Bee health: Determining the causes affecting honeybees' productivity (*Apis mellifera*)

Verónica R. Olate[1], Mayda Verde[1], Leslie Vallejos[1], Leonel Perez Raymonda[2], Maria C. Cortese[3], and Marnix Doorn[1*]

Affiliations:

[1] Center for Systems Biotechnology, Fraunhofer Chile Research, Avenida Del Cóndor #844, Piso 3, Huechuraba, Santiago, Chile

[2] Escuela Agrotécnica Libertador Gral San Martín, Universidad Nacional de Rosario, Ovidio Lagos 1000 2170 - Casilda, Santa Fe, Argentina

[3] Faculty of Veterinary Sciences, Universidad Nacional de Rosario, Bv. Ovidio Lagos 1000, S2170 Casilda, Santa Fe, Argentina.

*__Corresponding author at__:

Center for Systems Biotechnology, Fraunhofer Chile Research, Avenida Del Cóndor #844, Piso 3, Huechuraba, Postal code 8580704, Santiago, Chile. E-mail address: marnix.doorn@fraunhofer.cl (Marnix Doorn).

Abstract

1. Insects are responsible for the quantity and quality of one-third of all agricultural production worldwide through pollination. The quality of the pollination service and the safety of the honey production depends on the health and nutritional condition of the hives, which, for an important part is related to management practices.
2. This study aims to identify the stressors that lead to the loss of bee health and its consequences for the productivity of the hives. Different aspects related to management practices, productivity, clinical observations related to diseases, presence of health issues in the hives or in the apiaries, to the structure of the hives, weather and infestation rates by *Varroa* sp. mites were measured. The information was collected during two field surveys in 53 apiaries in the Province of Santa Fe, Argentina.
3. The results show correlations among many of the management practices, health condition and productivity of the hive, with most importantly the change of the bee queen, the disinfection of the beekeeping material and the number of combs in the brood chamber.
4. Although honey production is important in the region, the hive structure was deficient and inadequate during both surveys. Due to its dependence on management by the beekeeper, this suggests that a holistic approach can improve the hive structure, increasing the honey production.

RESUMEN

1. Se estima que los insectos polinizadores son responsables de la cantidad y calidad de un tercio de la producción agrícola a nivel mundial. La calidad del servicio de polinización y la inocuidad en la producción de miel depende de las condiciones sanitarias y nutricionales de las colmenas, lo que en gran parte, está asociado a las prácticas de manejo.
2. El objetivo de este estudio es identificar los estresores que provocan la pérdida de salud de las abejas y sus consecuencias para la productividad de las colmenas. Se evaluaron diferentes aspectos relacionados a las prácticas de manejo, productividad, observaciones clínicas relacionadas a enfermedades, presencia de brechas sanitarias en las colmenas o en los apiarios, estructura de las colmenas, condiciones climáticas y las tasas de infestación por *Varroa* sp. La información fue recopilada durante dos encuestas de campo en 53 apiarios de la Provincia de Santa Fe, Argentina.
3. Los resultados muestran que las distintas prácticas de manejo, la condición de salud y la productividad de la colmena se correlacionan en mayor medida con el cambio de abeja reina, la desinfección del material apícola y el número de panales en la cámara de cría.
4. A pesar de que la producción de miel es importante en la región, la estructura de la colmena fue decifiente e inadecuada en ambos monitoreos. El manejo de las colmenas es dependiente del apicultor, por lo tanto, una mirada holística puede mejorar la estructura de la colmena, incrementando la productividad de las colmenas.

KEYWORDS:

Apis mellifera, Bee health, Beekeeping, Honey, Honeybee, *Varroa* sp.

1. INTRODUCTION

Insects are responsible for the quantity and quality of one-third of all agricultural production worldwide through pollination. This is equivalent to approximately 235 to 577 billion dollars per year, which is comparable to the gross domestic product of a country like Argentina (Heiblum 2019). Although crop yield and quality depend on both the abundance and diversity of pollinators, in the particular case of the honeybees, they can be confined and managed in hives, which allows them to be transported (Moritz et al., 2005; Pirk et al., 2017). This enables honey production in different territories and to meet the demand for pollinators during bloom. (Isaacs, 2017, Klatt et al., 2014; Klein et al., 2007; Potts et al., 2010; Aizen et al., 2008; Aizen et al., 2009). The quality of the pollination service and the safety of the honey production depends on the health and nutritional condition of the hives, which in turn depends for an important part on management practices (Doorn et al., 2015).

According to the OIE (2019), there is a critical relationship between animal health and animal welfare. An animal or an animal population is healthy when it approaches its maximum productive potential. Animal welfare is expressed when a population grows fattens and reproduces (Verde et al., 2013). Different factors affect the health of *Apis mellifera*, and consequently on the amount of honey produced. Honeybees are challenged by environmental stresses that reduce colony survival (Dolezal et al., 2019). Over the last three decades, hives have been suffering from numerous health issues caused by the impact of climate change, landscape transformation with the introduction of exotic species that cause habitat changes, pollutants, toxins, pests, diseases and competition for resources (Braga et al., 2020; Pirk et al., 2017). Therefore, Cantalapiedra et al. (2017) note that bee health can be due to an undefined sum of causes.

Argentinian beekeepers contribute 20% to the word’s total honey exports (Consejo Federal de Inversiones, 2011). Country-wide the average honey production is estimated at 25 kg per hive per year. Productivity is highly variable throughout the territory due to the diversity of the ecosystems’ flowering plants on the one hand and different technological capacities of producers on the other (Carrasco et al., 2012; Aizen et al., 2009; Manuel-Navarrete et al., 2005). The Province Santa Fé represents 12% of national production (9,7 tons of honey), providing an estimated income of 21,7 million dollars per year (IPEC, 2020). Beekeeping is taken forward in a landscape where the intensive cultivation of oilseeds (soybean, sunflower and corn), wheat and sorghum is predominant. Beehive losses are estimated at around 34% (Requier et al., 2018). The losses are attributed to the indiscriminate use of agrochemicals and malnutrition (Lende, 2015). However, no publications were found that determine different factors that condition the bee health status in Santa Fé.

To determine the health of the managed honeybee populations requires the predisposing factors that provoke disease, even when these cannot be considered as direct causes. This study aims to identify the stressors that lead to the loss of individual and/or collective bee health in the Province of Santa Fe, Argentina. The health issues present in the apiaries are characterised and the monitored variables are related to the average kilogramme honey production per hive reported by the beekeepers. The data collected provide criteria that allow the formulation of strategies to enhance bee health and improve management practices. Using different criteria allows taking a holistic approach, aimed at re-establishing the dynamic balance of the hives. This forms the basis for a healthy bee population and the sustainability of the sector.

2. MATERIALS AND METHODS

2.1. Experimental design and hive selection

To test the hypothesis that bee health depends on multiple factors and is related to productivity, 53 apiaries located in the south of Santa Fe Province, Argentina, were studied during 2019. Santa Fé Province is located between parallels 28º and 34º South latitude and meridians 59º and 63º (Figure 1). The selection was based on criteria such as production, homogeneity of the agricultural-economic zones (Castignani, 2011), and the location near the access routes. Two field surveys were carried out. The first survey (53 apiaries, 265 hives) was taken forward between April and May, coinciding with the autumn. The second (49 apiaries, 241 hives) was taken forward between September and October, at the beginning of spring. Monitoring was carried out with the owner's consent, selecting five hives at random from each apiary. Hives with large amounts of dead adult bees at the entrance, with only dead bees inside and with decomposing brood or orphaned hives, were excluded. The selected hives were labelled with an alpha-numeric code. During the second monitoring, those hives that were not physically found due to abandonment or death were recorded and replaced. All the hives in the experiment were managed under the same conditions as the rest of the hives in the selected apiary.

2.2. Methodology for collecting field data

Field data were collected with surveys designed and validated for this purpose. The following aspects were considered: General information about the beekeeper and socio-environmental elements that may impact or are related to bee health; zootechnical and sanitary factors, which could predispose or be related to the development diseases affecting adult bees and/or their offspring; manifestations of clinical signs related to diseases affecting adult bees or their brood, and presence of health issues in hives and the apiaries a whole. Before intervening in the hives, the wind speed (km/h), the geographical location of the apiary (GPS), latitude (m.a.s.l.), temperature (°C), relative humidity (%RH) and the number of bees entering the hive entrance during one minute were recorded.

2.3. Infestation rate (IR%) by *Varroa* sp. mite

To complement the information obtained during the survey, the rate of infestation by the *Varroa* sp. mite was calculated using a standard method (Dietemann et al., 2013). For this purpose, a sample of about 300 adult bees was collected from frames with closed brood. The bees were preserved in hermetically sealed glass jars containing a hydroalcoholic solution (75% ethanol). The bottles were labelled and transported on ice (0ºC) to the laboratory for further analysis.

2.4. Structure of the hive

The structure of the hives was determined by the semi-subjective Liebefeld method, based on visual estimates by an observer (Delaplane et al., 2013). Briefly, all the combs of the selected hives were considered, according to the corresponding brood chamber (1, 2, etc.). During the review the following parameters were used: adult bee population, amount of open and closed brood, and the proportion of honey and pollen. The minimum unit of quantification used is 1/4 of one side of the frame and the sum of both sides (8/4) is equivalent to the result obtained for each frame (two sides of the comb).

2.5. Statistical analysis

To build a database for statistical analyses, the information was processed, weighted and entered according to the date of monitoring and the type of variable. Statistical and descriptive analyses were performed in the software IBM SPSS 22.0. Descriptive analyses are reported as a frequency or per cent of the total sample, and the arithmetic mean values ± SD, depending on each variable. To establish a possible correlation between variables, a bivariate Pearson's correlation analysis was carried out (95% confidence). The respective correlation coefficient (Pearson's r) and the significance value (p) were registered in each case. To find significant differences and to explain the variability between the studied apiaries and hives, according to the estimated production (Kg of honey per year), non-parametric tests were applied (Kruskal-Wallis or Mann-Whitney U tests, $\alpha = 0.05$). Finally, to obtain an estimated amount of honey produced according to the most relevant variables in this study, a predictive algorithm was applied (decision tree methodology, CHAID algorithm, $\alpha = 0.05$).

3. RESULTS

3.1. Field information

3.1.1. General characterisation of the participants

In total, 53 beekeepers with a diverse level of experience (Mean±SD = 19.2±14.6 years) (see Fig. S1-A in Supplementary information) were surveyed. The number of hives (Langstroth) per beekeeper fluctuates between 8 and 1100. The majority (58.5%) manages between 8 and 100 hives followed by 22.6% that manages between 110-200 hives, 9.6% manages 220-300 hives, while only a few beekeepers own more than 300 hives (Fig. S1-B). 60.4% of beekeepers manage their hives in one to three apiaries (Fig. S1-C). On average, each site has about 33 hives per apiary (Fig. S1-D), with a predominance of hives managed at one body in both monitors. All the beekeepers surveyed produce honey and a low percentage of the beekeepers move hives for pollination and transhumance in search of sources of nectar (9.4% and 5.7%, respectively) (Table 1). For most beekeepers (83.0%) the activity is not their primary source of income. About 62.3% of the beekeepers locate their apiaries near crops, at distances that in 60.3% of the cases do not exceed 100 m. Among the most frequent crops, soybean and corn are mentioned the most, and the main sources of nectar and pollen are corn, alfalfa, melliotus, white clover, thistle, soybean, eucalyptus and lotus. The 77.4% of the beekeepers indicated to have participated in training activities while 37.7% keep records of their activities.

3.1.2. Characterisation of zootechnical and animal health management

Table 1 shows that most beekeepers surveyed do not change bee queens (54.7%). Only 13.2% of the beekeepers change bee queen every year, the rest of the beekeepers changes the bee queen after two or more years. 77.0% of the beekeepers use the creation of nuclei as a method to multiply their hives. To compensate for the nutritional deficit during parts of the year, 94.4% of beekeepers feed supplements, most of them being energy supplements (77.4%) and a small proportion (17.0%) uses a mixture of protein and energy supplements. The formulations referred are diverse and most often prepared by the beekeepers themselves.

3.1.3. Clinical observations related to diseases

Fig. 2 shows that very few clinical observations for open brood were registered, whereas, several other clinical manifestations were observed in closed brood and hives. Spotted brood was observed in 29.2% together with the presence of detritus at the bottom of the hives (22.0%). Predators or pests were observed during both monitors (40 and 26%, respectively), but signs compatible with diarrhoea in adult bees (faeces in the tops and fronts of the hives) were almost unobserved.

Hive losses range around 10.6 ± 17.1 hives in the past year (Mean ± SD, Fig. S2). In this context, Fig. 3 shows that in 39.6% of the dead hives during the last year, beekeepers reported the presence of dead offspring inside the cells, while 17.0% reported dead bees in front of the hive entrance. In around 50% of the cases, no food reserves were found in the dead hives. The beekeepers reported possible causes for the losses like evasion, swarming or other unknown causes (58.5%) and 18.9% due to natural disasters. The number of death hives varies during the year, as shown in Fig. 4, where the months with the highest flow of nectar and pollen are also detailed, as well as the main interventions of the beekeeper concerning productive and animal health management.

3.1.4. Perception of health issues by beekeepers

Table 1 shows that 78.4% of the beekeepers suspect the presence of pests and diseases in their apiaries, with *Varroa* sp. being mentioned the most frequent (77.4%). Other diseases like American and European Foulbrood and *Nosema* spp are also suspected, however, only 11.3% confirms the suspicion by sending a sample to a laboratory. 52.8% of the beekeepers monitor the infestation rates by *Varroa* sp. mites. The beekeeper himself monitors and/or diagnosis the diseases in most cases (92.5%). Application of treatments against *Varroa* sp. mites is recurrent during the year. Oxalic acid is the most frequent treatment (79.2%), followed by Amitraz and Flumethrin (50.9% and 37.7%, respectively) and other less-used products like coumaphos and fluvalinate (Fig. S3). 54.7% of beekeepers disinfect the hives (frames, lids and bottoms), mostly by flaming (32.1%) and/or boiling water (20.8%), and 5.7% use caustic soda. Beekeeping materials are stored in a dedicated storage space by 60.4% of the beekeepers. The remaining 39.6% store materials in their apiary, move them to their homes or use other non-specific places. Honey is extracted in shared plants in 71.7% of the cases. As for the presence of other apiaries around, 79.2% of the beekeepers refer to the presence of other apiaries in the vicinity, in 66.1% at a distance of less than 2 km (Table 1).

3.2.Infestation rate (%IR) by *Varroa* sp. Mite

The %IR by *Varroa* sp. mite ranged from 0.00 and 41.62% (min. and max., respectively) (Table 2). There were statistically significant differences in the IR% by *Varroa* sp. mites between the two monitors (Mann-Whitney U test, $p = 0.000$; $\alpha = 0.05$) and the location of the apiaries (Kruskal-Wallis test, $p = 0.000$; $\alpha = 0.05$). A significant correlation between each monitor and %IR was observed (Pearson's r= -0.240, p= 0.000).

3.3. Structure of the hive and weather conditions

71% of the beekeepers manage just one body, the brood chamber. The rest (29%) grows the hives with a second body of which the majority (26%) uses ½ Langstroth box (Figure S4). Table 3 shows the values (Mean, SD, Min. and Max.) found in each monitoring for the number of frames, honeycomb sides with adult bees, closed and open brood, honey and pollen, as well as frame heads covered with bees and bees entering the hive per minute in the brood chamber. Parameters associated with weather conditions (temperature, %RH and wind speed) are also shown. Significant differences were found in some of these parameters ($p<0.05$, Mann-Whitney U test), according to the monitoring (Table S1). Significant correlations between variables (bivariate Pearson's correlation, 95% confidence) were also found (Table S2).

3.4.Productivity

According to information provided by beekeepers, honey production varies from 5 to 30 kg per hive (Figure S5), with an average of 17.7 ± 7.5 kg of honey per year (Mean ± SD). 61.5% of the beekeepers obtain yields between 10 and 20 kg/honey/hive/year, none withstanding the 26.4% which yield between 21 to 30 kg/honey/hive/year. Most of the beekeepers surveyed harvest once a year (57.7%), while those harvesting two and three times a year correspond to 36.5% and 5.8%, respectively. Significant differences in the productivity were found related to the change of the bee queen ($p= 0.013$, Mann-Whitney test), disinfection of the beekeeping material ($p=0.001$, Mann-Whitney test), number of combs in the brood chamber ($p= 0.000$, Kruskal-Wallis test), among other variables (Table S3).

In line with the significant differences, direct correlations were also found between the amount of honey produced and the formation of nuclei (Pearson's r= 0. 264, p= 0.000), the number of combs in the breeding chamber (Pearson's r= 0.251, p= 0.000), change of queen bee (Pearson's r= 0.124, p= 0.006) and disinfection of the beekeeping material (Pearson's r= 0.116, p= 0.010) (Table S4). The classification of beekeepers according to their productivity using these variables permitted the creation of classification tree (Fig. S6).

4. DISCUSSION

The present study was carried out in a geographic area where intensive large-scale agriculture is predominant, with soybean as the most widely cultivated crop (Pacheco, 2012). Intensification of agricultural systems causes loss of biodiversity and habitat fragmentation (Winfree et al., 2009). In this context, beekeeping is a complementary activity inserted in a productive ecosystem with a shortage of botanical species providing nectar and pollen and a high degree of human intervention. Nevertheless, the shortage of biodiversity, there were apiaries with up to 50 beehives.

According to the information provided by the participants, beekeeping is a dynamic activity, with both experienced and new beekeepers (1 to 54 years of experience). For the majority it is to a secondary source of income. Professional training is a vulnerable point, considering the available capacity building offer is not enough for most beekeepers. Also, a large percentage does not maintain records (Table 1), which is essential for the implementation of an adequate bee health programme. Keeping records guarantees the traceability and safety of the products or the quality of the pollination service (Spivak et al., 2017; Potts et al., 2016; Isaacs et al., 2017). The change of bee queens and nutrition are other determining variables in terms of yield and health status. Productivity is significantly higher when bee queens are changed annually, as shown in Tables S3, S4. According to Ricigliano et al. (2018), brood production by young queens is significantly higher than that of old queens, supplementing pollen gives an extra stimulus. Despite this, most beekeepers surveyed do not change bee queens regularly, creating a health risk.

The beekeepers reported that nectar and pollen flows are variable during the year (Fig. 4). The flow increases during September, at the beginning of spring, reaching its maximum point in December. Then, it decreases between January and April and stops during winter (May - July), which coincides with the observations of Giacobino (2015). Nectar and pollen shortages can lead to reductions in adult survival and hatching rates, causing a rapid depopulation of the colonies (Naug, 2009). Although beekeepers provide food supplements to compensate for nutritional deficiencies during periods of scarcity, the formulations seem not to be suitable. Energy supplements correspond to 77.4% of the nutrition, while only a few beekeepers provide protein supplements and always in combination with energy supplies (Table 1). Corby-Harris et al. (2019) and Dolezal & Toht (2018) both report that poor diet aggravates infectious processes, facilitating the action of pathogens and parasites that affect nutritional physiology and compromise the survival of the hive.

Productivity is also affected by the quantity and duration of the nectar flow (Farrar, 1937). Development and growth cycles of the colonies should be in harmony with the floral cycles. This allows anticipating harvest periods and periods of scarcity (Corby-Harris et al., 2019).

However, this seems not to be the case among the majority of beekeepers that participated in the survey.

Concerning the hive structure, most hives had just one body (brood chamber) with up of nine frames in both monitors. In most cases, the tenth frame is replaced by a feeder (Dolittle) to provide an energy supplement (sugar syrup). The number of frames have a direct correlation (p<0.01) with the number of adult bees and the quantity of honey, as well as the productivity, but an inverse correlation with the %IR (Table S2). These correlations infer that those hives containing ten frames maintain better productivity indices, honey reserves and bee population, and lower infestation rates by *Varroa* sp. mites.

The results of the first monitor show fewer honeycomb sides with closed brood, open brood and pollen while a higher mean of honeycomb sides with honey. (Table 3). The number of bees entering the hive per minute, temperature, relative humidity and wind speed were also different in both monitors. The first monitor (before winter) was characterised by lower temperatures and wind speed, higher relative humidity (%) and a low number of bees entering the hive/min (Mean = 10 bees). In contrast, the second monitor (beginning of spring) had a higher number of bees entering the hive/min (Mean = 33 bees), accompanied by higher temperatures and less relative humidity. All differences in these parameters were significant (p<0.05, Mann-Whitney U test), as shown in Table S1. In addition, significant correlations between the number of adult bees (Pearson's r = 0.130, p = 0.003), closed (r = 0.659, p = 0.000) and open brood (r = 0.531, p = 0.000) and also, nutritional reserves of honey (r = -0.291, p = 0.000) and pollen (r = 0.168, p = 0.000) (Table S2). In this sense, the number of bees entering the hive per minute corresponds to a good parameter to estimate, in a generic way, how the hive is shaped inside.

The main clinical manifestations of diseases were found in the closed brood. Spotted brood, accumulation of residues at the bottom of the hive and dark honeycombs or deep-set/perforated capping were found mainly during the second monitor. Presence of predators or pests was observed in both monitors, however in a greater proportion during the first one. The presence of these signs reveals the loss of the internal dynamic balance of the hive, which in turn is subject to the efficiency of the environmental and health management made by the beekeeper during the production process. (Verde et al., 2013). Loss of hives (closed to 10 hives/year) is mainly related to abandonment swarming or unknown causes and to the absence of feed reserves (Fig. 3). According to Fig. 4, the highest mortality of hives occurs during the winter months (June-August), when there is no availability of nectar and pollen, along with the little or hive management activities of the beekeepers.

Based on the perception of health issues by beekeepers, most of them presume the presence of *Varroa destructor* in their apiaries, which is logical, considering that the mite has a cosmopolitan distribution (Rosenkranz et al., 2010) and it is one of the most relevant diseases affecting honeybees (OIE, 2019). However, only half of the surveyed beekeepers monitor infestation rates and just a little percentage confirms the suspicion of diseases in specialised laboratories. This allows the development of outbreaks of diseases with unpredictable consequences.

The results showed highly variable infestation rates (%) by *Varroa* sp. mites between the location of apiaries and per monitors. The highest rates were encountered during the first monitor, with values from 0.00 to 41.62% (Table 2). The %IR showed relevant correlations with the structure of the hive and weather conditions (Table S2 and S4), which means that infestation rates are lower if the hive has an adequate composition in terms of the number of frames, adult bees, closed/open brood, in association with higher temperature and less relative humidity. On the other hand, the application of treatments against *Varroa* sp. mites also plays an important role in the variability of infestation rates, since it is a recurrent fact during the year (Fig. S3) Without a regional epidemiological strategy to control of varroosis, as well as the lack of knowledge about the infestation rates before the application of treatments. The infestation with Varroa has economic repercussions like low productivity, loss of hives; and the cost to control (DeGrandi-Hoffman et al., 2016).

Giacobino (2015) determined a damage threshold by mites of 3% in the studied territory, which shows the need for treatments against the mite to avoid hive loss during the winter. Within the most frequent products, oxalic acid (organic) stands out, followed by amitraz and Flumethrin which are synthetic chemicals. In terms of productivity, honey yield per hive is the easiest to quantify and to relate to bee health (Potts et al., 2016; Ollerton, 2017). In line with the results, introducing the most significant variables affecting the productivity (Table S3, S4) in a classification algorithm (Fig. S6), it shows that the amount of honey produced can reach up to 22.7 Kg/honey/year considering key aspects.

5. CONCLUSION

The survey shows that beekeeping is a complementary activity inserted in a productive ecosystem with limited nutritional supply and a high degree of human intervention. The amount of hives per apiary and the closeness of the apiaries and extensive crops not favourable to bees as reported by the beekeepers may indicate more hives than the ecosystem can sustain. Nevertheless, honey production is still important in the territory. The amount of honey harvested is variable and directly related to the practices of each beekeeper. The structure of the hive was found to be generally inadequate. This can be attributed to a large extent to deficiencies in the management of the apiaries and lack of planning of productive and economic objectives. For example, brood chambers made up of nine combs, bee queens are not renewed, and the type of nutritional supplements do not provide a balanced diet. The growth of the hives is not in tune with the floral cycles. As a result, hives enter the wintering with a deficient structure (feed reserve, number of combs with open brood, closed brood and adult bee population), and emerge weakened if they manage to survive winter. On the other hand, the infestation rates by *Varroa* sp. mites are a latent risk, despite the beekeepers apply treatments almost constantly during the year.

All of these factors lead to a series of unfavourable events modulated by the beekeeper, the main intermediary between the bee and the ecosystems. For that reason, the different factors leading to loss of health in bees have to be evaluated in a holistic and multidimensional way, including all aspects which the individual and collective health status of the bees depends.

ACKNOWLEDGEMENTS

The authors thank the beekeepers of Santa Fe (Argentina), for their trust and allowing them access to their apiaries, the monitors, for their valuable fieldwork and Dr Alvaro González for mapping the apiaries.

FUNDING INFORMATION

Funding for this study was provided by the Bee Care Centre of Bayer A.G.

Conflict of interest. The authors declare that they have no potential conflict of interest concerning the study in this paper.

AUTHORS' CONTRIBUTIONS

VRO performed statistical analysis and organised the manuscript. MV contributed to the design of the monitoring method, training of the monitors and the interpretation of the results from the point of view of veterinarian sciences. LV contributed to the organisation of the monitoring activities, training of the monitors and processing the collected data. LPR helped for the coordination of the local team, the fieldwork and the interpretation of the results in the local context. MCC performed the digitalisation and organisation of the earliest version of the data and contributed to the interpretation of the data in the local context. MD collaborated in the coordination of the monitoring activities, analysis of the results and the organisation of the manuscript.

REFERENCES

Aizen, M., Garibaldi, L., Cunningham, S., Klein, A. (2008). Long-term global trends in crop yield and production reveal no current pollination shortage but increasing pollinator dependency. *Current Biology*, *18(20)*, 1572-1575. https://doi.org/10.1016/j.cub.2008.08.066

Aizen, M.A., Garibaldi, L.A., Dondo, M. (2009). Soybean expansion and agriculture diversity in Argentina. *Ecologia Austral*, *19*, 45-54. Available Online in:

https://bibliotecadigital.exactas.uba.ar/download/ecologiaaustral/ecologiaaustral_v019_n01_p045.pdf (Accessed on November 2020).

Aizen, M., Garibaldi, L., Cunningham, S., Klein, A. (2009). How much does agriculture depend on pollinators? Lessons from long-term trends in crop production. *Annals of Botany*, *103*, 1579-1588. https://doi.org/10.1093/aob/mcp076

Braga, A.R., Gomes, D.G., Rogers, R., Hassler, E.E., Freitas, B.M., & Cazier, J.A. (2020). A method for mining combined data from in-hive sensors, weather and apiary inspections to forecast the health status of honey bee colonies. *Computers and Electronics in Agriculture*, *169*, 105161. https://doi.org/10.1016/j.compag.2019.105161

Cantalapiedra, J.J, Puerta J.L., Yllera, M.d.M., Blanco, I., Fernández, M.E. (2017) Bienestar Animal. Salud y enfermedad en relación con el comportamiento. Available Online in: https://www.researchgate.net/publication/334612499 (Accessed on November 2020).

Corby-Harris, V., Bowsher, J.H., Carr-Markell, M., Carroll, M.J., Centrella, M., Cook, S.C., Couvillon, M., DeGrandi-Hoffman, G., Dolezal, A., Jones, J.C., Mogren, C.L., Otto, C.R.V., Lau, P., Rangel, J., Schürch R. & St. Claire, A. (2019). Emerging themes from the ESA symposium entitled "Pollinator nutrition: lessons from bees at individual to landscape levels.". *Bee World 96(1)*, 3-9. https://doi.org/10.1080/0005772X.2018.1535951 Carrasco, A.E, Sánchez, N.E, Tamagno, L.E. (2012). Modelo agrícola e impacto socio-ambiental en la Argentina: monocultivo y agronegocios. AUGM-Comité de Medio Ambiente Serie Monográfica Sociedad y Ambiente: Reflexiones para una nueva América Latina (1st Ed.). AUGM-Comité de Medio Ambiente. ISSN 2314-1743. Available Online in:

http://sedici.unlp.edu.ar/bitstream/handle/10915/24722/Documento_completo__.pdf?sequence=3&isAllowed=y

Castignani, H. (2011). Zonas agroeconómicas homogéneas Santa Fe. Estudios socioeconómicos de la sustentabilidad de los sistemas de producción y recursos naturales. INTA, EEA, Rafaela, Santa Fe. ISSN 1851-6955. Available Online in: https://inta.gob.ar/sites/default/files/script-tmp-inta-_zah_santa_fe.pdf

DeGrandi-Hoffman, G., Ahumada, F., Zazueta, V., Chambers, M., Hidalgo, G., DeJong, E. (2016). Population growth of *Varroa destructor* (Acari: Varroidae) in honey bee colonies is affected by the number of foragers with mites. *Experimental and Applied Acarololgy, 69,* 21-34. https://doi.org/10.1007/s10493-016-0022-9

Delaplane, K.S., van der Steen, J., Guzman-Novoa, E. (2013). Standard methods for estimating strength parameters of *Apis mellifera* colonies. *Journal of Apicutural Research 52(1),* 1-12. https://doi.org/10.3896/IBRA.1.52.1.03

Dietemann, V., Ellis, J. D., & Neumann, P. (2013). The COLOSS BEEBOOK Volume II, Standard methods for *Apis mellifera* pest and pathogen research: Introduction. *Journal of Apicutural Research, 52(4),* 1-4. https://doi.org.10.3896/ibra.1.52.4.16

Dolezal A.G., Toth A.L. (2018). Feedbacks between nutrition and disease in honey bee health. *Current Opinion in Insect Science, 26,* 114.119. https://doi.org/10.1016/j.cois.2018.02.006

Dolezal, A.G., St. Clair, A.L., Zhang, G., Toth, A.L., O'Neal, M.E. (2019). Native habitat mitigates feast–famine conditions faced by honey bees in an agricultural landscape.

Proceedings of the National Academy of Sciences of the United States of America, *116(50)*, 25147-25155. https://doi.org/10.1073/pnas.1912801116

Doorn, M., Monsalve, S., Rivas, M., Rodríguez, P., Rodríguez, S., Van Lookeren, G., Verde, M. (2015). Polinización y agricultura sustentable. Fraunhofer Chile. Available Online in: https://www.fraunhofer.cl/content/dam/chile/es/documents/Newsite/FOLLETO_SEMINARIO_POLINIZACION.pdf (Accessed on November 2020).

Farrar C.L. (1937) The influence of colony populations on honey production. *Journal of Agricultural Research*, *54(12)*, 945-954. Available Online in: https://naldc.nal.usda.gov/download/IND43969007/PDF (Accessed on November 2020).

CONSEJO FEDERAL DE INVERSIONES (2011). La Apicultura Argentina y sus regiones. Una visión panorámica. Available Online in: http://biblioteca.cfi.org.ar/wp-content/uploads/sites/2/2017/06/apicultura-argenitna.pdf (Accessed on November 2020).

Giacobino, A. (2015). Estudio de los factores de riesgo asociados a la presencia y difusión de la Varroosis en la Provincia de Santa Fe. Tesis de Doctorado en Ciencias Biologicas. Facultad de Ciencias Exactas, físicas y naturales Universidad Nacional De Córdoba. Córdoba, Argentina. Available Online in: http://hdl.handle.net/11086/12837 (Accessed on November 2020)

Heiblum, A. (2019). Abejas: insectos polinizadores. INCYTU. Oficina para la Información Tecnológica para el Congreso de la Unión, México. Nota nro 31. Available Online in:

https://www.foroconsultivo.org.mx/INCyTU/index.php/notas/135-31-abejas-insectos polinizadores-2 (Accessed on November 2020).

Isaacs, R., Williams, N., Ellis, J., Pitts-Singer, T.L., Bommarco, R., Vaughan, M. (2017). Integrated Crop Pollination: Combining strategies to ensure stable and sustainable yields of pollination-dependent crops. *Basic and Applied Ecology, 22*, 44-60. https://doi.org/10.1016/j.baae.2017.07.003

IPEC (2020). Comercio Exterior - Exportaciones con origen en la provincia de Santa Fe - 2018 al 2020. Available Online in: http://www.estadisticasantafe.gob.ar/contenido/exportaciones-con-origen-en-la-provincia-de-santa-fe/ (Accessed on November 2020).

Klatt, B.K., Holzschuh, A., Westphal, C., Clough, Y., Smit, I., Pawelzik, E., Tscharntke, T. (2014). Bee pollination improves crop quality, shelf life and commercial value. *Proceedings of the Royal Society B, 281*, 20132440. https://doi.org/10.1098/rspb.2013.2440

Klein, A-M., Vaissiére, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B, 274, 303-313*. htpps://dx.doi.org/10.1098/rspb.2006. 3721.

Lende, S.G. (2015) Soybean model in Argentina (1996-2014), an example of accumulation by dispossession. *Mercator, Fortaleza, 14(3)*, 7-25. https://doi.org/10.4215/RM2015.1403. 0001

Moritz, R. F. A., Härtel, S., & Neumann, P. (2005). Global invasions of the western honeybee (*Apis mellifera*) and the consequences for biodiversity. *Écoscience, 12(3)*, 289-301. https://doi.org/10.2980/i1195-6860-12-3-289.1

Naug, D. (2009). Nutritional stress due to habitat loss may explain recent honeybee colony collapses. *Biological Conservation, 142(10)*, 2369-2372. https://doi.org/10.1016/j.biocon.2009.04.007

Manuel-Navarrete, D., Gallopín, G., Blanco, M., Díaz-Zorita, M., Ferraro, D., Herzer, H., Laterra, P., Morello, J., Murmis, M.R., Pengue, W., Piñeiro, M., Podestá, G., Satorre, E.H., Torrent, M., Torres, F., Viglizzo, E., Caputo, M.G., Celis, A. (2005). Análisis sistémico de la agriculturización en la pampa húmeda argentina y sus consecuencias en regiones extrapampeanas: sostenibilidad, brechas de conocimiento e integración de políticas. División de Desarrollo Sostenible y Asentamientos Humanos. CEPAL. ONU. Publicación de las Naciones Unidas. ISSN impreso 1564-4189, ISSN electrónico 1680-8886 ISBN: 92-1-322828-7. Available Online in: https://repositorio.cepal.org/bitstream/handle/11362/5656/1/S051013_es.pdf (Accessed on November 2020).

OIE. (2019). Código Sanitario para los Animales Terrestres. Título 7. Capítulo 7.1. Introducción a las recomendaciones para el bienestar de los animales. Available Online in: https://www.oie.int/fileadmin/Home/esp/Health_standards/tahc/current/chapitre_aw_introduction.pdf (Accessed on November 2020).

Ollerton, J. (2017). Pollinator diversity: distribution, ecological function, and conservation. *Annual Review of Ecology, Evolution, and Systematics, 48*, 353-376. https://doi.org/10.1146/annurev-ecolsys-110316-022919

Pacheco, P. 2012. Soybean and Oil Palm Expansion in South America: A Review of Main Trends and Implications. Working Paper 90. CIFOR. Available Online in: https://www.cifor.org/publications/pdf_files/WPapers/WP90Pacheco.pdf

Pirk, C.W.W., Crewe, R.M., Moritz, R.F.A (2017). Risks and benefits of the biological interface between managed and wild bee pollinators. *Functional Ecology*, *31*, 47-55. https://doi.org/10.1111/1365-2435.12768

Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W.E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution, 25(6)*, 345-353. https://doi.org/10.1016/j.tree.2010.01.007 Potts S.G., Imperatriz-Fonseca V., Ngo, H.T., Aizen M.A., Biesmeijer J.C., Breeze, T.D., Dicks, L.V., Garibaldi, L.A., Hill, R., Sttele, J., Vanbergen, A.J. (2016). Safeguarding pollinators and their values to human well-being. *Nature, 540*, 220-229. https://doi.org/10.1038/nature20588

Requier, F., Antúnez, K., Morales,C.L., Sánchez, Aldea Sánches, P., Castilhos, D., Garrido, P.M., Giacobino, A., Reynaldi, F.J., Rosso Londoño, J.M., Santos, E., & Garibaldi, L.A. (2018). Trends in beekeeping and honey bee colony losses in Latin America. Journal of Apicultural Research, 57(5), 657-662. https://doi.org/10.1080/00218839.2018.1494919

Ricigliano, V.A., Mott, B.M., Floyd, A.S., Copeland, D.C., Carroll, M.J., & Anderson, K.E.(2018). Honey bees overwintering in a southern climate: longitudinal effects of nutrition and queen age on colony-level molecular physiology and performance. *Scientific Reports, 8,* 10475. https://doi.org/10.1038/s41598-018-28732-z

Rosenkranz, P., Aumeier, P., & Ziegelmann, B. (2010). Biology and control of *Varroa destructor*. *Journal of Invertebrate Pathology, 103*, S96-S119. https://doi.org/10.1016/j.jip.2009.07.016

Spivak, M., Browning, Z., Goblirsch, M., Lee, K., Otto, C., Smart, M., Wu-Smart, J. (2017). Why does bee health matter? The science surrounding honeybee health concerns and what we can do about It. Available Online in: https://www.cast-science.org/wp-content/uploads/2018/12/QTA20171_Bee_Health_565CB839D149E.pdf (Accessed on November 2020).

Verde, M., Demedio, J., Gómez, T. (2013) Apicultura, salud y producción. Guía técnica para el apicultor. Instituto de Medicina Veterinaria, Ministerio de agricultura, Cuba. ISBN:978-959-7190-21-9. Available Online in: https://eac.unr.edu.ar/wp-content/uploads/archivos/Apicultores%20y%20salud%2013-11-2013%20digital_compressed.pdf

Winfree, R., Aguilar, R., Vasquez, D.P., LeBunn, G., Aizen, M.A. (2009). A meta-analysis of bees' responses to anthropogenic disturbance. *Ecology, 90(8)*, 2068–2076. https://doi.org/10.1890/08-1245.1

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

DATA AVAILABILITY STATEMENT

We declare all data supporting the conclusions of this work are available within the article and/or its Supplementary Information. Upon reasonable request, further information related to this work can be requested from the corresponding author.

Tables

Table 1. Main results derived from the applied survey.

Variable	**Percentage of beekeepers (%)**		
	Yes	**No**	**Observations**
Does the beekeeper produce honey?	100.0	0.0	
Pollination	9.4	90.6	
Hive migration	5.7	94.3	
Is beekeeping the main source of income?	17.0	83.0	
Near crops	62.3	37.7	Distance: 0.1-100 m: 60.3% 100.1-500 m: 24.6% >1000 m: 5.7%
Training activities	77.4	22.6	Annually: 39.6% Biannual: 18.9% More than two years: 18.9%
Records of their productive activities	37.7	62.3	
Change of bee queens	45.3	54.7	Annually: 13.2% Biannual: 9.4% More than two years: 22.7%
Creation of nuclei	77.0	23.0	
Food supplements	94.4	5.6	Energy food: 77.4% Protein food: 0.0% Both: 17.0%

Does the beekeeper suspect a pest or disease in his apiary?	78.4	21.6	Which one? *Varroa* sp. 77.4% Foulbrood 13.2% *Nosema* spp. 3.8% Ants: 1.9%
Does the beekeeper monitor *Varroa* sp.?	52.8	47.2	
Who monitors or diagnoses diseases or pests?	-	-	Beekeeper: 92.5% A specialist: 3.8 % Both: 1.9% None: 1.9%
Does the beekeeper confirm suspicions by sending a sample to a laboratory?	11.3	88.7	
Hives disinfection	54.7	45.3	Flaming: 32.1% Boiling water: 20.8% Caustic soda: 5.7% Others: 22.6%
Are beekeeping materials stored in a dedicated storage space?	60.4	39.6	
Does the beekeeper have a plant for honey extraction?	54.7	45.3	Single plant: 26.4% Shared plant: 71.7%
Presence of other apiaries in the vicinity	79.2	20.8	Distance: < 1 Km: 26.5% 1.0-2.0 Km: 39.6% > 2.0 Km: 13.1% None: 20.8%

Table 2. *Varroa* sp. mite infestation rate, according to the monitoring and location. Results are presented as the Mean, SD and Maximum per each category. The minimum values were omitted because they were equal to zero in all cases.

	Location (Department)	Mean	SD	Maximum
First monitoring	Casilda	1.38	3.08	15.22
	Constitución	3.36	5.54	29.64
	Iriondo	4.67	7.30	32.29
	Rosario	5.20	9.00	41.62
	San Jerónimo	3.77	7.91	34.40
	San Lorenzo	2.39	4.99	22.26
	Total First monitoring	**3.38**	**6.40**	**41.62**
Second monitoring	Casilda	0.09	0.24	0.97
	Constitución	0.80	2.21	10.86
	Iriondo	2.58	3.16	12.18
	Rosario	1.65	2.78	11.36
	San Jerónimo	0.56	1.85	9.14
	San Lorenzo	0.68	1.53	7.25
	Total Second monitoring	**0.97**	**2.18**	**12.18**
Total	Casilda	0.74	2.28	15.22
	Constitución	2.15	4.47	29.64
	Iriondo	3.86	6.09	32.29
	Rosario	3.54	7.02	41.62
	San Jerónimo	2.17	5.92	34.40
	San Lorenzo	1.54	3.79	22.26
	Total	**2.24**	**5.03**	**41.62**

Table 3. Structure of the hive in the brood chamber or first body and parameters associated with weather conditions and observations of the hive. Values are presented as Mean, standard deviation (SD), minimum (Min.) and maximum (Max.) for each case.

		Found frames	Honeycomb sides with:					Frame heads with bees	Bees entering the hive/min	Temperature (°C)	%RH (%)	Wind speed (Km/h)
			Adult bees	**Closed brood**	**Open brood**	**Honey**	**Pollen**					
First monitor	Mean	9	5.92	0.47	0.19	6.38	0.76	3.07	9.85	22.35	62.10	2.57
	SD	1	2.80	0.80	0.30	3.70	0.94	2.39	11.97	3.52	18.19	4.14
	Min.	8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.00	25.00	0.00
	Max.	10	15.50	5.00	1.75	16.50	6.25	9.00	63.00	30.80	99.00	20.00
Second monitor	Mean	9	5.90	4.00	1.93	2.76	1.07	2.42	33.27	28.44	33.27	2.93
	SD	1	3.12	2.59	1.27	2.61	0.89	2.39	26.50	5.40	10.80	3.65
	Min.	5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	17.30	17.00	0.00
	Max.	10	15.25	12.50	5.75	12.00	4.50	9.00	99.00	46.00	68.00	17.00

Figures

Figure 1. Map of Santa Fe Province, Argentina, showing the location of the studied apiaries.

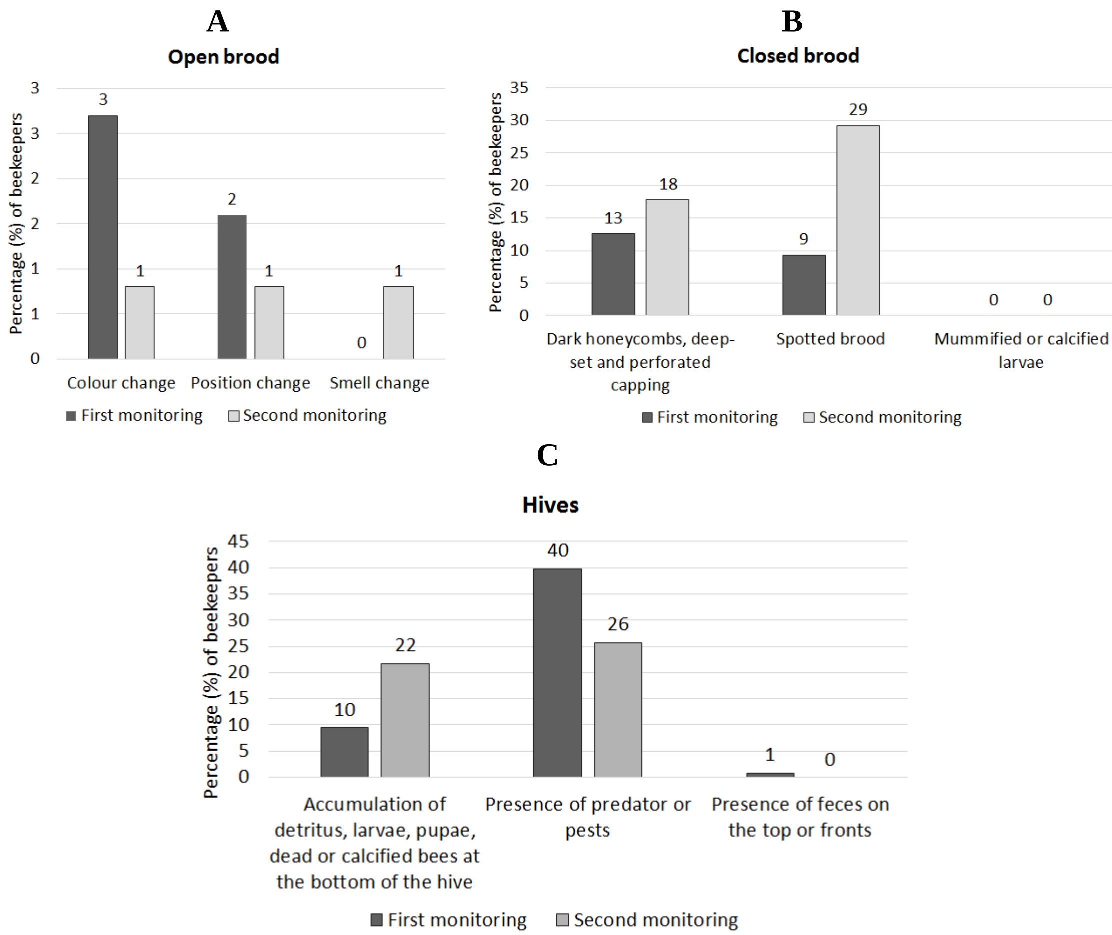


Figure 2. The manifestation of clinical signs related to diseases. Observations for (A) Open brood, (B) Closed brood and (C) Hives.

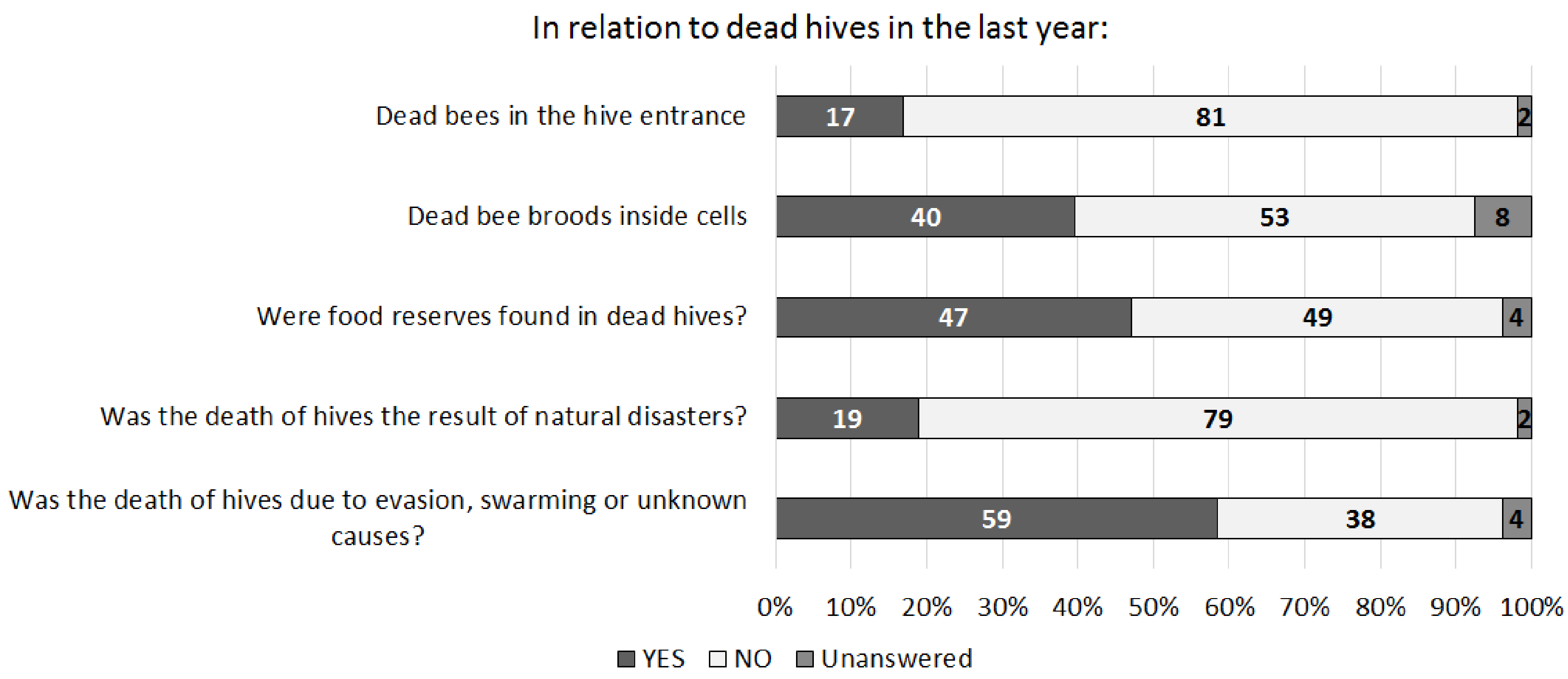
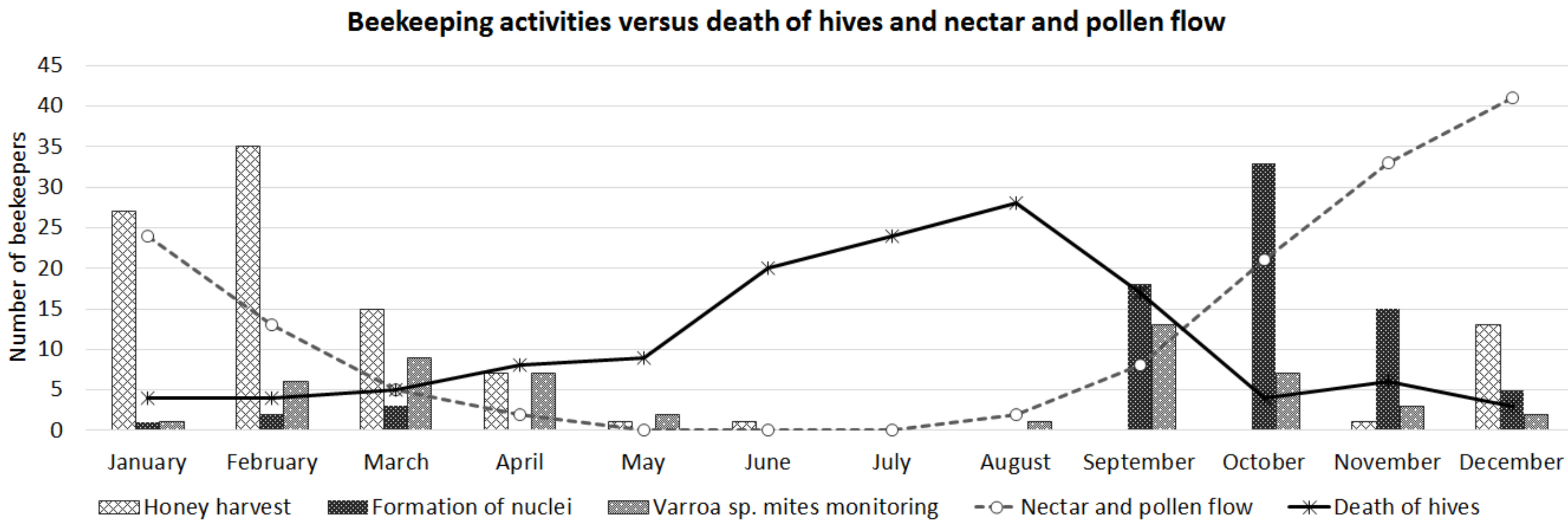


Figure 3. Observations related to dead hives in the last year.



598 **Figure 4.** Description of the main beekeeping activities versus the presence of dead beehives, according to the month in one year.