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Key management practices to prevent high infestation levels of *Varroa destructor* in honey bee colonies at the beginning of the honey yield season

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ABSTRACT

Varroa destructor is considered one of the main threats to worldwide apiculture causing a variety of physiological effects at individual and colony level. Also, *Varroa* mites are often associated with several honey bee viruses presence. Relatively low levels of Varroa during the spring, at the beginning of the honey yield season, can have a significant economic impact on honey production and colony health. Winter treatments against Varroa and certain management practices may delay mite population growth during following spring and summer improving colonies performance during the honey yield season. The aim of this study was to identify risk factors associated with the presence of *Varroa destructor* in late spring in apiaries from temperate climate. A longitudinal study was carried out in 48 apiaries, randomly selected to evaluate *V. destructor* infestation level throughout the year. The percentage of infestation with *V. destructor* was assessed four times during one year and the beekeepers answered a survey concerning all management practices applied in the colonies. We used a generalized linear mixed model to determine association between risk of achieving 2% infestation on adult bees at the beginning of the honey yield season and all potential explanatory variables. The complete dataset was scanned to identify colonies clusters with a higher probability of achieving damage thresholds throughout the year. Colonies that achieved ≥2% of infestation with *V. destructor* during spring were owned by less experienced beekeepers. Moreover, as Varroa populations increase exponentially during spring and summer, if the spring sampling time is later this growth remains unobserved. Monitoring and winter treatment can be critical for controlling mite population during the honey production cycle. Spatial distribution of colonies with a higher risk of achieving high Varroa levels seems to be better explained by management practices than a geographical condition.

1. Introduction

Varroa destructor (Anderson and Trueman, 2000) is considered one of the main threats to worldwide apiculture (Genersch, 2010). Damage caused by *Varroa* mite includes body weight loss, malformation, and weakening of the bees (Duay et al., 2003; Marcangeli et al., 1992; Garedew et al., 2004; Amdam et al., 2004). Also it was suggested that the presence of Varroa increases the number of viruses that can be detected in a colony (Mondet et al., 2014).

The control of *V. destructor* populations is important as infestation leads to increased honey production costs, and hence periodic treatment against Varroa is essential to avoid colony collapse (Rosenkranz et al., 2010). Also, the presence of mites indirectly affects the profitability of beekeeping given the cost of replacing colony losses (vanEngelsdorp and Maixner, 2010).

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Relatively low levels of Varroa during the spring, at the beginning of the honey yield season, can have a significant economic impact on honey production (Currie and Gatien, 2006). Usually, colonies that have reached more than 2% (2 mites per 100 adult bees) of infestation level during spring should be treated to prevent significant losses (Currie and Gatien, 2006). Moreover, to avoid achieving high levels of mites on adult bee at the beginning of the nectar flow is important since bee brood availability provide ideal conditions for mite reproduction. This may result in collapsing colonies before autumn treatment (Goodwin and Van Eaton, 2001). Organic treatments against Varroa at the end of winter may delay population growth during summer and therefore help in maintaining Varroa infestation levels below the threshold of damage in the following autumn (Giovanezzo and Dubreuil, 2011).

In addition, management practices may directly or indirectly affect the percentage of infestation with *V. destructor* (Boecking and Genersch, 2008; Giacobino et al., 2014). Together, management practices, environmental conditions, and socio economic factors circumscribe the spatial-temporal distribution of colonies with high Varroa infestation levels. In epidemiology, to associate risk factors to the spatial distribution of an infection is critical to understand a possible clustered disease (Pfeiffer et al., 2008).

Given the multiplicity and complex association of factors affecting the presence of *V. destructor* during the spring, it is essential to address the problem from an epidemiological approach. A longitudinal study is one in which the same individual is observed several times (Cook and Ware, 1983), based on the experience of the population in due course (Delgado Rodríguez and Llorca Díaz, 2004). Within the family of longitudinal studies, transition models perform a regression of the present disease circumstances (e.g. healthy/non healthy) as result of the past condition including present and past exposition to risk factors (Delgado Rodríguez and Llorca Díaz, 2004). This sort of study allows associating the percentage of parasitism registered in honey bee colonies during spring with the percentages of parasitism previously registered on the same colonies and the exposure to different environmental and management variables. The aim of this study was to identify risk factors associated with the presence of *Varroa destructor* at the beginning of the honey yield season (spring) in apiaries from temperate climate.

2. Materials and methods

2.1. Study design and sample size

A longitudinal study was carried out from February to December 2013 in Santa Fe province, east-central Argentina. Summers are warm and humid throughout the province with daily average temperature of 30 °C in the south of the province and 34 °C in the northwest. In the south, winter temperatures range from 15 °C during day to 4 °C at night, whereas in the north, from 21 °C to 9 °C, respectively. However, from the beekeeping viewpoint there is not a broodless period and therefore brood is presented during the entire year (Marcangeli et al., 1992; Giacobino et al., 2015). Thus "winter" is defined between the end of the autumn acaricide treatment and the beginning of the following nectar flow.

Approximately 95% of the Argentinean honey production is exported (Blengino, 2014). Beekeeping in this region is represented by beekeepers that own fewer than 200 colonies, distributed in several apiaries. Honey yield is performed exclusively during summer, once or twice per season and colonies are normally treated against Varroa mites during autumn. Almost all beekeepers apply a commercial acaricide with a few exceptions that may use homemade formulations (Giacobino et al., 2015). On the other hand, late winter–early spring treatment is less common and depends on each year conditions (sanitary and economically). Usually, apiaries receive some carbohydrate supply during autumn and spring (frequently sucrose syrup) and less frequently some pollen substitutes (Giacobino et al., 2014). Also, during spring some beekeepers replace their colony queens (buying new queens from commercial queen producers) and make nuclei from their colonies before nectar flow start.

Santa Fe province has 3735 apiaries officially registered distributed in four regions (Giacobino et al., 2014). During 2012, we first informally contacted the beekeepers in the annual conference meetings that took place in different cities from Santa Fe province. We explained comprehensively the purpose and importance of the survey. The number of apiaries within each region was determined according to the proportional distribution and randomly chosen (Moher et al., 2010). The selected beekeepers were contact again either personally or by telephone to confirm they participation in the study. Although there was no economic incentive, at the end of the study each beekeeper received a detailed report with the study results.

A total of 48 apiaries were sampled consistent with the amount of apiaries in Santa Fe province n = 3735; 95% confidence level; precision = 14.05% and 74% of expected prevalence of colonies with >3% of Varroa adult infestation during autumn (Department of Agriculture from Santa Fe province, 2008). Within each apiary, a minimum of 6 colonies or 10% of total colonies has been randomly selected to evaluate *V. destructor* infestation level throughout the year (Lee et al., 2010).

2.2. Data collection

The percentage of infestation with *V. destructor* was assessed four times in the entire study period (the same colonies were sampled in each apiary during the four moments): prior to and after autumn acaricide treatment (following last honey yield); during winter (July–August) and at the beginning of the next honey yield season (middle spring). Each time, approximately 250 bees were collected from both sides of three unsealed brood combs in a jar containing 50% ethanol and drop of soap. The mites were separated from the bees by pouring the jar content into a sieve with a mesh size of 2 mm (Dietemann et al., 2013). The intensity of mite infestation 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). In addition, the numbers of bees and brood, pollen, and honey cells of all colonies were estimated according to the Liebefeld method (Imdorf and Gerig, 2001).

During each visit to the apiaries the beekeepers answered a management survey (available upon request). The first questionnaire (prior to autumn acaricide treatment) gathered information about: general apiary traits (i.e. geographic location, number of colonies, average honey production and winter mortality per year), commonly performed management practices (carbohydrates and protein diets, monitoring of mite levels in the colonies measured by the beekeepers, queen replacement, making nuclei, colonies migration) and acaricide treatment against Varroa mites (active ingredient, date of treatment, chemical rotation during the last four years). During the following visits we recorded all the information concerning management practices applied during the year. Table 1 shows a summary of the management practices variables registered in all surveys. The response variable for all the analysis was the Varroa infestation level at the beginning of the honey yield season during spring (≥2%; <2%), according to Currie and Gatien (2006).

Table 1
Summary of variables derived from the questionnaire and assessed as potential risk factors for colonies with more than 2% of Varroa infestation on adult bees at the beginning of the honey yield season.

General Management on the apiary	Variable description
Region	Geographic location within Santa Fe province: North, South, Central and Riverside
Size of the apiary	Number of colonies within each apiary
Average Winter mortality	Percentage of colonies lost in the apiary in the last three years
Average honey yield	Honey harvested per colony (kg.) in the last three years
Migratory beekeeping	If normally colonies are moved during winter If yes: to which crops, when and how long
Protein diet	Feed colonies during autumn or spring with natural pollen, supplements or substitutes
Carbohydrate supply	Feed colonies during autumn or spring with sucrose syrup or high fructose corn syrup
Colony multiplication	Colonies within the apiary that has been used for nuclei production during last spring
Frequency of requeening	How frequently (in years) a queen is replaced in each colony by the beekeepers
Percentage of requeening	The proportion of colonies within each apiary in which queen is replaced by the beekeeper during one season
Annual comb replacement	How many combs per colony are replaced by new ones per year
Wooden ware disinfection	Do you normally disinfect the wooden ware before storage it after yield season? If disinfection takes place, how?
Autumn treatment against Varroa mites	Active substance that they used Date of treatment Rotation of chemical substance during last 4 treatments
Monitoring varroa infestation level	Checking for the% of varroa infestation level in adult bees, prior to and after treatment
Late winter–Early spring treatment	Active substance that they used Date of treatment

2.3. Statistical analysis

We compared the adult bee population size and the amount of brood, pollen, and honey cells over the entire experimental period between colonies with ≥2% and with <2% at the beginning of the honey yield season (outcome from now on) using a generalized linear mixed model (GLMM) including temporal pseudo-replication as colonies were measured four times.

We first evaluated separately all potential explanatory variables associated with the outcome (univariate analysis), using two GLMM according to spatial (apiary) and temporal (repeated measures) data distribution. On the one hand we evaluated the management practices variables using a GLMM with apiary as random effect as all colonies from the same apiary are uniformly managed. On the other hand we use a GLMM with apiary and colony as random effects to evaluated the effect of Varroa mites level measured during the former visits, prior to and after autumn acaricide treatment and during winter (here colony is also included as random factor because the same colony was measured several times). All variables with a significance value $P < 0.15$ were selected to be included in the final multivariable model after confounding revision.

We used a multivariable GLMM to determine association between risk of achieving >2% infestation and the significant factors previously tested, including apiary and colony as random effects.

Table 2
Honey bee categories according to the percentage of infestation with *Varroa destructor* on adult bees throughout the year 2013.

Season	Low infestation with Varroa mites on adult bees (Non-cases)	High infestation with Varroa mites on adult bees (Cases)	Source
Autumn (prior to acaricide treatment)	≤3%	>3%	Giacobino et al. (2014)
Autumn (after acaricide treatment)	≤1%	>1%	Giacobino et al. (2015)
Winter (July–August)	≤1%	>1%	Giacobino et al. (2015)
Middle spring (Beginning of the honey yield)	≤2%	>2%	Currie and Gatien (2006)

The outcome variable (<2%; ≥2%) was assumed to follow a binomial distribution with a logit link function:

$$\text{Varroa infestation at the beginning of the honey yield season} \sim X_1 + X_2 + X\ldots_n + (1|\text{apiary}) + (1|\text{colony}),\ \text{family} = \text{binomial}.$$

where $X_1 + X_2 + X\ldots_n$ were fixed effect factors selected after univariate analysis and confounding revision and (1|apiary) and (1|colony) were the random intercepts. Variables with a significant odds ratio, calculated using the Wald test ($P < 0.05$), were maintained in the model. There are often complex interrelationships between variables being studied, which can lead to confounding effects. We tested confounding by evaluating change in odds ratio after including the potential confounders in the multivariable model. With each variable removed from the model, the coefficient of significant variables was checked and if it resulted in more than 20% change in estimates, the variable was retained in the model to account for its confounding effect (Chowdhury et al., 2012).

We evaluated a priori of the multivariable analysis for potential collinearity by testing that a variable is not in the causal pathway using causal diagrams: we tested correlation between all explanatory variables before including them in the multivariable model. Besides we evaluated variables that may explain the same potential effect (for instance winter treatment/winter treatment product) and only choose the one with the lowest P-value. Finally, interactions between all explanatory variables (2 ways) were evaluated in the final model.

All the statistical analyses were carried out using *lme4, Lattice* and *Matrix* packages from R software (version 2.15.3).

2.4. Space-time analysis

Honey bee colonies classified as high and low Varroa infestation levels were considered as "cases" and "non-cases", respectively (Table 2) and a Bernoulli distribution was assumed. The complete dataset was scanned to identify colonies clusters with a higher probability of achieving damage thresholds throughout the year. Clusters were detected using the Space-time retrospective Scan Statistics method. The analysis defines also a time interval for each possible location and cluster size. Thus an infinite number of overlapping cylinders of different size and shape along the study region are obtained (Kulldorff et al., 1998). The likelihood ratio for each proposed cluster, with upper limit set scanning over 50% of the study population and 50% of the study period was calculated. Spatial-temporal analysis was performed using the software *SaTScan* version 9.2 (available at www.satscan.org) and the cluster output was included in a map produced with QGIS version 2.2.0-Valmiera (available at www.qgis.org).

Table 3
Sampling time of adult bee population and amount of brood, pollen, and honey cells during 2013 according to treatment schedule and honey flow (n: 280 honey bee colonies distributed in 48 apiaries).

Month	Season	Beekeeping Schedule	Adult bees population (±S.D)	Brood Cells (±S.D)	Pollen Cells (±S.D)	Honey Cells (±S.D)
January	Summer	Honey flow	–	–	–	–
February	Summer	Honey flow	–	–	–	–
March	Autumn	Samples Prior to acaricide treatment	19005 (±3049)	33166 (±12298)	5729 (±4695)	19852 (±10976)
April						
May		Samples After acaricide treatment	15667 (±3905)	9582 (±8096)	4111 (±4874)	24449 (±12360)
June	Winter with		–	–	–	–
July	present brood	Samples Winter	13844 (±3662)	14771 (±11588)	3932 (±4326)	15420 (±9479)
August						
September	Spring	Samples Beginning of season	22998 (±10835)	45609 (±16950)	6606 (±4138)	22400 (±26783)
October			–	–	–	–
November						
December	Summer	Honey flow	–	–	–	–

3. Results

The percentage of infestation at the beginning of the 2013 honey yield season was 0.93% ± 2.07% (mean ± SD). The prevalence of honey bee colonies with values ≥2% infestation in middle spring was 16.8% (280 honey bee colonies distributed in 48 apiaries). A total of 32 apiaries (67%) were treated against Varroa during or at the end of winter and 72.7% of the beekeepers choose oxalic acid to do it. Average bee population and number of brood cells at the beginning of this season were 22998 ± 10835 bees and 45609 ± 16950 cells, respectively. There were not statistically significant differences in population of adult bees ($z = -0.199$; $P = 0.84$); brood cells ($z = 0.017$; $P = 0.99$) and the pollen ($z = -0.163$; $P = 0.87$) and honey ($z = 0.023$; $P = 0.98$) reserves all over the study between colonies with ≥2% and with <2% of infestation with *V. destructor* at the beginning of the honey yield season (Table 3).

From all potential risk factors separately analyzed (Tables 4 and 5), seven significant variables ($P < 0.15$) were selected to be included in the multivariable analysis. More experienced beekeepers showed less probability of having colonies ≥2% ($P = 0.054$) as well as those who declared to replace their colonies queens ($P = 0.04$) and to make nuclei ($P = 0.077$). Colonies treated during winter were less likely of achieving ≥2% at the beginning of the honey yield season ($P = 0.014$). In addition, the presence of apiaries closer than 1500 m and sampling time at beginning of season were linked to Varroa levels ($P = 0.109$; $P < 0.001$, respectively). Also, colonies that achieved more than 2% during honey yield season were more likely to have more than 1% infestation with Varroa on adult bees during previous winter ($P = 0.014$).

Four out of the seven selected variables were significant in the final multivariable model. Honey bee colonies that had ≥2% during the spring were monitored during late spring (OR = 9.9; $P < 0.001$) (Table 6).

Alternatively, colonies that had <2% during the spring were owned by more experienced beekeepers (OR = 0.33; $P = 0.01$); who also frequently replaced their colonies queens (OR = 0.085; $P < 0.001$), and treated the colonies against *V. destructor* during winter (OR = 0.25; $P = 0.002$) (Table 6).

No confounding effect was found when non significant variables were removed from the multivariable analysis as magnitude of change in significant odds ratio was lower than 20%. However, a significant interaction (OR = 52.99; $P < 0.001$) was found between queen replacement and Varroa level on adult bees during winter and therefore was included in the final model (Table 6). In apiaries where no queen replacement is performed similar proportion (about 30%) of colonies had ≥2% during the spring independently of the infestation with *V. destructor* showed during previous winter ($P = 0.83$). In contrast, only 7.9% of the colonies owned by beekeepers who declared to replace the queens reached 2% of infestation with Varroa during spring when winter Varroa infestation was less than 1% ($P = 0.001$).

Six honey bee colonies cluster were detected for the time interval from autumn to middle spring during 2013 (Table 7), for a total of 280 colonies distributed in 48 apiaries (Fig. 1). The total number of cases in the study area was 138 (16.4%) similar to the prevalence of honey bee colonies with values ≥2% infestation in middle spring reported in this study.

Four out of the six significant detected clusters had colonies with a higher probability of achieving damage thresholds throughout the year (high Varroa level cluster). Two of them included three apiaries, one included two apiaries and in the last cluster just one apiary was included. On the other hand, the two low Varroa level clusters identified were bigger including four and seven apiaries respectively.

4. Discussion

Varroa destructor is one of the leading causes of *Apis mellifera* colonies losses (Guzmán-Novoa et al., 2010; Smith et al., 2013) and loss of honey production (Murilhas, 2002; Medina Flores et al., 2011). Sanitary control, especially at the beginning of the honey yield season is essential to avoid the exponential growth of mites population in late summer and early autumn, when probably the mite population reaches a significant damage threshold (Le Conte et al., 2010).

In this study, four variables associated with the percentage of infestation with Varroa mites at the beginning of the honey yield season were identified: (1) winter treatment, (2) queen replacement, (3) beekeeping experience and (4) sampling time at beginning of season. In addition, risk of achieving more than 2% during spring when Varroa winter levels were under 1% was reduced by queen replacement performance.

4.1. Risk factors

A significant association between Varroa level on adult bees during spring and queen replacement was found. This is in line with the previously suggested role of queen replacement as a potential risk factor associated with treatment failure (Giacobino et al., 2015) and with higher mite infestations during autumn (Giacobino et al., 2014). The age and quality of the queen are two highly leading conditions influencing brood rearing, growth rate and honey production (Akyol et al., 2007). It was reported that honey bee colonies with queens up to one-year-old produced up to 40% more honey

Table 4
Distribution of management practices analyzed using a generalized linear mixed model (univariate analysis) for potential association with *Varroa destructor* infestation level at the beginning of the honey yield season during spring 2013 (≥2%; <2%).

Variable	Level	No Cases[a](Total number)	Odds Ratio (95% CI)[b]	*P*-Value
Geographical zone	North	20 (47)	Reference	0.973
	Centre	24 (153)	0.25 (0.12–0.52)	
	South	3 (68)	0.06 (0.02–0.23)	
	Riverside	0 (12)	–	
Beekeeping experience	≤10 years	35 (140)	Reference	0.054
	>10 years	12 (125)	0.29 (0.14–0.59)	
Number of colonies per apiary	Continuous	–	–	0.765
Protein diet	No	31 (153)	Reference	0.651
	Yes	16 (127)	0.57 (0.29–1.09)	
Kind of Protein diet	No or pollen patties	44 (256)	Reference	0.870
	Natural pollen	3 (24)	0.68 (0.20–2.41)	
Carbohydrate diet	No	4 (11)	Reference	0.466
	Sucrose syrup	39 (250)	0.32 (0.09–1.15)	
	HFCS	4 (19)	0.47 (0.09–2.43)	
Queen replacement	No	25 (98)	Reference	0.04
	Yes	22 (188)	0.35 (0.19–0.67)	
% of colonies with queen replacement per apiary	≤50%	40 (251)	Reference	0.506
	>50%	7 (29)	1.68 (0.67–4.19)	
Making nuclei	No	15 (43)	Reference	0.077
	Yes	32 (237)	0.29 (0.14–0.60)	
Old combs replacement per colony per year	≤3 combs	45 (254)	Reference	0.551
	>3 combs	2 (26)	0.39 (0.09–1.70)	
Hives wooden ware disinfection	No	12 (105)	Reference	0.428
	Yes	35 (175)	1.94 (0.96–3.93)	
Monitoring before acaricide treatment	No	1 (32)	Reference	0.168
	Yes	46 (248)	7.06 (0.94–53.05)	
Monitoring after acaricide treatment	No	13 (54)	Reference	0.283
	Yes	34 (226)	0.56 (0.27–1.15)	
Autumn treatment product	Amitraz	3 (48)	0.25 (0.07–0.85)	0.149*
	Coumaphos	0 (22)	–	
	Flumethrin	44 (210)	Reference	
Autumn treatment date	Feb./March	41 (243)	Reference	0.716
	Apr./May.	5 (31)	0.95 (0.34–2.61))	
Winter treatment	No	29 (92)	Reference	0.014
	Yes	19 (187)	0.26 (0.13–0.49)	
Winter treatment date	No	29 (92)	Reference	0.292
	July/August	6 (104)	0.14 (0.05–0.36)	
	September	9 (47)	0.54 (0.23–1.27)	
Winter treatment product	No	28 (92)	Reference	0.048**
	Synthetics	2 (51)	0.09 (0.02–0.41)	
	Organics	17 (136)	0.33 (0.17–0.64)	
Apiaries closer than 1500 m	No	2 (45)	Reference	0.109
	Yes	45 (228)	5.29 (1.23–22.65)	
Sampling time at beginning of season	Aug./Sept.	6 (141)	Reference	<0.001
	Nov./Dec.	41 (138)	9.51 (3.88–23.29)	

[a] Cases: honey bee colonies with ≥2% of percentage of infestation with *V. destructor* at the beginning of honey yield season 2013–2014.
[b] 95% confidence interval.
* Excluded: level of variables with 0 cases.
** Excluded: *Chi-square* approximation is not suitable when the expected values in any of the cells are below 5.

Table 5
Generalized linear mixed model for Varroa mites level (measured during autumn and winter) analyzed for potential association (univariate analysis) with *Varroa destructor* infestation level at the beginning of the honey yield season during spring 2013 (≥2%; <2%).

Variable	Level	Cases[a] (%)	*P*-Value
Varroa level on adult bees before autumn treatment	<3%	14.1	0.169
	≥3%	19.3	
Varroa level on adult bees after autumn treatment	<1%	17.4	0.223
	≥1%	13.3	
Varroa level on adult bees during winter	<1%	14.5	0.014
	≥1%	28.3	

[a] Cases: honey bee colonies with ≥2% of percentage of infestation with *V. destructor* at the beginning of honey yield season 2013–2014.

Table 6
Generalized lineal mixed model for risk factors (multivariable analysis) associated with *Varroa destructor* infestation level at the beginning of the honey yield season during spring (≥2%; <2%) in 280 honey bee colonies distributed in 48 apiaries.

	AIC	BIC	Loglik	Pr(>Chisq)
Null model (only intercept)	211.94	219.21	−103.968	**0.003**
Full model	187.76	220.48	−84.88	
Fixed Effects	**Level**	**OR[a]**	**95% CI[b]**	**Pr(>|z|)**
Varroa level on adult bees during winter	<1%	Reference	–	–
	≥1%	0.27	0.04–1.59	0.15
Winter acaricide treatment	No	Reference	–	–
	yes	0.25	0.11–0.61	**0.002**
Queen replacement	No	Reference	–	–
	yes	0.09	0.03–0.23	**<0.001**
Beekeeping experience	≤10 years	Reference	–	–
	>10 years	0.33	0.14–0.78	**0.01**
Sampling time at beginning of season	Aug./Sept.	Reference	–	–
	Nov–Dec	9.99	3.67–27.16	**<0.001**
Queen replacement*Varroa level on adult bees during winter	No*<1%	Reference	–	–
	yes *≥1%	52.99	5.39–520.56	**<0.001**

Bold values signifies statistical significance $p < 0.05$.
[a] Odds Ratio.
[b] 95% confidence interval.

Table 7
Summary of honey bee colonies space-time distribution (Cluster detection), for low and high rate clusters throughout the year (n: 48; maximum spatial cluster size: 50% of population at risk; number of replications: 999).

Cluster Order	No of apiaries included	Radius (km)	RR[a]	Time interval	*P*-value
1	3	20.18	4.30	After autumn acaricide treatment	0.005
2	2	5.51	3.29	Winter-Beginning of harvest season	0.006
3	1	0	6.28	After autumn acaricide treatment	0.011
4	3	28.91	3.05	Winter-Beginning of harvest season	0.03
5	4	16.89	0	Winter-Beginning of harvest season	0.031
6	7	74.37	0	After acaricide treatment	0.031

[a] RR: Relative risk.

compared to two-year old queens (Akyol et al., 2007). In addition, the role of the queen in the worker bee population renewal and losses caused by Nosemosis is essential to maintain colony homeostasis (Botías et al., 2012). To periodically replace the queen is important in professional beekeeping to maintain strong, healthy, and productive colonies (Invernizzi et al., 2006). Moreover, when no queen replacement was performed similar proportion of the honey bee colonies exceeded the damage threshold during spring (2% on adult bees) even when showed low infestation with Varroa all through winter. Likewise, when queen replacement was performed, 28% of the colonies with ≥2% on adult bees were more likely to have more than 1% infestation during winter but a smaller fraction of the colonies showed high infestation during spring when winter infestation was under 1%.

Colonies with less than 2% of spring Varroa infestation were frequently owned by more experienced beekeepers suggesting that "management itself" is a risk factor. Although we try to disaggregate "management" in order to recognize core practices (acaricide treatment, queen replacement, monitoring, etc.), we include the beekeeping experience as a multidimensional variable that accounted for those aspects that are hard to quantify (for instance time spending in the apiary, experience detecting the presence of diseases in colonies, etc).

On the other hand, the probability of exceeding the damage threshold increases significantly as the spring progresses into summer, suggested by the significant effect of the sampling time at beginning of season. These results emphasize the importance of early monitoring as a key tool for controlling mite population during the honey production cycle (Imdorf et al., 2003). A low infestation level in spring may reflect the successful use of an acaricide during the winter (Ward et al., 2008). During spring, once nectar flow started and the honey yield season began it is not longer possible to use a chemical control treatment. Therefore, beekeepers must wait to treat their colonies until honey yield is over, during early autumn, when it is likely that Varroa populations already reached damaging level. Apiaries should be monitored regularly to ensure that treatments successfully keep mite levels below the damage threshold (Currie and Gatien, 2006; Boecking and Genersch, 2008).

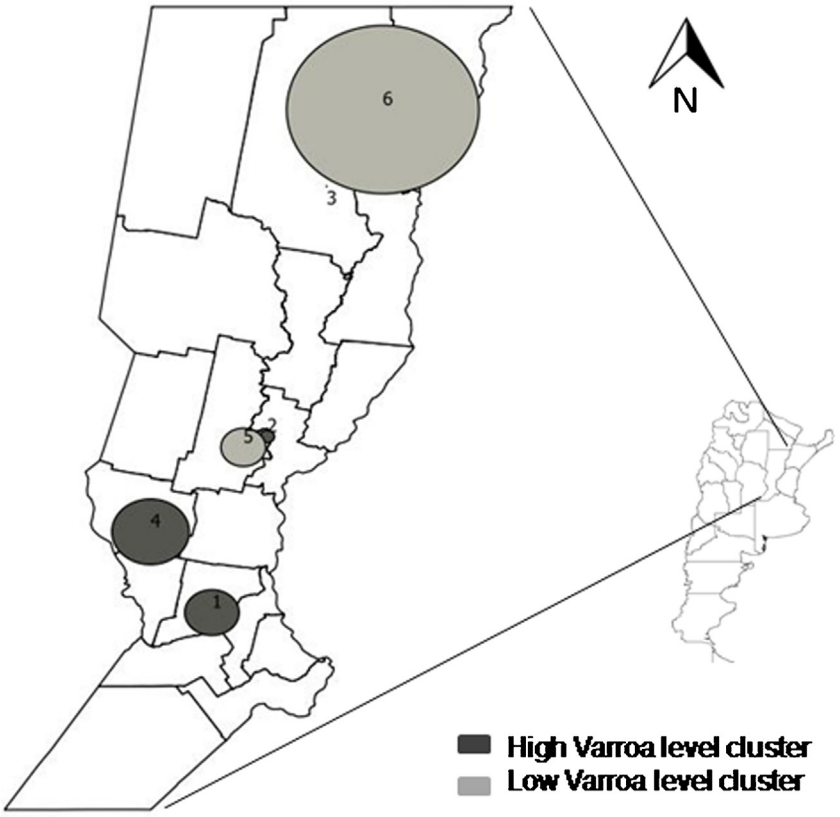

Fig. 1. Space-time *cluster* distribution of honey bee colonies with high probability (high Varroa level) and low probability (low Varroa level) of achieving damage thresholds for percentage of Varroa infestation throughout the year.

Having an accurate estimate of population size of *V. destructor* in honey bee colonies is an important tool in an Integrated Pest Management program (Branco et al., 2006).

We used only the adult bee infestation as a measure of the mite infestation level per colony mainly because the number of samples to analyze and the distances from apiaries to the laboratory act like a restriction condition. Although brood amount affects significantly Varroa mite population growth (Wilkinson and Smith, 2002), both high and low infested colonies showed similar amounts of sealed brood cells during the entire study. Thus, we considered that adult bee infestation data was a good estimator of the infestation level in the colonies despite its restrictions. Similarly, threshold justification for the spring season was based on Canada results published by Currie and Gatien (2006) which are different from Argentinean conditions. On the one hand, spring information was not available from our region conditions so we searched for other countries thresholds as a secondary option. On the other hand, we selected it considering that proposed spring value was twice the winter threshold and that brood amount increases significantly during spring and summer. Moreover, as the colonies are not treated throughout nectar flow is preferable to be cautious in controlling mites before honey yield season.

4.2. Space-time analysis

The identification and reporting of areas with the highest apparent incidence is called disease cluster alarm. It determines the location and traits of these cluster groups and provides information about conditions that are favorable for the development and spread of the disease (Pfeiffer et al., 2008).

Two of the four high Varroa level clusters were detected in the "after autumn acaricide treatment" time interval and the other two along winter and the beginning of the next yield season. These clusters include colonies that had been treated with homemade acaricides and therefore they showed a high proportion of cases after treatment. Commonly, unfavorable economic conditions may result in the beekeepers using homemade products, usually in an uncontrolled concentration (vanEngelsdorp and Maixner, 2010). This contributes to both contamination of the bee products and resistance development (Wallner, 1999; Wallner and Fries, 2003; Higes et al., 2010).

Also, high Varroa level clusters were linked to the absence of winter acaricide treatment and the presence of apiaries closer than 1500 m and consequently presented a high proportion of cases during or after winter. Risk of achieving ≥1% of infestation with Varroa during winter increased since it is likely that a high density of honey bee colonies within flight range would increase the invasion pressure (Frey and Rosenkranz, 2014). Moreover, acaricide failure in a region with crowded colonies along with the absence of a monitoring assessment might result in massive re-invasion pressure (Imdorf et al., 2003), increasing the risk of colony damage and selecting for more virulent forms of the pathogen (Fries and Camazine, 2001; Frey and Rosenkranz, 2014).

Two low Varroa level clusters were identified, one of them for the "after autumn acaricide treatment" time interval and the other along winter and the beginning of the next yield season. These clusters include colonies that were treated with oxalic acid during winter and monitored after treatment to evaluate its effectiveness (Boecking and Genersch, 2008). This treatment is essential for keeping Varroa population under 1% of infestation on adult bees as most mites which are likely to appear in the next year population are killed (Rashid et al., 2012), avoiding further damage to the colonies (Giovanezzo and Dubreuil, 2011). Combining monitoring of the colonies and the use of organics products at the end of winter

can help maintaining Varroa population below damage threshold during spring (Rashid et al., 2012).

Spatial distribution of colonies with a higher risk of achieving high Varroa levels seems to be better explained by management practices than a geographical condition. We found that High Varroa level cluster were distributed across the entire study region. The lack of coordinated strategies and comprehensive treatment as well as control methods that are unsuccessfully applied or delayed, lead to significant colony losses (Boecking and Genersch, 2008). Systematic monitoring and proper management practices implementation alongside acaricide treatments could reduce the negative impact of *Varroa destructor* during spring, a key period of the honey production cycle. Further research based on longitudinal studies should be conducted in order to review the correlation between Varroa infestation level and honey yield throughout the years.

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