SANITARY AND NUTRITIONAL CHARACTERIZATION OF HONEYBEE COLONIES IN *EUCALYPTUS GRANDIS* PLANTATIONS

CARACTERIZACIÓN SANITARIA Y NUTRICIONAL DE COLONIAS DE ABEJAS MELÍFERAS EN FORESTACIONES DE *EUCALYPTUS GRANDIS*

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ADDITIONAL KEYWORDS

Apis mellifera. *Nosema ceranae*. *Varroa destructor*. Pollen. Honey production.

PALABRAS CLAVE ADICIONALES

Apis mellifera. *Nosema ceranae*. *Varroa destructor*. Polen. Producción de miel.

SUMMARY

In Uruguay, many beekeepers transport their colonies to *Eucalyptus grandis* plantations at the end of the summer and autumn, obtaining important honey harvests. However, at the end of the flowering period the colonies become extremely weakened undergoing high levels of mortality. Nutritional and health problems could explain the weakening of colonies. In order to find out the causes for this weakening, colonies of the same size were taken to an *E. grandis* plantation, split up in three groups differentiated by the availability of pollen. Throughout the flowering period we registered: the botanical origins and crude protein content of the incoming pollen, the body protein of the bees, the infection by *Nosema ceranae* and the infestation of *Varroa destructor*, the brood area and the production of honey. The most important findings were: i) the sustained decline in botanical diversity of pollen as the flowering period of *E. grandis* advanced until only pollen from this species remained; ii) pollen from *E. grandis* presented crude protein values close to 30%, but these gradually diminished reaching values lower than 20% towards the end of the flowering period; iii) those colonies which initially counted on pollen reserves presented bees with higher body protein a few days after settling in the plantation and lower levels of infection with *N. ceranae* during most of the flowering period; iv) pollen availability did not affect levels of infection by *V. destructor*, size of the brood area or honey production. Bee's nutritional deficit during *E. grandis* flowering could generate adequate conditions for the multiplication of *N. ceranae*. At the end of the *Eucalyptus*' flowering period colonies presented on average more than 90% of foraging workers infected with *N. ceranae* and 12% infection of adult bees with *V. destructor*. Incidence of both pathogens in weakened bees could explain colony losses.

RESUMEN

En Uruguay muchos apicultores trasladan sus colonias a las forestaciones de *Eucalyptus grandis* al final del verano y en otoño obteniendo importantes cosechas de miel. Sin embargo, cuando finaliza la floración las colonias se encuentran muy debilitadas, sobreviniendo una elevada mortalidad. Problemas nutricionales y sanitarios podrían explicar el debilitamiento de las colonias. Para averiguar las causas del debilitamiento se llevaron colonias de igual tamaño a una forestación de *E. grandis*, separadas en tres grupos diferenciados por la disponibilidad de polen. A lo largo del periodo de floración se registró: el origen botánico y el contenido de proteína cruda del polen que ingresaba en las colmenas, la proteína corporal de las abejas, la infección por *Nosema ceranae* y la infestación por *Varroa destructor*, el área de cría y la producción de miel. Los resultados más importantes hallados fueron: i) la sostenida disminución de la diversidad botánica del polen a me-

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dida que transcurría el periodo de floración de *E. grandis* hasta quedar únicamente el polen de esta especie; ii) el polen de *E. grandis* presentó valores de proteína bruta cercanos al 30%, pero fue disminuyendo paulatinamente hasta alcanzar un valor inferior a 20% al final de la floración; iii) las colonias que contaban inicialmente con reservas de polen presentaron abejas con mayor proteína corporal pocos días después de llegar a la forestación y menor infestación de *N. ceranae* durante la mayor parte del periodo de floración; iv) la disponibilidad de polen no incidió en la infestación de *V. destructor*, el área de cría y la producción de miel. El déficit nutricional de las abejas durante la floración de *E. grandis* podría generar las condiciones adecuadas para la multiplicación de *N. ceranae*. Al final de la floración del eucalipto las colonias presentaron promedialmente más del 90% de las abejas pecoreadoras infectadas con *N. ceranae* y 12% de *V. destructor* en las abejas adultas. La incidencia de ambos patógenos sobre las abejas debilitadas podría explicar la pérdida de colonias.

INTRODUCTION

Trees of the genus *Eucalyptus* are very important for apiculture due to their great value as sources of nectar and pollen.

In Uruguay, commercial *Eucalyptus* plantations had a rapid development since the implementation in 1988 of the National Plantation Plan, with extensive plantations of *E. grandis*, *E. globulus globulus*, *E. saligna*, *E. globulus maidenii*, *E. dunii*, and *E. viminalis* (Brussa, 1994). According to the latest survey from the Uruguayan Dirección General Forestal-MGAP (2010), large scale plantations of *Eucalyptus* reach up to 630 000 has, which has resulted in a profound change in the structure of agriculture, economy and landscape in the country.

Beekeepers exploit *Eucalyptus* plantations, especially those of *E. grandis*, by transporting their beehives to the edges of the plantations in February and March where they form large apiaries.

Provided climate conditions are favourable, honey production in these woodlands can reach up to 50 kg/beehive. However, at the end of the flowering period, the colonies are very weakened and will undergo great losses unless they are quickly removed from the plantations.

Two factors, which may be linked, appear as probable cause of the weakening and death of the colonies. Firstly, there may be a nutritional deficit in bees due to deficiencies in *Eucalyptus* pollen. Pollen is a nutritional component that provides proteins, lipids, minerals and vitamins. It is ingested mainly by adult bees, especially during their first 10 days of life, to complete the chitinization of the exoskeleton, accumulate fatty acid reserves and initiate the development of the hypopharyngeal glands, whose secretion is a basic component in the diet of young larvae. Deficiencies, or low nutritional contents of pollen results in weaker, generally smaller honeybees, with a reduced capability of feeding the brood and a shorter life expectancy. The nutritional value of pollen is mainly determined by its protein contents, its concentration of essential aminoacids threonine, valine, methionine, leucine, isoleucine, phenylalanine, lysine, histidine, arginine and tryptophan, and their digestibility by bees (De Groot, 1953; Haydak, 1970; Crailsheim *et al*., 1992; Herbert, 1992; Keller *et al*., 2005). According to Kleinschmidt and Kondos (1976) pollen that is valuable for honeybees must contain at least 20% of crude protein. In this regard, Stace (1996) reported that crude protein found in pollen of different *Eucalyptus* species in Australia varies between 16 and 33% and the vast majority contains less than 4% of isoleucine, the minimum quantity required by honeybees (De Groot, 1953). In *E. grandis* plantations, honeybees are able to collect pollen from these trees in great quantities but they cannot rely upon other sources of pollen that could compensate for a possible nutrient deficiency, especially when it is well into the autumn.

The second probable cause of mortality

in honeybee colonies is *Nosema* disease. Over decades it was believed that the only agent causing this disease was the microsporidian *Nosema apis*. However, recently it was found that *N. ceranae*, whose original host is the Asian honeybee *Apis cerana* (Fries *et al*., 1996), jumped to European honeybees a few years ago and is currently present all around the world (Higes *et al*., 2006; Klee *et al*., 2007; Huang *et al*., 2007; Chen *et al*., 2008; Higes *et al*., 2009; Invernizzi *et al*., 2009; Giersch *et al*., 2009; Fries, 2010). In Uruguay Invernizzi *et al*. (2009) only found *N. ceranae* in honeybees collected from different regions of the country and determined that this species was present before 1990. Both *Nosema* species reproduce in the epithelial cells of the honeybee's ventricle affecting its digestive functions, leading to malnutrition, physiological ageing and reduced longevity. *Nosema* disease also reduces the nurse bees' hypopharyngeal glands, resulting in a reduction in the production of royal jelly with the consequent deterioration in the diet of larvae. When the queen is infected, the workers often substitute it and eventually the colonies may end queenless (Bailey and Ball, 1991; Fries, 1997; Fries, 2010). The presence of *N. ceranae*, apparently more virulent than *N. apis* (Higes *et al.*, 2007; Martín-Hernández *et al*., 2007; Paxton *et al*., 2007; Higes *et al*., 2008) is followed with attention as it could explain the high losses of colonies that have occurred over the last few years mainly in the northern hemisphere, although this is a very controversial assertion (Cox-Foster *et al*., 2007; Chen *et al*., 2008; Gómez Pajuelo *et al.*, 2008; Invernizzi *et al*., 2009; Forsgren and Fries, 2010).

In Uruguay, Invernizzi *et al*. (2011) indicate that *Nosema* disease is present in very high proportions among colonies established in *Eucalyptus* plantations. This information identifies *Nosema* disease as an essential factor to consider as causing population and colony loss in those colonies transported to *Eucalyptus* plantations.

In addition to the two factors mentioned earlier, others could be associated to the weakening of honeybee colonies, such as brood diseases or infection by the parasitic mite *Varroa destructor* (Bailey and Ball, 1991).

The aim of this work is to determine the availability and nutritional value of pollen during the flowering period of *Eucalyptus* trees, and analyzing the incidence of the different availability of pollen in the nutrition of adult honeybees, in their sanitary condition and in the production of honey.

MATERIALS AND METHODS

PREPARATION OF THE COLONIES PREVIOUS TO THEIR TRANSPORTATION TO THE *EUCALYPTUS* PLANTATION

A total of 31 healthy colonies in Langstroth hives with young queens that had not received antibiotics or acaricides were selected from an apiary on the 26th of February 2004 in the locality of Sarandí Grande, department of Florida. The colonies were divided in three groups according to pollen availability: group S, without pollen (11 colonies) had five combs containing brood with a wax sheet and a honeycomb on each side; group P, with pollen reserves (10 colonies) were constituted like the previous ones but instead of the two wax sheets on each side they had two combs with more than 75% of the cells occupied by pollen; group PP, with reserves and pollen supplement (10 colonies) were exactly the same as the previous ones but received pollen supplement once they were established in the *Eucalyptus* plantation. Three pollen samples were taken from the combs with reserves to determine their botanical origin and protein content.

In order to measure the infestation levels by *V. destructor*, a sample of approximately 200 bees located over the brood combs was taken from each colony on the 29th of February. Samples were transported to the

laboratory in 10% formalin where the foretic mites were counted. These samples of bees were also used to determine the presence or absence of *N. ceranae*[*], as foraging workers (which have more chances of being infected) were not possible to collect previous to the relocation of the apiary in the plantation. With this purpose, the abdomens of 50 bees were macerated in 15 ml of water and a drop of the suspension was taken for exhaustive observation at 400x magnification searching for spores. This analysis was intended to determine only the presence or absence of *Nosema* disease in the colonies, since the disease is not normally present during February in the region where the apiary was located.

Activities conducted in the plantation

Colonies were relocated to an *E. grandis* plantation in the department of Durazno, approximately 40 km north of Sarandí Grande, on the 3rd of March. The apiary was located in a large clear area of approximately 1 ha in the centre of the plantation.

The first flowering *Eucalyptus* were observed on the 6th of March while an inspection carried out on the 2nd of May revealed a few remaining flowering trees, and the honey robbing by the bees as the hives were opened was very intense. These dates were considered the beginning and the end of the *Eucalyptus*' flowering period.

The presence of the queen in each colony was verified during all visits to the apiary and hive bodies for honey storage were added when necessary. Broodnests did not undergo any type of handling that would imply an exchange or modifications in the position of the combs.

The brood area was visually registered on the 3rd and the 28th of April, taking one complete frame side as a unit.

Colonies from group PP received pollen patties during the flowering period of *Eucalyptus* on the 13th and 24th of March and on the 3rd, 13th and 24th of April. Patties were made with commercial pollen from Spain and sugar syrup. A pattie of 300 g was placed over the top of the broodnest frames containing 160-180 g of pollen. A sample of the pollen utilized was taken to determine its botanical origin and nutritional value.

The pollen collected by the bees was sampled on the 6th, 13th and 24th of March and on the 3rd, 13th and 28th of April. For this purpose, 10 pollen traps were set in the colonies of an apiary located 40 m away from the experimental apiary. The pollen obtained in the traps in each sampling was mixed to subsequently determine its botanical origins and crude protein content of each species.

Bee samples from broodnests were taken on the 13th of March, 2nd of May and 7th of July to analyze their body protein contents.

In order to determine the infection of colonies by *N. ceranae*, samples of foraging workers were taken on the 6th of March, 13th of April, 11th of May and 21st of July. For each colony, 30 bees were individually analyzed following the criteria set by Pickard and El.Shemy (1989). For this purpose, the abdomen of each bee was macerated in 0.5 mL of water and a drop of the obtained suspension was observed under a microscope at 400x magnification registering the number of spores found in 10 fields.

The infection of the colonies by *V. destructor* was determined after the flowering period of *Eucalyptus* (11th of May and 7th of July) using the procedure mentioned above.

The colonies' honey production was estimated on the 2nd of June by counting the number of complete honeycombs in each hive and multiplying it by 1,5 kg (average weight of each half frame).

The apiary was removed from the plantation on the 30th of July.

Palynologycal analysis

In order to determine the botanical origin

[*]Recently, bee samples from this study, infected with *Nosema*, were analyzed using molecular tecniques (PCR-RFLP). Only *N. ceranae* was detected. This information is available in Invernizzi *et al.* (2009).

of the pollen samples, a representative quantity was taken from each one, which was subsequently homogeneized in glacial acetic acid and acetolized for observation under optical microscope. The residues were mounted in gelatine-glycerine with formol (Faegri and Iversen, 1975). The identification of pollen grains was carried out using 1000x magnification. The microscopic preparations of the samples were analyzed quantitatively by counting at least 1200 pollen grains per slide per sample (Lieux, 1972).

Determination of protein content in pollen and honeybees

The samples of adult bees and corbicular pollen were dried at 60ºC until constant weight was verified. For ground residues, protein content was quantified using the Kjeldahl acid digestion technique (N x 6,25) (Somerville, 2001).

RESULTS AND DISCUSSION

Botanical origins and crude protein contents in different types of pollen

Palynologycal analysis of pollen reserves found in colonies from groups P and PP showed that in all three samples, lotus pollen (*Lotus corniculatus*) was the most represented followed by white clover pollen (*Trifolium repens*). Both types of pollen together constituted more than 80% of each sample. Regarding the proteic value of pollen reserves, the three samples analyzed presented 27.9%, 27.2% and 26.8% of crude protein (CP). The small difference in the values obtained would indicate that pollen reserves from all colonies from groups P and PP contain approximately 27% of CP. The pollen mix used to supplement colonies from group PP was constituted by more than 20 botanical species, but more than 90% was integrated by two species of *Cistus* sp, one Brassicaceae, sunflower (*Heliantus annuus*) and corn (*Zea mays*). This mix contained 23.4% of CP. Therefore, pollen reserves and the mix utilized as proteic supplement surmount the minimum required for a given pollen to be considered valuable for the nutrition of honeybees (20%) as proposed by Kleinschmidt and Kondos (1976).

The most important types of pollen found during the *Eucalyptus*' flowering period belonged to *Eucalyptus*, gerardia (*Agalinis purpurea*), carqueja (*Baccharis trimera*) and chircas (*Baccharis* spp.) **(table I)**. Gerardia pollen was dominant in the first sample while *Eucalyptus* and carqueja pollen were present in small proportions. *Eucalyptus* pollen was represented in higher proportion in both the second and third sample followed by carqueja and chircas, respectively. Instead, pollen from the three samples collected in April came almost exclusively from *Eucalyptus*. This shows a steady decrease in pollen's botanical diversity as the flowering period of *Eucalyptus* advances. The absence of accompanying flora to the *Eucalyptus* may

Table I. *Availability of pollen resources during the flowering period of E. grandis*. (Disponibilidad de recursos de polen durante el periodo de floración de *E. grandis*).

Date	*Eucalyptus* (%)	Gerardia (%)	Carqueja (%)	Chircas (%)	Others (%)
6th March	16.2	67.3	10.7	0	5.7
13th March	54.0	0	36.3	0	9.7
24th March	57.6	0	0	42.3	0.1
3rd April	92.2	0	0	7.8	0.0
13th April	99.6	0	0	0	0.4
28th April	96.1	0	0	0	3.9

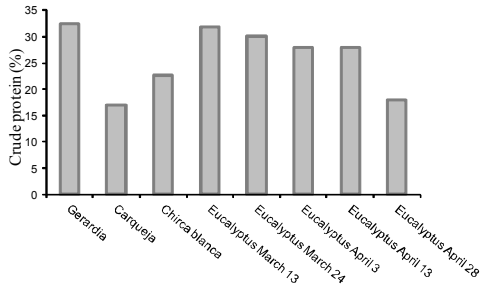


Figure 1. *Crude protein content in the different types of pollen available during the flowering period of* E. grandis. (Contenido de proteína bruta en los diferentes tipos de polen disponibles durante el periodo de floración de *E. grandis*).

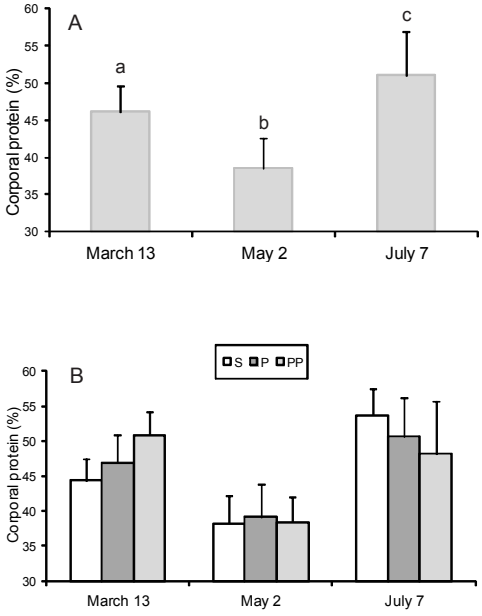
have a negative impact in the nutrition of the honeybees due to deficiencies in certain specific nutrients in *Eucalyptus* pollen.

The offer and succession of the different types of pollen found in this work are possibly also found in *E. grandis* plantations in other areas of the country, as the botanical species found have a wide distribution, especially carqueja and chircas.

The CP contents of the main types of pollen collected in the pollen traps are shown in **figure 1**. Pollen from *Eucalyptus* from the last five samplings were analyzed separately. Among the accompanying flora to the *Eucalyptus*, exploited by the honeybees in the days subsequent to the establishment of the apiary, pollen from gerardia and carqueja stand out due to their respective high and low content of CP. Regarding pollen from *Eucalyptus*, it was found that at the start of the flowering period the CP content is very good (>30%), but this value gradually decreases in the subsequent three records, sharply falling in the last one (<20%) at the end of the flowering period. As mentioned earlier, this value of CP is considered insufficient by Kleinschmidt and Kondos (1976) for a satisfactory nutrition in honeybees. The CP contents in pollen of a given species can vary due to changes in soil humidity, fertility and ambient temperature (Herbert, 1992; Somerville, 2001). This last factor, which varies considerably as the autumn progresses, could explain the decrease in CP towards the end of the flowering period.

BODY PROTEIN CONTENT IN ADULT BEES

Body protein content in adult honeybees decreased sensibly between the beginning and end of the flowering period **(figure 2.A)**. The highest value, however, occurred in July in spite of the low bee population and



A: Considering the totality of colonies in the apiary. B: In colonies of each treatment group. Abbreviations: S: colonies without pollen; P: colonies with pollen reserves; PP: colonies with pollen reserves and pollen supplement. Different letters indicate significant differences at the level $p<0.05$ for the Mann Whitney test. The lack of letters indicate no significant differences in any comparison.

Figure 2. *Body protein content in honeybees.* (Contenido de proteína corporal en las abejas).

poor sanitary conditions found in the colonies (see further ahead).

Stace (1996) proposes that the high income of nectar and temperatures below 20ºC, as it occurs frequently in *E. grandis* plantations, constitute situations of high stress for the colony and it requires a sufficient supply of pollen with more than 20% of CP for the body protein of the bees not to decrease. In this case, however, pollen availability does not seem to explain the changes in body protein since *Eucalyptus* trees provide abundant pollen throughout the whole period. Regarding CP content in *Eucalyptus* pollen, even if it decreases under 20% towards the end of the flowering period **(figure 1)**, it would not be the cause for the diminishing body protein of bees in May either, as this increases in July when the bees are still feeding off of this pollen of low nutritive value. The decrease of body protein in bees in May could be better explained by the bees wearing away during the intense foraging activity during the flowering period of *Eucalyptus*. Instead, the recovery of body protein in July was most likely favoured by the considerable reduction in the activity of bees.

The body protein content in the bees did not change between groups in colonies S, P and PP for all three records **(figure 2.B)**. The statistical analysis, however, presented marginal values in comparisons between bees from group S and groups P and PP in the first sampling ($p= 0,09$ for both cases). When grouping colonies from groups P and PP (this last group had still not received the pollen supplement) significant differences were found with colonies from group S ($U= 48$; $p<0,04$). These differences would indicate that the elimination of pollen reserves in group S had a negative impact in the body protein content of honeybees during the first days in the plantation. This effect was possibly diluted later, when all colonies used the abundant fresh pollen from *Eucalyptus*.

INFECTION OF COLONIES BY *NOSEMA CERANAE* AND *VARROA DESTRUCTOR*

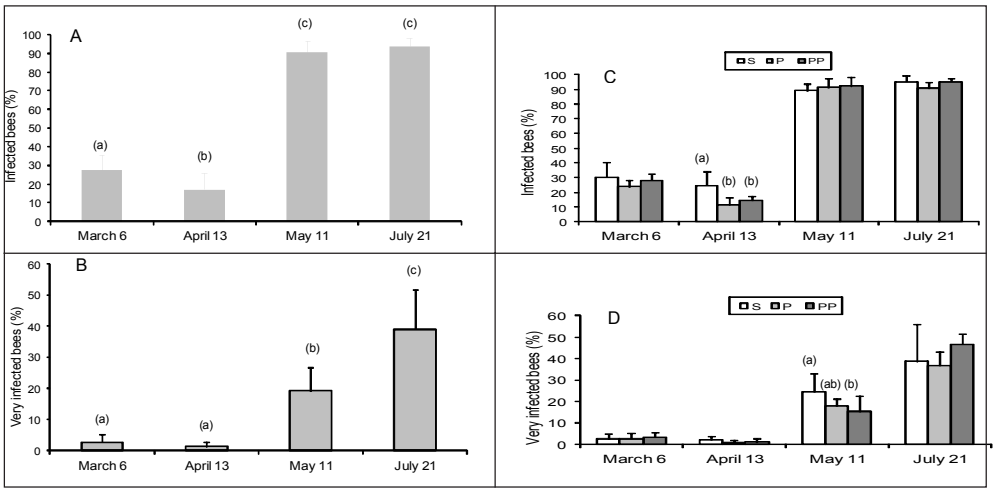
Nosema ceranae

The analysis of bees colected from the broodnests (sample used to determine infection by *V. destructor*) before transferring colonies to the *Eucalyptus* plantations showed practically no *N. ceranae* in the colonies. Only one colony from group PP presented two spores after detailed inspection of the slide. Even when the probability of finding infection in nurse bees is lower than in foraging workers (Fries, 1997), the total absence of spores in a sample of the abdomen macerates of 50 honeybees would indicate that *N. ceranae*, if present, would have insignificant levels in the colonies.

When foraging workers were analysed individually in the plantation, two clearly distinct situations were found among the infected bees. While some bees presented low levels of infection (less than 10 spores in 10 fields of the slide), other bees showed high levels of infection (more than 20 spores per field). Thus, bees belonging to the second group contained at least 200 times more spores than those from the first group. No bees were found presenting an intermediate amount of spores between the two groups. This result could be a consequence of the microsporidium's explosive way of multiplication.

Colonies were analyzed for *Nosema* disease initially considering all infected bees (IB) and subsequently attending to very infected bees (VIB) only.

Infection of bees by *N. ceranae* presented extensively throughout the apiary after only three days of relocating the colonies to the plantation, with an average of 27% of IB **(figure 3.A)**. Surprisingly, the proportion of IB decreased in the middle of the flowering period only to increase sharply reaching values over 90% towards the end of the period and for two months after. When analyzing the incidence of



A: Infected bees considering the totality of colonies in the apiary. B: Very infected bees considering all colonies in the apiary. C: Infected bees in colonies of different treatment groups. D: Very infected bees in colonies of different treatment groups. In the 21st of July sampling, colonies from group PP were not compared to those from groups S and P as only two colonies remained. S: colonies without pollen; P: colonies with pollen reserves; PP: colonies with pollen reserves and pollen supplement. Different letters indicate significant differences ($p<0.05$) for the Mann Whitney test. The lack of letters indicate no significant differences in any comparison.

Figure 3. *Infection of colonies by* N. ceranae. (Infección de colonias por *N. ceranae*).

Nosema disease considering only the VIB, the increment occurs at the end of the flowering period and reaches approximately 40% of the bees in July **(figure 3.B)**. Thus, *Nosema* disease appears to affect adult bees and is tightly linked to the exploitation of *Eucalyptus* plantations. In Victoria, Australia, Langridge (1961) found that *Nosema* disease in colonies foraging in *E. hemipholia*, *E. sidaeroxylon* and *E. albens* is notably increased in March-April when weather conditions change abruptly.

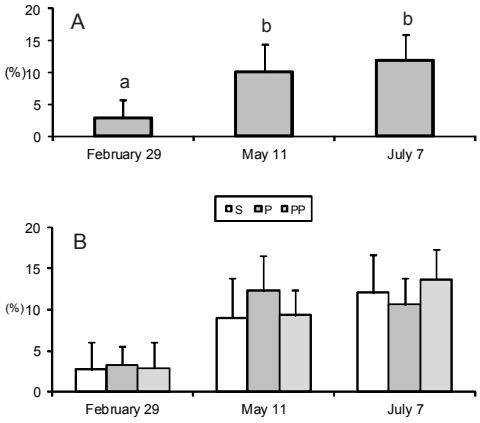
Comparing the presence of *N. ceranae* in the colonies undergoing the three different treatments, it was found that at the middle of the flowering period (13th of April) colonies from group S presented more IB than those from groups P and PP **(figure 3.C)** Since great quantities of pollen entered the hives during that period, especially from *Eucalyptus*, and presented CP values higher than 25% **(figure 1)**, the increase found in the infection cannot be attributed to a lack of nutritious pollen. More likely, the availability of pollen from diverse botanical origins seems to be the cause of lower levels of infection in colonies from groups P and PP. Thus, colonies belonging to these two groups benefited from the consumption of *Eucalyptus* pollen in conjunction with pollen from their reserves (and the pollen supplement in colonies from group PP). Similar differences were found in the subsequent sampling considering the VIB **(figure 3.D)**, which could be due to an increase in the infection of individual bees during the time lapse between the two samplings. Recently Alaux *et al*. (2010) found that pollen botanic diversity induced higher glucose oxidase activity compared with monofloral diets,

including protein-richer diets. Nevertheless, it did not improve the expression of other components of humoral and cellular responses possibly involved in the *Nosema* spp. resistance (Antúnez *et al*., 2009).

The lack of differences between the colonies from the three groups in the first sampling (colonies from group PP had still not received the supplement of pollen) could be attributed to the fact that during that period of time all colonies relied upon pollen from diverse botanical origins with a CP average over 20% (**table I**, **figure 1**). This result was repeated in the last two samplings if we only consider the IB, and only in the last one for the VIB (**figure 3.C** and **3.D**). The explanation in these cases could be the almost exclusive use of *Eucalyptus* pollen by all colonies due to the exhaustion of pollen reserves, and the suspension of pollen supplies to colonies from group PP (the last supply was on the 24th of April). Fries (1995) propose that protein deficiency is one of the causes for increments in *Nosema* disease, especially when honeybees make use of the nectar flows in autumn and winter. This researcher indicates that the infection could be reduced by supplying pollen to the colonies or relocating them to places with a good pollen offer. The results obtained in this study show that the increased botanical diversity of pollen, as well as its quantity and protein content, also contribute to reduce the incidence of *N. ceranae*. In this way, the pathogen could act as opportunist, taking advantage of bee's nutritional stress for its multiplication.

Varroa destructor

In each of the three samplings the presence of *V. destructor* in the different colonies was highly variable **(figure 4.A)**. In the first one, carried out before relocating the colonies to the *Eucalyptus* plantation, the average infection was of 3%, oscillating between 0 and 10.6%. These values are normal in this region for colonies that were not treated with acaricide. The presence of foretic mites was multiplied by more than three times towards the end of the flowering period of *Eucalyptus* and then it increased slightly until it reached an average value of 12% (the statistical analysis in the comparison of last two samplings presented a marginal value p= 0.072). The increment in Varroa infection, even if registered in important proportions, does not differ greatly from that usually registered in untreated colonies which are not relocated to the plantations remaining in low activity during autumn and winter. Nevertheless, the symptoms commonly associated with damage caused by *V. destructor*, such as important quantities of bees presenting deformed wings, spotted pattern of brood or pupae in uncapped cells either whole or cannibalized, were not found during the



A: Considering the totality of colonies in the apiary. B: In colonies of different treatment groups. Abbreviations: S: colonies without pollen; P: colonies with pollen reserves; PP: colonies with pollen reserves and pollen supplement. Different letters indicate significant differences at the level α= 0.05 for the Mann Whitney test. The lack of letters indicate no significant differences in any comparison.

Figure 4. *Infestation of colonies by* V. destructor. (Infestación de colonias por *V. destructor*).

flowering period. These symptoms only appeared as late as June and July in many colonies.

The level of infestation was not associated with the different availability of pollen for colonies from the three groups **(figure 4.B)**. Anyhow, the first sampling was carried out only three days after forming the three groups and the other two samplings when the flowering period had already ended. Therefore, the way in which pollen availability could affect the amount of varroa present in the colonies is difficult to infer from this study.

BROOD AREA

All colonies arrived at the *Eucalyptus* plantation with five complete frames of brood (10 faces of comb). At the middle of the flowering period the colonies had incremented their brood area in approximately 20%, possibly in response to the high nectar flow, but towards the end of the period this was reduced under its initial size **(figure 5.A)**. Neither of the two records carried out in the plantation showed differences in the breeding area of colonies under different treatments **(figure 5.B)**, indicating that the availability of pollen was not a limiting factor for brood development.

HONEY PRODUCTION

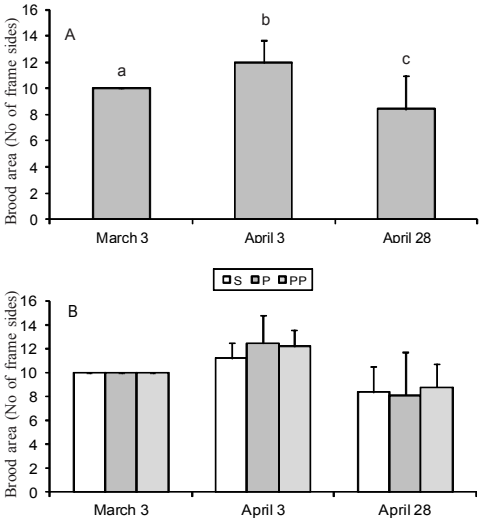
Honey production in the apiary was of 30.8 ± 14.9 kg/colony and no significant differences were found between the colonies in the three groups (Kruskal-Wallis test: H= 1.75; p= 0.416), therefore pollen availability would not affect this variable.

SITUATION OF THE COLONIES IN JULY

Upon inspection on the 7th of July it was found that one colony from group P and three from group PP had lost their queens. The 27 remaining colonies had weakened, having found 20 colonies with less than four frames covered with bees and only 7 with more than 5 frames covered. The last visit to the apiary was carried out on the 21st of July finding only 19 colonies alive: 9 from group S, 6 from group P and 4 from group PP.

FINAL CONSIDERATIONS

Relocating the colonies to *Eucalyptus grandis* plantations bears risks since bees become weak rapidly reducing their corporal protein. This situation favors *N. ceranae* infectation, which appears in all colonies without exception, in addition to incrementing the load of *V. destructor*. Bee's nutritional stress during *E. grandis* flowering period, added to the presence of both parasites, could explain the frequent loss of colonies. Both pathogens could explain the frequent loss of colonies. The use of



A: Considering the totality of colonies in the apiary. B: In colonies of different treatment groups. Abbreviations: S: colonies without pollen; P: colonies with pollen reserves; PP: colonies with pollen reserves and pollen supplement. Different letters indicate significant differences at the level $\alpha = 0.05$ for the Mann Whitney test. The lack of letters indicate no significant differences in any comparison.

Figure 5. *Brood area of colonies.* (Área de cría de las colonias).

conventional chemical treatments to control the health of colonies is very difficult because colonies are being used to produce honey since the spring and the risks of leaving chemical residues in the honey are very high. In the case of *V. destructor* the use of organic products may be a good solution but for *N. ceranae* there are no alternatives to the use of fumagiline. The results of this study, however, indicate that the efficient management of pollen of good nutritional value (and eventually a substitute) could attenuate the infection of *N. ceranae*.

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