Environment or beekeeping management: What explains better the prevalence of honey bee colonies with high levels of *Varroa destructor*?

Agostina Giacobino [a,*], Adriana Pacini [a], Ana Molineri [a], N. Bulacio Cagnolo [b], J. Merke [b], E. Orellano [b], E. Bertozzi [b], G. Masciangelo [b], H. Pietronave [b], M. Signorini [a]

[a] *Consejo Nacional de Investigaciones Científicas y Técnicas, Instituto Nacional de Tecnología Agropecuaria EEA Rafaela, Ruta 34 Km 227, Rafaela 2300, Santa Fe, Argentina*
[b] *Instituto Nacional de Tecnología Agropecuaria EEA Rafaela, Ruta 34 Km 227, Rafaela 2300, Santa Fe, Argentina*

ARTICLE INFO

ABSTRACT

Varroa destructor is one of the major threats to honey bee colonies. The mite abundance in the colonies is affected by environmental conditions as well as by beekeeping management. The aim of this study was to recognize the main drivers associated with autumn *V. destructor* infestation in honey bee colonies when different regions from Argentina are compared. A total of 361 colonies distributed in five Argentinean eco-regions were examined to evaluate *Varroa* mite infestation rate during autumn and *Nosema* sp. presence. Regions were different regarding annual temperature, precipitation and especially vegetation landscape. In addition, beekeeping management practices were obtained from a checklist questionnaire answered by the beekeepers. The prevalence of colonies with high infestation level was lower in semi-arid Chaco followed by humid and transition Chaco regions. Also, colonies that were positive for *Nosema* sp. showed a higher *Varroa* infestation rate. The "environmental" effect was stronger compared with the influence of secondary drivers associated with beekeeping activities. As well, a significant association between *V. destructor* infestation rates and *Nosema* presence was identified. Under contrasting natural conditions, environment seems a predominant driver on *Varroa destructor* infestation level in honey bee colonies.

1. Introduction

Beekeeping is threatened by multiple drivers including honey bee diseases and environmental factors. Among them, *Varroa destructor* (Anderson and Trueman, 2000) is considered one of the main threats to worldwide apiculture (Genersch, 2010) and a key player for winter colony losses, particularly as a promoter of opportunistic viral infections (Dainat et al., 2012). Beekeeping practices and surrounding environment are responsible for the presence and dissemination of parasites like *Varroa* mites (Boecking and Genersch, 2008; Giacobino et al., 2014; Kraus and Velthuis, 1997; Moretto et al., 1991; Rosenkranz et al., 2010).

On the one hand, previous studies show that management practices, mainly queen replacement and nutritional supplementation, allow keeping lower *V. destructor* infestations (Giacobino et al., 2014) and improved treatment effectiveness (Giacobino et al., 2015). On the other hand, the amount and quality of forage sources have globally declined (vanEngelsdorp and Meixner, 2010), especially given that changes in land-use have reduced the diversity of flowering plants (Kremen et al., 2007). Like in neighboring countries such as Bolivia, Brazil, and Paraguay, in Argentina the agricultural frontier is expanding from traditional productive central provinces such as Santa Fe to the forest and natural grassland landscape of Chaco provinces in the northeast of the country (Pengue, 2005). Mite infestation could increase by reduced food sources, as robbing is a route of disease horizontal transmission that occurs when there is little available foraging opportunities in the field (Fries and Camazine, 2001). Moreover, pollen nutrition can play an important role in the development of disease because poor nutrition may result in a less robust "defense system" (Vandame and Palacio, 2010).

Varroa destructor epidemiology is complex as multiple factors are involved simultaneously. Are good beekeeping practices enough to control diseases when environment resources are limited? In contrast, could the "quality" of more flowering diverse regions correct poor management strategies against *Varroa* mites in the colonies?

The aim of this study was to recognize the main drivers associated with autumn *V. destructor* infestation in honey bee colonies when different eco-regions from Argentina are compared.

* Corresponding author.
E-mail addresses: agostinagiacobino@hotmail.com, giacobino.agostina@inta.gob.ar (A. Giacobino), pacini.adriana@inta.gob.ar (A. Pacini), amolineri83@gmail.com (A. Molineri), bulacio.natalia@inta.gob.ar (N. Bulacio Cagnolo), merke.julieta@inta.gob.ar (J. Merke), orellano.emanuel@inta.gob.ar (E. Orellano), bertozzi.ezequiel@inta.gob.ar (E. Bertozzi), masciangelo.german@inta.gob.ar (G. Masciangelo), pietronave.hernan@inta.gob.ar (H. Pietronave), signorini.marcelo@inta.gob.ar (M. Signorini).

2. Materials and methods

2.1. Study design and sample size

A cross sectional study was carried out from late February to early June 2015 (autumn), in north-central Argentina. Sampling time was about three months and was defined between honey yield ending and before autumn acaricide treatment (for the colonies where treatment was applied). A total of 64 apiaries (361 colonies), owned by different beekeepers, were sampled (total population in the study region: 5300 apiaries; 90% confidence level; precision 10%; 74% of expected prevalence of colonies with >3% of autumn mites infestation). Apiaries were randomly chosen following stratified randomization procedures (computerized random numbers) (Moher et al., 2010). Within each apiary, a minimum of six colonies or 10% of the total colonies were randomly selected to evaluate V. destructor infestation (Lee et al., 2010).

Five regions were defined based on the ecoregions (Arzamendia and Giraudo, 2004; Burkart et al., 1999; Riveros, 2009) and land use categorization (Giorgi et al., 2008) including the nectar flow period and beekeeping management schedule: south and central Santa Fe, humid, transition, and semi-arid Chaco (Table 1; Fig. 1).

2.2. Data collection

Adult bees were examined to diagnose the presence of varroa mites in all the selected colonies. In each colony, approximately 250 bees were collected from both sides of three unsealed brood combs in a jar containing 50% ethanol. The mites were separated from the bees by adding a drop of soap, shaking for 20 s and pouring the jar content into a sieve with a mesh size of 2 mm (Dietemann et al., 2013). The mean abundance of V. destructor on adult bees was calculated dividing the number of mites counted by the number of bees in the sample to determine the proportion of infested individuals and multiplying by 100 to obtain the percentage of infestation per colony (Dietemann et al., 2013). We categorized all colonies in high Varroa level: >3%; low Varroa level: ≤3% (3 mites per 100 bees) considering the definition of autumn threshold levels of V. destructor infestation on adult bees (SENASA, 2007; Genersch et al., 2010; Bulacio Cagnolo, 2011; Lodesani et al., 2014). Worker honey bee samples were collected from the hive entrance using a portable vacuum device to diagnose the presence of Nosema sp. A minimum of 60 bees was gathered and placed in labeled plastic flasks containing 60 ml of 96° alcohol. Spore suspensions were prepared by adding 60 ml of distilled water to crushed abdomens of 60 randomly-selected individuals of each colony. Nosema sp. spores/bee (transformed to $\log_{10}$) were determined using light microscopy $40\times$ and haemocytometer. For each sample the number of spores in 80 haemocytometer squares (5 groups of 16 squares) was countered (Cantwell, 1970; Fries et al., 1984). All colonies were categorized as Nosema presence/absence according to this results. In addition, the number of adult bees and number of cells with sealed brood, pollen,

and honey of all colonies were estimated according to the Liebefeld method (Imdorf and Gerig, 2001).

Potential explanatory variables were obtained from a checklist questionnaire concerning management practices answered by the beekeepers. The questionnaire included questions with reference to geographic location, number of colonies, carbohydrates and protein diets, monitoring of mite levels in the colonies measured by the beekeepers, queen replacement, making nuclei, colonies migration and treatment against Varroa mites.

2.3. Statistical analysis

Univariate analysis (with apiary as random effect) was conducted for selecting explanatory variables potentially associated with autumn mite infestation level (high: >3%; low: ≤3%) and those having P-value ≤ 0.15 were selected for multivariable analysis (Dohoo et al., 1996). Only the explanatory variable with the highest P-value was selected for the multivariate model when two of them may have explained similar results and were statistically associated (collinearity evaluation).

Multivariable logistic regression analyses with random effect (using a generalized linear mixed model) were performed to evaluate the effect of the selected explanatory variables on the binary outcome variable autumn infestation with Varroa mites (high: >3%; low: ≤3%). A manually conducted backward elimination strategy was followed by removing one variable at a time with the highest P-value. With each variable removed from the model, the coefficient of significant variables was checked and if it resulted in >20% change in estimates, the variable was retained in the model to account for its confounding effect (Chowdhury et al., 2012).

3. Results

The mean size of each apiary was 40 ± 27 colonies (mean ± S.D.) and most beekeepers have been in the activity for >10 years (12 ± 8 years). The mean V. destructor infestation in the colonies prior to autumn treatment was 7.13 ± 8.7%. The higher infestation was observed in central Santa Fe (10.31 ± 11.58%) followed by south Santa Fe (8.08 ± 9.97%), transition Chaco (7.16 ± 7.99%), humid Chaco (5.66 ± 3.82%) and semi-arid Chaco (3.08 ± 2.79%) (K: 33.95; df: 4, N = 361; P < 0.001). Pairwise comparison between regions are presented in Table 1. The mean colony size was 18,786 ± 3517 adult bees and 26,461 ± 14,126 brood cells per colony. We found no significant correlation between autumn percentage of mite infestation and colony size (n = 318; r = 0.081; P = 0.15); number of pollen cells (n = 313; r = −0.104; P = 0.067) and number of honey cells and (n = 313; r = −0.077; P = 0.17). Although we found a significant correlation between infestation intensity and number brood cells the correlation coefficient was low and the P value was influenced by the sample size (n = 318; r = −0.155; P = 0.006).

Table 1
Region characterization based on annual mean temperature and precipitation, land use and floral resources.

Region	Annual temperature (°C)	Annual precipitation (mm)	Climate	Main Land use	Nectar/pollen flow	Varroa infestation (%)
South Santa Fe	18	600–1100	Temperate	Soy, corn, and wheat	Short (less than three months)	8.08 ± 9.97[ab]
Central Santa Fe	17–18	800–900	Temperate	Dairy farms and wintering animals on alfalfa pastures	Intermediate (three–four months)	10.31 ± 11.58[a]
Humid Chaco	23	>1200	Sub-tropical without dry season	Small farmstead, livestock or forest and rice production	Long (between 9 and 10 months)	5.66 ± 3.82[b]
Transition Chaco	23 to 24	<1000	Sub-tropical with dry season	Cereals, oleaginous, and cottonseed crops mixed with livestock production	Long (between 9 and 10 months)	7.16 ± 7.99[b]
Semi-arid Chaco	23	550–800	Semi-arid	Forest production	Long (between 9 and 10 months)	3.08 ± 2.79[c]

Different letters showed significant differences at P < 0.05.
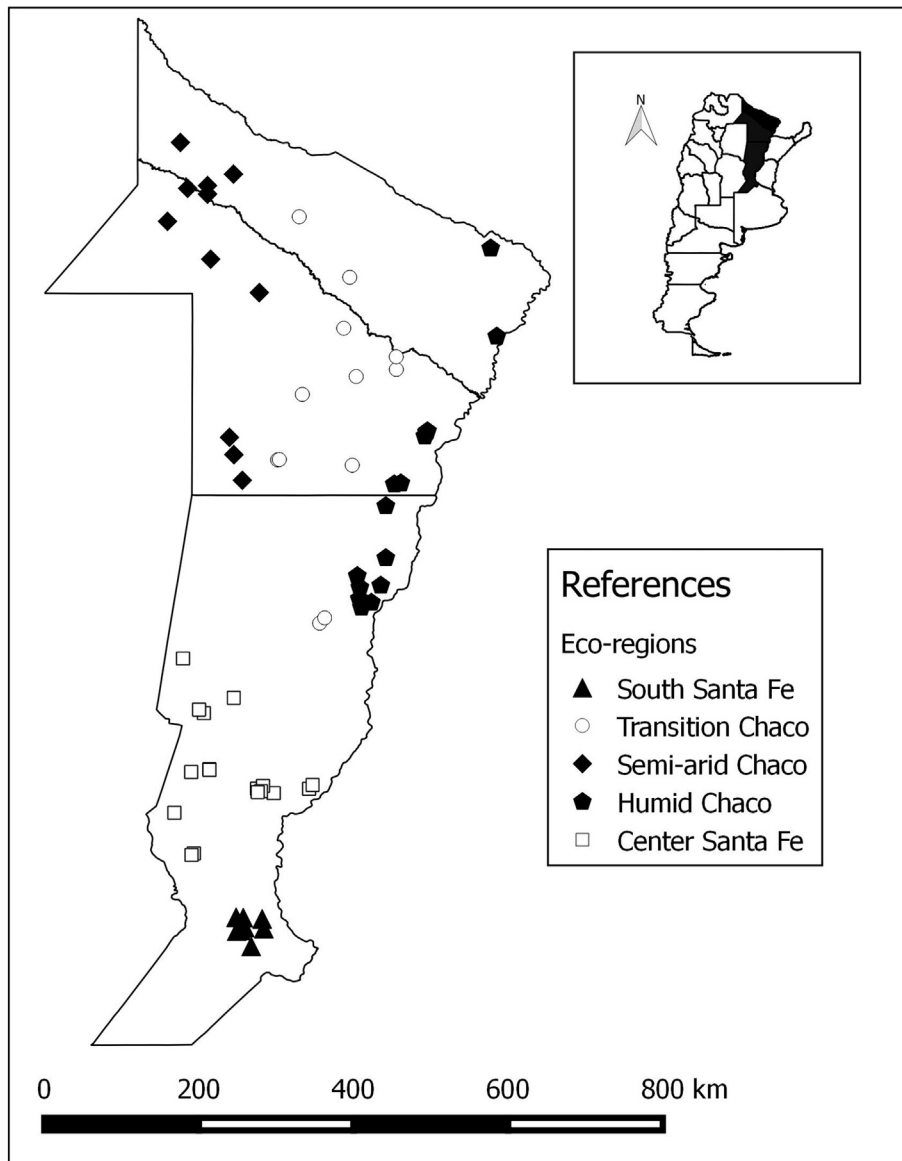
Fig. 1. Apiaries location and distribution according to eco-regions of Argentina.

The prevalence of colonies with high infestation (>3%) of *V. destructor* was 57.1% (220 of the 361 examined colonies). All apiaries received some carbohydrate supply (sucrose or high fructose corn) at least once a year. Pollen substitute and multivitamins supplementation were used only by 36.1% and 23.4% of beekeepers respectively. *Nosema* sp. was detected in 181 out of the 361 colonies (47.1%).

Five out of the 15 potential explanatory variables tested were selected after the univariate analysis to be included in the multivariable analysis (selected variables had a significance value $P < 0.15$; Table 2). The variables region and surrounding vegetation were associated ($P < 0.0001$). Therefore, only the region was included in the final model.

The final multivariable model revealed two variables associated with the prevalence of colonies with high infestation (>3%) of *V. destructor* (Table 3). The apiary random-effect was significant ($P = 0.002$). Significantly less colonies with >3% of *Varroa* infestation were detected in semi-arid Chaco followed by humid and transition Chaco ($P = 0.043$). Colonies that were positive for *Nosema* sp. showed a higher *Varroa* infestation rate (OR = 2.15; 95% CI: 1.11–4.16; $P < 0.024$).

3.1. Confounding effect of region

The carbohydrate diet period, the kind of protein diet and the colonies migration were associated with the region. Only 20% of the beekeepers from both Santa Fe regions declared to feed carbohydrate to their colonies during autumn whereas almost all beekeepers normally feed carbohydrates during autumn in Chaco ($P < 0.001$). Alternatively, 53.8% of the beekeepers from humid Chaco, 37.5% from south Santa Fe and 36.1% from semi-arid Chaco used patties or pollen to feed the colonies while only 23% and 30% used patties in transition Chaco and central Santa Fe, respectively ($P < 0.001$). Finally, only 13.2% of apiaries in humid Chaco had transported the colonies during winter (migrating colonies), while all beekeepers from semi-arid Chaco, transition Chaco and south Santa Fe had fixed colonies. On the other hand, only 5.9% of central Santa Fe apiaries had migrating colonies ($P < 0.001$).

Other recommended beekeeping practices like queen replacement were also associated with a regional effect. Close to 50% of beekeepers declared to replace annually the queen in their colonies in both Santa Fe regions and in transition Chaco but only between 20 and 27% of the

Table 2
Explanatory variables evaluated for potential association with *Varroa destructor* infestation level during autumn season (>3%; ≤3%) in 361 honey bee colonies distributed in 64 apiaries.

Variable	Level	# Cases[a] (%)	P-Value
Geographical region	South Santa Fe	34 (70.8)	0.021[b]
	Central Santa Fe	71 (69.6)	
	Humid Chaco	44 (57.7)	
	Transition Chaco	44 (62.9)	
	Semi-arid Chaco	26 (41.3)	
Number of colonies per apiary	Continuous	–	0.853
Kind of Protein diet	No	139 (59.9)	0.112
	Pollen	3 (50)	
	Commercial patties	19 (46.3)	
	Homemade patties	58 (72)	
Carbohydrate diet	Sucrose syrup/Honey	212 (61)	0.872
	HFCS	7 (58.3)	
Carbohydrate diet period	autumn	77 (51)	0.032
	Other than autumn	142 (68.3)	
Synthetic vitamins supplementation	No	156 (57.7)	0.235
	Yes	57 (68.7)	
Queen replacement	No	84 (60.9)	0.884
	Yes	135 (61)	
Frequency of queen replacement in the apiary	No/>2 years	158 (63.9)	0.335
	Every year	61 (54.5)	
Splitting colonies	No	14 (58.3)	0.934
	Yes	205 (61.1)	
Old combs replacement per colony per year	No	21 (52.5)	0.300
	<3 combs	74 (66.1)	
	3 combs	69 (55.2)	
	>3 combs	55 (66.7)	
Colonies migration	No	218 (61.7)	0.056
	Yes	1 (16.7)	
Regular Autumn treatment[c]	No	34 (63)	0.793
	Yes	180 (60.1)	
Apiaries closer than 1500 m	No	76 (65)	0.535
	Yes	142 (60.1)	
Surrounding vegetation	Forest and grasslands	102 (56.4)	0.135[b]
	grasslands and crops	75 (63.3)	
	Only crops	35 (72.9)	
Nosema sp. presence	No	83 (50.9)	0.006
	Yes	121 (68.4)	

HFCS: high fructose corn syrup.
[a] >3% autumn infestation with *Varroa* mites.
[b] Collinearity between geographical region and surrounding vegetation.
[c] Treat their colonies once per autumn with amitraz or flumethrin during 40 days.

beekeepers does it in humid and semi-arid Chaco, respectively (P = 0.002).

As expected, we also found an association between region and apiaries surrounding vegetation. >20% of Santa Fe apiaries where bounded exclusively by crops like soy, corn, and wheat and between 40 and 60% by a mixed crop-grassland environment. At the same time, both Santa Fe regions presented 70% of prevalence of colonies >3% of *Varroa* infestation (Table 2). In contrast, >40% apiaries from Chaco regions were delimited by forest and natural grassland and <15% of apiaries were surrounded exclusively by crops. Apiaries from these regions showed a lower prevalence of colonies >3% compared to Santa Fe apiaries (Table 2; Fig. 2).

4. Discussion

4.1. Regional effect

More than half of the honey bee colonies evaluated exceeded the autumn threshold level of *V. destructor* infestation on adult bees (3 mites per 100 bees) (Genersch et al., 2010; Lodesani et al., 2014; Giacobino

Table 3
Final multivariable logistic regression model (backward selection) for *Varroa destructor* infestation level during autumn season (>3%; ≤3%) in 361 honey bee colonies distributed in 64 apiaries.

Random effect	Estimate	Z	95% CI[a]	P-value
Apiary	1.75	3.11	0.93–3.28	0.002
Fixed effects	Level	Exp B (Odds Ratio)	95% IC Exp B	P-Value
Geographical region	South Santa Fe	2.99	0.62–14.43	0.043
	Central Santa Fe	3.79	1.04–13.77	
	Humid Chaco	2.57	0.69–9.57	
	Transition Chaco	3.14	0.77–12.87	
	Semi-arid Chaco(Ref.)	–	–	
Nosema sp. presence	No (Ref.)	–	–	0.024
	Yes	2.15	1.11–4.16	

[a] 95% confidence interval.

et al., 2015). There was a regional effect on *Varroa destructor* infestation level, as Chaco regions showed significantly less prevalence of colonies >3%. The "environmental factor" comprises multiple variables such as floral resources, temperature and humidity. This complex environmental effect confounded the effect of secondary drivers like carbohydrate diet period, protein diets supplementation, and colonies migration.

Formerly we found that the prevalence of colonies with infestation ≥3% was more related with beekeeping management (Giacobino et al., 2014, 2016a). Strategies for the successful chemical control of *Varroa* in the colonies are essential in temperate climates together with appropriate management practices to improve the effectiveness of the treatment concept (Giacobino et al., 2015). Monitoring and winter treatment can be critical for controlling mite population during the honey production cycle (Giacobino et al., 2016a). In contrast, in the present study we observed an "environmental effect" when more contrasting regions were included (different climatically conditions and surrounding vegetation).

Nutritional supplementation may help keeping lower *V. destructor* infestations (Giacobino et al., 2014). These apicultural techniques that limit or reduce mite population growth are often regarded as complementary to other control options (Wallner and Fries, 2003). However, although all Santa Fe beekeepers treat their colonies once per autumn with amitraz or flumethrin (using plastic strips during 40 days) and approximately 50% of them declared to replace frequently their colonies queen, both regions had higher prevalence of colonies > 3% compared to Chaco. Furthermore, the highest (south Santa Fe) and lowest (semi-arid Chaco) *Varroa* infested regions showed a similar proportion of beekeepers that use pollen patties to feed their colonies, similar colony density surrounding their apiaries and neither of the apiaries from these regions had migrating colonies.
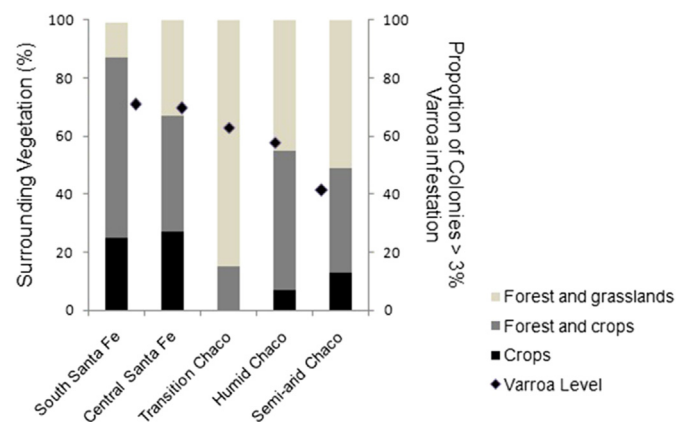

Fig. 2. Kind of surrounding vegetation and *Varroa destructor* infestation rate in honey bee colonies distributed in five diverse eco-regions.

Alternatively, as our results suggested, an environmental effect on *V. destructor* population dynamics has been previously reported (Harris et al., 2003) underlying the strong predominance of location on disease prevalence (Meixner et al., 2014). Moreover, previous studies indicated that colonies from subtropical climate also showed lower DWV amounts than colonies from temperate climate (Giacobino et al., 2016b). The flowering fluctuation affects the development of the bee colonies and this, in turn, influences the proliferation of the parasite (García Fernández, 1997). The yield-intensive crops have increased in recent years and have negatively affected beekeeping as it reduced pollen availability (Vandame and Palacio, 2010). Here we found that apiaries from regions where crops were entirely or partially predominant (Santa Fe) showed more prevalence of colonies >3% of mite infestation on adult bees. Nutritional stress due to scarce resources may increase susceptibility to disease (Field et al., 2002). Moreover, the environment (temperature and humidity) also exerted a strong effect on the relative success of grooming behavior (Currie and Tahmasbi, 2008) and therefore in the relative *Varroa* infestation on adult bees. Colony life histories, driven by environmental conditions, have a significant influence on *Varroa* infestation rates so mite infestation level fluctuates across regions (Meixner et al., 2015).

There are some restrictions to strongly link environmental factors with the observed regional differences as the study was achieved on a single year, mainly due to some logistical issues (the long distances and sampling coordination). Multiple temporal-spatial studies would be needed to answer whether the environment or the beekeeping management explains better the prevalence of *Varroa* in the colonies. However, this is a first attempt to understand the complexity involved, including a large number of colonies distributed in contrasting regions.

4.2. Nosema sp. association

Our results showed that the presence of Nosema was linked to colonies with high *Varroa* infestation level. Particularly, an interaction between *V. destructor* and *Nosema* sp. has been reported previously in Argentina as *Varroa*-parasitized colonies showed higher Nosema counts in temperate climate (Mariani et al., 2012). Similarly, in Uruguay, at the end of the Eucalyptus flowering period colonies that presented >90% of foraging workers infected with *N. ceranae* also presented 12% infection of adult bees with *V. destructor* (Invernizzi et al., 2011). Also, Botias et al. (2012) reported a negative effect of Nosema infection on *Varroa* control as acaricide treatment efficacy might be reduced probably due to the behavioral alterations in the colonies affected by Nosemosis. The question remaining is which is the role of the region in this association as the prevalence of *N. ceranae* and *N. apis* seem to be strongly affected by environmental conditions (Meixner et al., 2015), particularly by pollen diversity and quality (Di Pasquale et al., 2013). Furthermore, is the association different between Nosema species? As former studies suggested that *Varroa* mites and *N. apis* coexist but develop independently of one another (Orantes Bermejo and García Fernández, 1997).

Finally, mite infestations may be lower because of the presence of Africanized bees (De Jong, 1984; Rosenkranz, 1999) in Chaco regions that are closer to Brazil than Santa Fe, yet this point was not evaluated in our study. However, a stronger effect of environment compared to the bees' genotype on *Varroa* infestation rates was suggested by the COLOSS genotype- environment interactions experiment in Europe (Meixner et al., 2014). Additionally, studies regarding parasites distribution in African bees showed that *Varroa* infestation rates might be explained by differences in climate or floral resources rather than due to differences in honey bee genetic background (Muli et al., 2014). Though, further studies should be conducted in order to evaluate the effect of the host genetics on *Varroa* infestation levels in different regions and under different beekeeping practices.

5. Conclusion

The prevalence of colonies with high *Varroa* infestation (>3%) was more associated with beekeeping management practices than with a geographical effect when nutritional resources were similar between study regions (Giacobino et al., 2014). However, when the geographical range was expanded to more contrasting natural conditions such as Chaco regions; occurrence of *Nosema* sp. and *Varroa* mites seems to be associated and environment comes out as the main predominant driver on *Varroa destructor* infestation level.

Conflicts of interest statement

There are no conflicts of interest to be declared.

Acknowledgements

References

Anderson, D.L., Trueman, J.W.H., 2000. *Varroa jacobsoni* (Acari: Varroidae) is more than one species. Exp. Appl. Acarol. 24, 165–189.

Arzamendia, V., Giraudo, A.R., 2004. Usando patrones de biodiversidad para la evaluación y diseño de áreas protegidas: las serpientes de la Provincia de Santa Fe (Argentina) como ejemplo. Rev. Chil. Hist. Nat. 77, 335–348.

Boecking, O., Genersch, E., 2008. Varroosis: the ongoing crisis in bee keeping. J. Consum. Prot. Foo. Saf. http://dx.doi.org/10.1007/s00003-008-0331-y.

Botias, C., Martín-Hernández, R., Barrios, L., Garrido-Bailón, E., Nanetti, A., Meana, A., Higes, M., 2012. *Nosema* spp. parasitization decreases the effectiveness of acaricide strips (Apivar®) in treating varroosis of honey bee (*Apis mellifera iberiensis*) colonies. Environ. Microbiol. Rep. 4 (1), 57–65.

Bulacio Cagnolo, N., 2011. Manejo Integrado de *Varroa destructor* (Acari: Varroidae) en colonias de *Apis mellifera* (Hymenoptera: Apidae) en el centro oeste de la provincia de Santa Fe. (PhD Thesis). Facultad de Ciencias Naturales, Universidad Nacional de Mar del Plata, Argentina.

Burkart, R., Bárbaro, N.O., Sánchez, R.O., Gómez, D.A., 1999. Ecoregiones de la Argentina, Buenos Aires. Administración de Parques Nacionales Online:. http://www.sib.gov.ar/archivos/Eco-Regiones_de_la_Argentina.pdf (accesed on 10 Dec. 2012).

Cantwell, G.E., 1970. Standard methods for counting *Nosema* spores. Am. Bee J. 110 (6), 222–223.

Chowdhury, S., Sandberg, M., Themudo, G.E., Ersboll, A.K., 2012. Risk factors for *Campylobacter* infection in Danish broiler chickens. Poult. Sci. 91 (10), 2701–2709.

Currie, R.W., Tahmasbi, G.H., 2008. The ability of high- and low-grooming lines of honey bees to remove the parasitic mite *Varroa destructor* is affected by environmental conditions. Can. J. Zool. 86, 1059–1067.

Dainat, B., Evans, J.D., Chen, Y.P., Gauthier, L., Neumann, P., 2012. Predictive markers of honey bee colony collapse. PLoS One 7 (2), e32151. http://dx.doi.org/10.1371/journal.pone.0032151.

De Jong, D., 1984. Africanized honey bees in Brazil: forty years of adaptation and success. Bee World 77, 67–70.

Di Pasquale, G., Salignon, M., Le Conte, Y., Luc, P., Belzunces, L.P., Decourtye, A., Kretzschmar, A., Suchail, S., Brunet, J.L., Alaux, C., 2013. Influence of pollen nutrition on honey bee health: do pollen quality and diversity matter? PLoS ONE 8 (8): e72016. http://dx.doi.org/10.1371/journal.pone.0072016.

Dietemann, V., Nazzi, F., Martin, S.J., Anderson, D.L., Locke, B., Delaplane, K.S., Wauquiez, Q., Tannahill, C., Frey, E., Ziegelmann, B., Rosenkranz, P., Ellis, J.D., 2013. Standard methods for *Varroa* research. In: Dietemann, V., Ellis, J.D., Neumann, P. (Eds.), The COLOSS BEEBOOK, Volume II: Standard Methods for *Apis mellifera* Pest and Pathogen Research. J. Apic. Res. 52(1). http://dx.doi.org/10.3896/IBRA.1.52.1.09.

Dohoo, I.R., Ducrot, C., Fourichon, C., Donald, A., Humik, D., 1996. An overview of techniques for dealing with large numbers of independent variables in epidemiologic studies. Prev. Vet. Med. 29, 221–239.

Field, C.J., Johnson, I.R., Schley, P.D., 2002. Nutrients and their role in host resistance to infection. J. Leukoc. Biol. 71, 16–32.

Fries, I., Ekbohm, G., Villumstad, E., 1984. *Nosema apis*, sampling techniques and honey yield. J. Apic. Res. 23 (2), 102–105.

Fries, I., Camazine, S., 2001. Implications of horizontal and vertical pathogen transmission for honey bee epidemiology. Apidologie 32, 199–214.

García Fernández, P., 1997. Influence of the environment and the host on parasitization by Varroa jacobsoni Oud. The varroosis in the Mediterranean region. Zaragoza CIHEAM 21, 33–47.

Genersch, E., 2010. Honey bee pathology: current threats to honey bees and beekeeping. Appl. Microbiol. Biotechnol. 87, 87–97.

Genersch, E., von der Ohe, W., Kaatz, H., Schroeder, A., Otten, C., et al., 2010. The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. Apidologie 41, 332–352.

Giacobino, A., Bulacio, C.N., Merke, J., Orellano, E., Bertozzi, E., Masciangelo, G., Pietronave, H., Salto, C., Signorini, M., 2014. Risk factors associated with the presence of Varroa destructor in honey bee colonies from east-central Argentina. Prev. Vet. Med. 115, 280–287.

Giacobino, A., Bulacio, C.N., Merke, J., Orellano, E., Bertozzi, E., Masciangelo, G., Pietronave, H., Salto, C., Signorini, M., 2015. Risk factors associated with failures of Varroa treatments in honey bee colonies without broodless period. Apidologie http://dx.doi.org/10.1007/s13592-015-0347-0.

Giacobino, A., Molineri, A., Bulacio Cagnolo, N., Merke, J., Orellano, E., Bertozzi, E., Masciangelo, G., Pietronave, H., Pacini, A., Salto, C., Signorini, M., 2016a. Key management practices to prevent high infestation levels of Varroa destructor in honey bee colonies at the beginning of the honey yield season. Prev. Vet. Med. 131:95–102. http://dx.doi.org/10.1016/j.prevetmed.2016.07.013.

Giacobino, A., Molineri, A., Pacini, A., Fondevila, N., Pietronave, H., Rodríguez, G., Palacio, A., Bulacio Cagnolo, N., Orellano, E., Salto, C., Signorini, M., Merke, J., 2016b. Varroa destructor and viruses association in honey bee colonies under different climatic conditions. Environ. Microbiol. Rep. 8:407–412. http://dx.doi.org/10.1111/1758-2229.12410.

Giorgi, R., Tosolini, R., Sapino, V., Villar, J., León, C., Chiavassa, A., 2008. Zonificación Agroeconómica de la provincia de Santa Fe. Vol. 110. INTA, Argentina, pp. 215–224 (ISSN 0325-9137).

Harris, J.W., Harbo, J.R., Villa, J.D., Danka, R.G., 2003. Variable population growth of Varroa destructor (Mesostigmata: Varroidae) in colonies of honey bees (hymenoptera: Apidae) during a 10-year period. Environ. Entomol. 32 (6), 1305–1312.

Imdorf, A., Gerig, L., 2001. Course in Determination of Colony Strength. Swiss Bee Research Centre. Swiss Federal Dairy Research Institute, Liebefeld, CH-3003 Bern.

Invernizzi, C., Santos, E., García, E., Daners, G., Di Landro, R., Saadoun, A., Cabrera, C., 2011. Sanitary and nutritional characterization of honeybee colonies in Eucalyptus grandis plantations. Arch. Zootec. 60 (232), 1303–1314.

Kraus, B., Velthuis, H.H.W., 1997. High humidity in the honey bee (Apis mellifera L.) brood nest limits reproduction of the parasitic mite Varroa jacobsoni Oud. Naturwissenschaften 84, 217–218.

Kremen, C., Williams, N.M., Aizen, M.A., Gemmill-Herren, B., LeBuhn, G., Minckley, R., Packer, L., Potts, S.G., Roulston, T., Steffan-Dewenter, I., Vázquez, D.P., Winfree, R., Adams, L., Crone, E.E., Greenleaf, S.S., Timothy, H., Keitt, T.H., Klein, A.M., Regetz, J., Rickett, T.H., 2007. Pollination and other ecosystem services produced by mobile organisms: a conceptual framework for the effects of land-use change. Ecol. Lett. 10, 299–314.

Lee, K.V., Moon, R.D., Burkness, E.C., Hutchison, W.D., Spivak, M., 2010. Practical sampling plans for Varroa destructor (Acari: Varroidae) in Apis mellifera (Hymenoptera: Apidae) colonies and apiaries. J. Econ. Entomol. 103 (4), 1039–1050.

Lodesani, M., Costa, C., Besana, A., Dall'Olio, R., Franceschetti, S., Tesoriero, D., Vaccari, G., 2014. Impact of control strategies for Varroa destructor on colony survival and health in northern and central regions of Italy. J. Apic. Res. 53 (1), 155–164.

Mariani, F., Maggi, M., Porrini, M., Fuselli, S., Caraballo, G., Brasesco, C., Barrios, C., Principal, J., Eguaras, M., 2012. Parasitic interactions between Nosema spp. and Varroa destructor in Apis mellifera colonies. Zootec. Trop. 30 (1), 81–90.

Meixner, M.D., Francis, R.M., Gajda, A., Kryger, P., Andonov, S., Uzunov, A., et al., 2014. Occurrence of parasites and pathogens in honey bee colonies used in a European genotype-environment interactions experiment. J. Apic. Res. 53 (2):215–229. http://dx.doi.org/10.3896/IBRA.1.53.2.04.

Meixner, M.D., Kryger, P., Costa, C., 2015. Effects of genotype, environment, and their interactions on honey bee health in Europe. Curr. Opin. Insect. Sci. 10, 177–184.

Moher, D., Hopewell, S., Schulz, K., Montori, V., Gøtzsche, P., Devereaux, P., Elbourne, D., Egger, M., Altman, D., 2010. ConSoRT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. BMJ 340:c869. http://dx.doi.org/10.1136/bmj.c869.

Moretto, G., Gonçalves, L.S., De Jong, D., Bichuette, M.Z., 1991. The effects of climate and bee race on Varroa jacobsoni Oud infestations in Brazil. Apidologie 22, 197–203.

Muli, E., Patch, H., Frazier, M., Frazier, J., Torto, B., Baumgarten, T., Kilonzo, J., Ng'ang'a, K.J., Mumoki, F., Masiga, D., Tumlinson, J., Grozinger, C., 2014. Evaluation of the distribution and impacts of parasites, pathogens, and pesticides on honey bee (Apis mellifera) populations in East Africa. PLoS One 9 (4), e94459. http://dx.doi.org/10.1371/journal.pone.0094459.

Orantes Bermejo, F.J., García Fernández, P., 1997. Nosema disease in the honey bee (Apis mellifera L.) infested with Varroa mites in southern Spain. Apidologie 28 (3–4), 105–112.

Pengue, W.A., 2005. Transgenic crops in Argentina: the ecological and social debt. B. Sci. Technol. Soc. 25 (4), 314–322.

Riveros, F., 2009. El gran Chaco. http://www.fao.org/ag/agp/agpc/doc/counprof/spanishtrad/argentina_sp/granchaco/GranChaco_sp.htm (accessed on December 2015).

Rosenkranz, P., 1999. Honey bee (Apis mellifera L.) tolerance to Varroa jacobsoni Oud in South America. Apidologie 30 (2–3), 159–172.

Rosenkranz, P., Aumeier, P., Ziegelmann, B., 2010. Biology and control of Varroa destructor. J. Invertebr. Pathol. 103, 96–119.

SENASA (Animal Health National Service), 2007. Situación actual de Varroosis (accessed on 18th March, 2013). http://www.senasa.gov.ar/Archivos/File3824-varroosis-aituacion-actual-argentina.pdf.

Vandame, R., Palacio, M.A., 2010. Preserved honey bee health in Latin America: a fragile equilibrium due to low-intensity agriculture and beekeeping? Apidologie 41, 243–255.

vanEngelsdorp, D., Meixner, M.D., 2010. A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. J. Invertebr. Pathol. 103, 80–95.

Wallner, K., Fries, I., 2003. Control of the mite Varroa destructor in honey bee colonies. Pestic. Outlook http://dx.doi.org/10.1039/b301510f.