of pollinating insects by up to 30% (Linares *et al.*, 2021). It is also possible that there is a more abundant offer of nectar during the spring. This coincides with Medina-Flores *et al.* (2019), who assessed the same flowering seasons as in our study and observed a more abundant honey reserve during the spring than in the fall.

The abundance of reserves derived from a greater availability of nectar in the field could have limited the expression of HB and thus no differences were observed in the level of HB once the flowering seasons began. This phenomenon could be related to the distribution of tasks within the colony since the hygiene bees are able to interchange the activity of collecting nectar and hygienic behavior depending on the needs of the colony (Momot & Rothenbuhler, 1971). In contrast, Janmaat & Winston (2000) consider that the abundance of nectar and pollen is a factor that could motivate the expression of HB because it generates a greater need for storage space.

The brood solidness (BS) is a qualitative variable that can contribute to the evaluation of the health status of the colony. The results show that BS was equally consistent during both seasons (Figure 3b) and correlated positively with ABP (+0.40), which is reasonable since BS is an indicator of brood abundance and homogeneity, as well as of the egg-laying performance of the queen. A disperse pattern is sign of a problem in oviposition due to low sperm quality, egg death, etc. (EFSA-AHAW Panel, 2016), and it is an efficient predictor of the future adult population.

Regarding the variable stored pollen (POL), the colonies had statistical differences between seasons as of day 30 of monitoring (Figure 2c), and it was higher during the

spring. At the same time, a significant correlation was observed between CH and POL (+0.65). This is logical since both products (nectar and pollen) are available in the field at the same time during flowering and are the mainstays for feeding the colony. The larger quantity of pollen stored by the colonies during the spring could be associated to the type of species that are flowering. Smart *et al.* (2017) mentions that even when the supply of plant resources in flower is highly diverse, the bees tend to concentrate their efforts on gathering a few species, either because the preferred species are more abundant or because they supply specific nutrients that the colony needs at a given moment. Therefore, although the summer season may offer a greater diversity, it is possible that the species of interest for the *A. mellifera* colonies were less abundant and, during the spring, the colonies showed greater preference for the species that were flowering and gathered a larger volume of pollen.

It is difficult to speculate on the positive correlation between the quantity of capped honey (+0.58) and pollen (+0.62) stored in the brood chamber and the level of *V. destructor* infestation. The level of infestation had only a slight numerical, non-statistical difference during the second half of monitoring, despite the significant increase in the quantity of honey and pollen reserves as of day 30. In contrast, the negative correlation observed between CH and the adult bee population (-0.56) is supported by the behavior of both variables. For example, during summer, the significant increase in population as of day 15 led to a significant reduction in the quantity of CH as of day 30 since the larger population consumes more food and therefore the reserves decrease (Medina-Flores *et al.*, 2019). In the same sense, this interaction between the quantity of CH and ABP could explain to a certain extent the correlations between VDIP and CH and between VDIP and POL. Considering that the quantity of food reserves seems to be a good predictor of the size of the adult population, the reduction in the population derived from shorter longevity of the bees caused by *V. destructor* parasitosis during the larval stage (Aldea & Bozinovic, 2020) is reflected in the increase in food reserves. The above makes it clear that the characteristics of some *A. mellifera* colonies that seem to have an influence on the mite populations may not have any influence in other colonies; the differences may be even more marked under different environmental conditions. However, the development and implementation of strategies aimed to monitor populations of this parasite, identify their economic thresholds, develop adequate prevention techniques, and apply treatments step by step in accord with the needs of the colony would allow us to reach an effective integral management strategy to deal with *V. destructor*.

## CONCLUSIONS

Of the flowering seasons in the study area, summer seems to offer better conditions for the development of *A. mellifera* colonies, allowing significant growth of their population. Both the quantity of honey and pollen reserves and the adult population are the only variables associated with colony strength and seem to be affected by the flowering season, in such a way that the oviposition pattern will be determined by

intrinsic factors likely associated with the reproductive performance of the queen. Moreover, we confirm that the expression of hygienic behavior is affected by a large number of factors and so can vary significantly over time and even within the same flowering season. Although it correlated strongly and negatively with the level of *V. destructor* infestation, it cannot be considered a reliable predictor of the population dynamics of the mite. Likewise, even when the quantity of food reserves has an important positive correlation with the variable of interest, it is not necessarily a causal relationship.

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