Spanish Journal of Agricultural Research
20 (4), e0305, 15 pages (2022)
eISSN: 2171-9292
https://doi.org/10.5424/sjar/2022204-19634
INIA-CSIC

RESEARCH ARTICLE

OPEN ACCESS

Impact of nutritional and sanitary management on *Apis mellifera* colony dynamics and pathogen loads

Agostina Giacobino, Adriana Pacini, Ana Molineri, Natalia Bulacio-Cagnolo, Julieta Merke, Emanuel Orellano, Mónica Gaggiotti and Marcelo Signorini

Instituto de Investigación de la Cadena Láctea (INTA-CONICET). Ruta 34, Km 227, Rafaela. 2300 Santa Fe, Argentina.

Abstract

Aim of study: The aim of this study was to assess the impact of the mite control strategies combined with nutritional management on honey bee colony dynamics and survival during winter, the following spring, and summer.

Area of study: Santa Fe province in central Argentina.

Material and methods: We set two apiaries with 40 colonies each and fed one apiary with high fructose corn syrup (HFCS) and the other with sucrose syrup (SS). Within each apiary, we treated half the colonies against Varroa mites and half of these treated colonies also received a pollen patty. The other half of the colonies remained untreated and did not received pollen patties. All colonies were sampled for Varroa infestation level, *Nosema ceranae* abundance and colony strength seven times during a year (from summer 2016 to autumn 2017). We computed autumn mite invasion and colony losses at each sampling time.

Main results: Colonies fed with HFCS had more brood cells during the study that those fed with SS and treated colonies had fewer adult bees and Varroa infestation than untreated colonies. No significant effect of the protein supplementation was observed on any of the response variables. During 2017, SS colonies from all groups had significantly more mites drop counts than HFCS colonies.

Research highlights: Considering that a reduced frequency of application is desirable, our results suggested that nutrition management could enhance chemical treatment effectiveness since honey bees might profit from improved nutrition. However, a better understanding of the nutritional requirements of the colonies under field conditions is needed.

Additional key words: honey bees; *Varroa destructor*; beekeeping; chemical control; nutrition

Abbreviations used: GLZ (generalized linear model); HFCS (high fructose corn syrup); OI (overwinter index); SS (sucrose syrup).

Citation: Giacobino, A; Pacini, A; Molineri, A; Bulacio-Cagnolo, N; Merke, J; Orellano, E; Gaggiotti, M, Signorini, M (2022). Impact of nutritional and sanitary management on *Apis mellifera* colony dynamics and pathogen loads. Spanish Journal of Agricultural Research, Volume 20, Issue 4, e0305. https://doi.org/10.5424/sjar/2022204-19634

Received: 09 Jun 2022. **Accepted:** 10 Nov 2022.

Funding agencies/institutions	Project / Grant
Instituto Nacional de Tecnología Agropecuaria, Argentina	PNAPI Project Nº 1112042
	Specific Project Nº 1112042 "Estrategias multidisciplinarias para mitigar el efecto del nuevo contexto ambiental y productivo sobre la colmena"
ANPCyT	PICT 2016-1568

Competing interests: The authors have declared that no competing interests exist.

Correspondence should be addressed to Agostina Giacobino: giacobino.agostina@inta.gob.ar

Introduction

Traditionally in temperate climate, beekeeping management efforts are focused on enhancing colony strength and food stores during autumn, improving queen quality and protecting bees from *Varroa destructor.* This management is based on annual colony cycle that consider an overwintering state (distinct physiological and behavioral). The beginning and time duration of the overwintering period depends on environmental nutritional resources, physiological profile and climate conditions (Döke *et al*., 2015). Otherwise, overwintering success (for instance few colony losses) depends strongly on how beekeepers prepare their colonies during autumn (Smart *et al*., 2016). Concerning the honey bee health, both *V. destructor* presence and bee nutrition plus their interaction are key stressors specially linked to colony losses (Le Conte *et al*., 2010). In this context, some key aspects of the prevention of colony losses are linked to beekeeper background and management practices (Jacques *et al*., 2017; Giacobino *et al*., 2018).

Firstly, Varroa control in productive honey bee colonies is mandatory under temperate climate. Despite some limitations, synthetic and organic chemical substances are widely used (Rosenkranz *et al*., 2010). Acaricide not only efficiently control Varroa mites (Rosenkranz *et al*., 2010; Semkiw *et al*., 2013) but might also indirectly help mitigating *Nosema* sp. impact as a number of potential interactions has been revealed (Mariani *et al*., 2012; Little *et al*., 2016; Giacobino *et al*., 2017). Nevertheless, the impact of frequent introduction of a chemical on a colony weakened by pathogens might potentially diminish the lifespan of bees and compromise the immune system (Boncristiani *et al*., 2012). Thus, in line with Integrated Pest Management, alternative or complementary to application of chemicals methods are of particular interest (Dietemann *et al*., 2012; Al Toufailia *et al*., 2014: Lodesani *et al*., 2014; 2019).

Secondly, bee survival varies as function of feeding choices, including honey and sucrose syrup (Abou-Shaara, 2017). There is an increasing interest in artificial feeding (Dolezal & Toth, 2018) because honey bee nutrition is highly dependent on the floral resources that diminishes during periods of foraging dearth (Tsuruda *et al*., 2021). Most apiaries from temperate climate in Argentina received a carbohydrate supply during autumn (Giacobino *et al*., 2014) similar to the supplementation practice reported in the USA (Tsuruda *et al*., 2021) and Italy (Frizzera *et al*., 2020). Sucrose syrup (SS) and high fructose corn syrup (HFCS) are both alternatives to implement carbohydrate supplementation when sources of pollen and nectar are scarce. On the one hand, beekeepers buy HFCS because it is ready to administer and it is frequently cheaper than sucrose (reviewed in Wheler & Robinson, 2014). In addition, lower level of Varroa infestation were found in colonies fed with high HFCS (Giacobino *et al*., 2014). On the other hand, colonies fed with SS showed more brood production and more attraction to the syrup in comparison with HFCS fed colonies (Sammataro & Weiss, 2013).

Lastly, the well-known positive effect on bee survival of pollen availability suggests that a diet rich in pollen might be significant for the prevention of colony losses, especially by mitigating the negative impact of Varroa parasitism (Annoscia *et al*., 2017). An observational study pointed out that a diet based on natural pollen supplementation was associated with a reduced prevalence of colonies infested with Varroa mites (Giacobino *et al*., 2014). Furthermore, independently of whether a pollen diet can (Annoscia *et al*., 2017) or cannot compensate for negative effect of parasitism (Alaux *et al*., 2011) it might enhance the positive effect of Varroa control strategies.

Nutritional and health topics regarding honey bee were previously addressed, but few included field studies under semi-controlled conditions with a large number of colonies for several months in a row. Moreover, colony level approach is suitable for multiple stressors impact studies as colony measures are strongly indicative of apiary survival (Smart *et al*., 2016). The aims of this study were to: i)

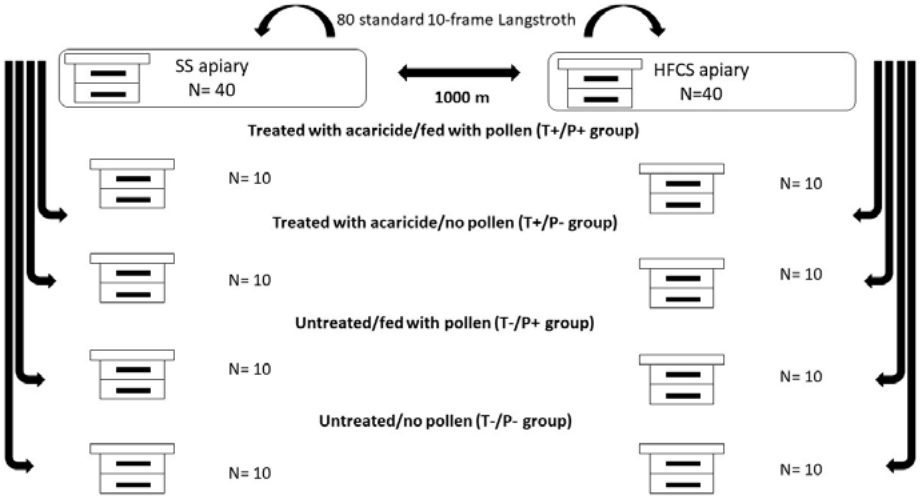

Figure 1. Apiaries and treatment distribution.

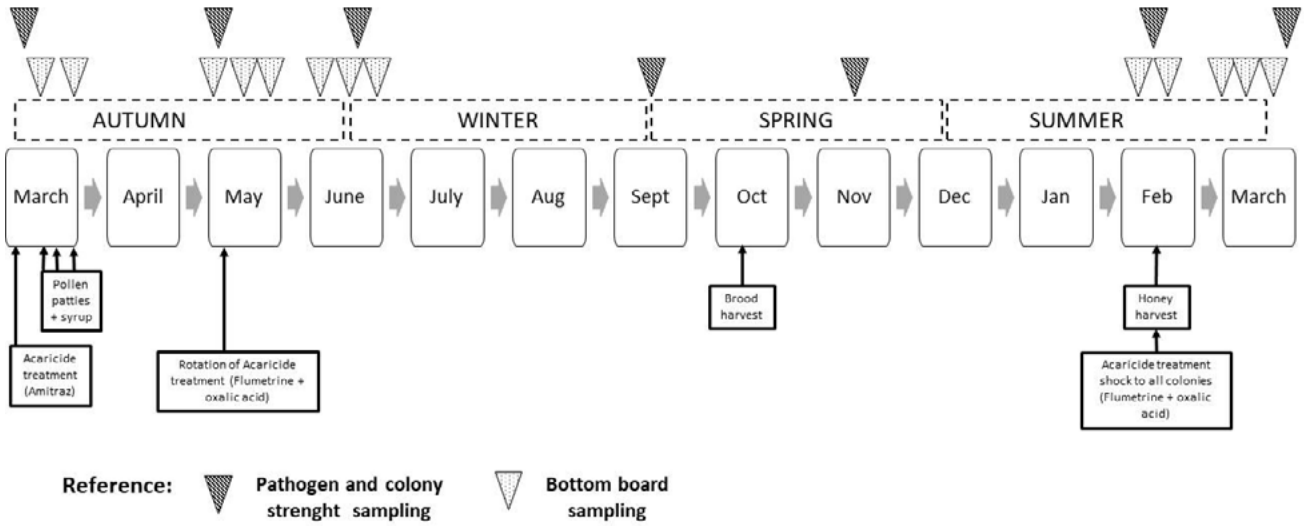

Figure 2. Timeline describing sampling time and treatment/feeding schedule.

verify the efficacy of different nutritional and mite control management strategies traditionally recommended for the overwintering season in temperate climates without broodless period; ii) evaluate the mite population dynamics in honey bee colonies under traditionally recommended management strategies for autumn season; and iii) assess the impact of nutritional management alternatives combined with autumn acaricide treatment on the strength and nutritional reserves of the honey bee colony during winter, the following spring, and summer.

Material and methods

Experimental setting

The study was conducted at the Experimental Station of National Institute of Agricultural Technology in Rafaela, Argentina (31°11' S latitude and 61°33' W longitude). We set an apiary with 80 nuclei (five frames each) in middle spring (October 2015). Each colony received a new, young, and openly matted queen of the same origin (sister queens). During the following summer (February 2016), when the nuclei reached sufficient population size, the 80 nuclei were transferred from their five-frame hives to 80 standard 10-frame Langstroth hives. All hives were equipped with a bottom board and distributed between two apiaries with half of the hives each and allocated 1000 m apart from each other, within the experimental station.

The number of colonies sampled was defined considering a treatment effect on the reduction on *V. destructor* infestation level of 30% (with 95% confidence level; precision=10% and standard error similar to the media of each group). Groups were standardized regarding honey bee population, amount of sealed brood cells, Varroa infestation level and *Nosema ceranae* abundance.

At the end of summer (late March 2016), one apiary was fed with high fructose corn syrup (HFCS apiary, n=40) and the other with sucrose syrup (SS apiary, n=40). Within each apiary, half of the colonies were continuously treated with an acaricide from March to June (T+, n=20), and the other half remained untreated (T-, n=20). In addition, half of the T+ colonies and half of the T- colonies within each apiary were supplemented with pollen (P+, n=10) and the other half were not (P- n=10). Overall, eight groups of 10 colonies each were defined, following a full factorial experimental design 2 × 2 × 2 (Fig. 1). Carbohydrate feeding and pollen supplementation was performed during three consecutive weeks. Once a week, 2 liters of HCFS or SS was added to the colonies using frame feeders. At the same time, P+ group received natural pollen patties (150 g) made with natural pesticide-free pollen mixed with the corresponding syrup. The HFCS pollen patties mixture had 15.3 % crude protein and SS pollen patties mixture had 15.08% crude protein. Weekly, unconsumed patties from the week before were removed and new patties were placed on the top bars of the frames. Treated colonies (T+) received amitraz (Amivar®) first (from March to May) and flumethrin (Flumevar®) + oxalic acid (Oxavar®) later (from May to June), in order to assess mite invasion rate (Fig. 2). This continuous application should have killed all invading mites (Frey & Rosenkranz, 2014).

Data collection

Colony strength measures and pathogens sampling

All colonies were sampled for Varroa infestation level and *N. ceranae* abundance estimation (see the schedule showed in Fig. 2). Colonies were sampled during March (before feeding and Varroa treatment), May, June, Sep-

tember, and November (2016). The following year (2017) all the surviving colonies were sampled in February and March. Approximately 250 honey bees were collected from both sides of three unsealed brood combs in a labeled plastic jar containing 50% ethanol (Dietemann *et al.*, 2013) to diagnose the presence of *V. destructor* in honey bee colonies during each sampling time. In the lab, the mites were separated from the bees by pouring the jar content into a sieve with a mesh size of 2 mm. The intensity of mite infestation on adult honey bees was calculated dividing the number of mites counted by the number of honey 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). Also, sticky bottom board mite drop counts were registered weekly from March to June 2016 and from February to March 2017 to estimate weekly mites invasion rates in the continuously treated colonies (T+ group) (Frey & Rosenkranz, 2014) (Fig. 2).

In order to establish *N. ceranae* presence, worker honey bee samples were collected from the hive entrance. A minimum of 60 bees were gathered and placed in labeled plastic flasks containing 60 mL of 96° ethanol. Spore suspensions were prepared by adding 60 mL of distilled water to crushed abdomens of 60 randomly selected individuals of each colony. *N. ceranae* spores/bee were determined using light microscopy 40× and hemocytometer. For each sample the number of spores in 80 hemocytometer squares (5 groups of 16 squares) was counted (Fries *et al*., 2013). Data set was Log_{10} transformed for statistical analysis purpose.

Additionally, the number of adult honey bees and the amount of sealed brood, honey and pollen cells of all colonies were estimated according to the Liebefeld method (Dainat *et al*., 2020) each time the samples were taken (Fig. 2). Colony losses were computed at each sampling time. Total losses (at the end of the study) were estimated, and winter mortality (colonies computed as death between May and September). The overwintering index (OI) per colony was also calculated as the number of spring bees divided by the number of previous autumn brood cells (Lodesani *et al*., 2014).

Honey bee hives products

Following the South hemisphere apicultural schedule, during October, frames fully covered with worker brood were gathered from colonies to make nuclei and avoid potential swarming (spring 2016). The number of harvested brood frames per colony was computed during October. In addition, honey yield from colonies was obtained during January-February (summer 2017) using a digital weighing scale Hook® AT-100 (0.001-150 kg). The honey yield (kg/colony) and brood harvest (number of frames with brood/colony) were registered in all colonies. At the end of the honey yield (2017) all colonies received a final acaricide treatment (flumethrin + oxalic acid). Each colony loss was recorded by date per apiary.

Table 1. Effect of acaricide treatment and nutritional management on colony strength, nutritional reserves and pathogen levels by means of generalized linear model (GLZ) for repeated measure (gamma distribution).

	Bee population		**Brood cells**		**Pollen cells**		**Honey cells**		**% Varroa infestation**		***N. ceranae* (spores/bee)**		**Bottom board count 2016**		**Bottom board count 2017**	
	p	**X**	***p***	**X**	***p***	**X**	***p***	**X**	***p***	**X**	***p***	**X**	***p***	**X**	***p***	**X**
Kind of syrup (HFCS/SS)	0.98		**0.05**	HFCS: 15756	0.163		0.66		0.99		0.10		0.85		**<0.001**	HFCS: 59
				SS: 13861												SS: 278
Acaricide treatment (Yes/No)	**0.01**	Yes: 14830	0.43		0.12		0.24		**<0.001**	Yes: 1.22%	0.81		**0.026**	Yes: 25	0.47	
		No: 16007								No: 3.15%				No: 15		
Protein supplementation (Yes/No)	0.48		0.83		0.24		0.47		0.303		0.10		0.92		0.15	
Syrup × Acaricide	0.63		0.68		0.15		0.77		0.16		0.623		**0.07**	HFCS: 20	0.52	
														SS: 31		
Syrup × Protein	0.82		0.76		0.35		0.51		0.58		0.41		0.55		0.57	
Acaricide × Protein	0.49		0.27		0.34		0.96		0.86		0.52		0.15		0.18	
Syrup × Acaricide × Protein	0.64		0.34		0.63		0.28		0.57		0.99		0.14		0.17	

p-values significant (<0.05) are highlighted in bold.

Statistical analysis

In order to evaluate the combined effect of acaricide treatment, protein supplementation and kind of syrup on annual colony strength (honey bee adult population and amount of sealed brood cells), nutritional reserves (number of cells contained pollen and honey) and pathogens abundance (percentage of Varroa on adult honey bees and *N. ceranae* abundance log) a generalized linear model (GLZ) for repeated measures (gamma distribution) was performed. In addition, a GLZ for repeated measures (Poisson distribution) was used for the comparisons of the weekly mite invasion rate (estimated by sticky bottom board mites drop counts) in all colonies, during autumn 2016 and 2017.

A GLZ with gamma distribution for *N. ceranae* abundance and percentage of Varroa on adult honey bees per sampling time was used to assess the effect of the treatments (kind of syrup × acaricide treatment × protein supplementation). Honey production (kg/colony) was compared in all groups with a factorial ANOVA 2 × 2 × 2. Similarly, dif-

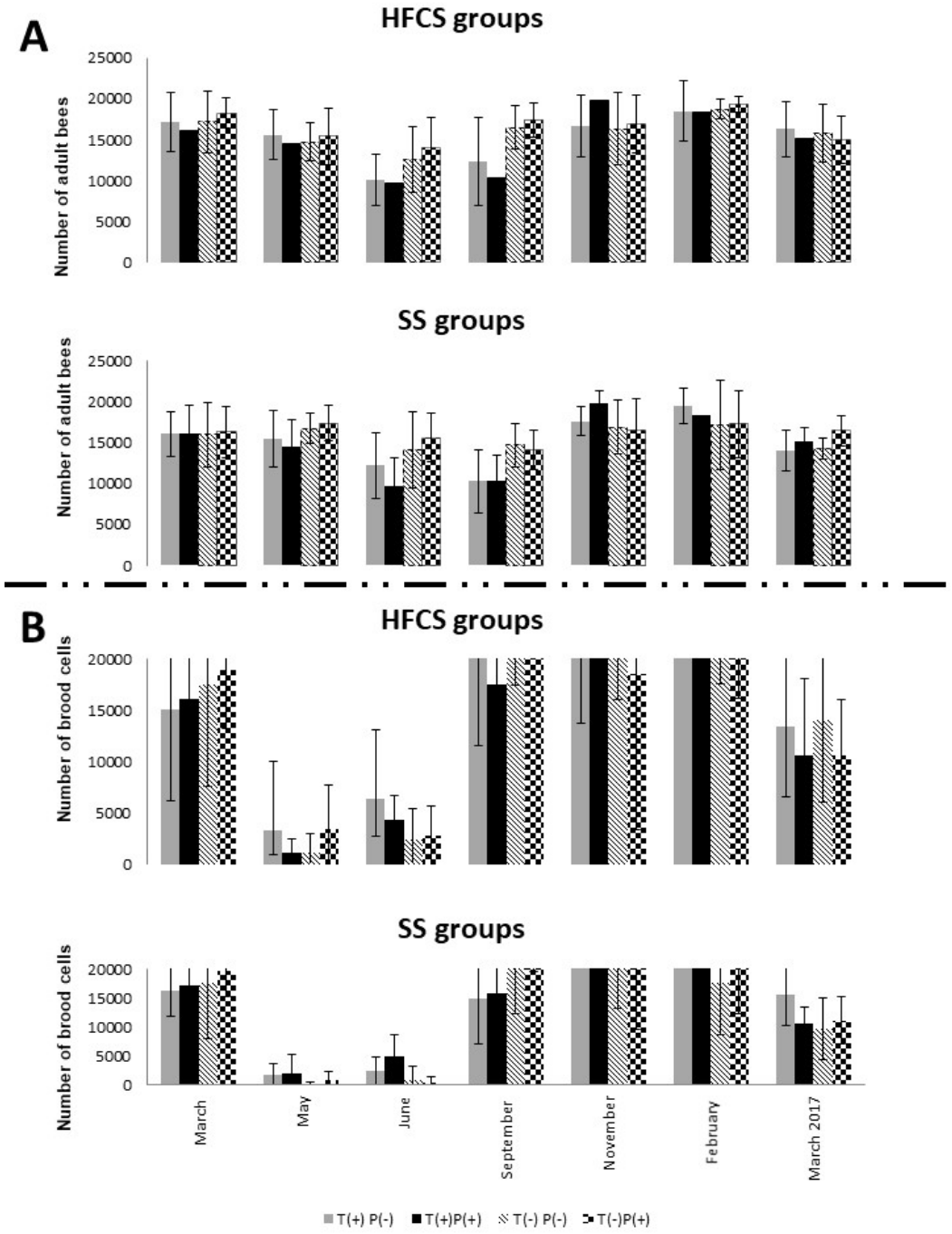

Figure 3. Colony strength (A for adult bee population and B for number of brood cells) from sucrose syrup (SS) and high fructose corn syrup (HFCS) apiaries for colonies fed with (P+) and without (P-) pollen patties and for colonies treated (T+) and non-treated (T-) with acaricide.

ferences in brood harvest (frames fully covered with brood harvested in spring and summer) between groups was analyzed by means of GLZ (gamma distribution). Cox Regression (total survival weeks: 55) was performed in order to analyze the total colony losseson. Winter mortality and over wintering index were compared using GLZ (binomial distribution) and factorial ANOVA respectively.

Results

Effect of acaricide treatment and nutritional management on colony strength, nutritional reserves and pathogen levels

Colonies fed with HFCS had more brood cells during the study than those fed with SS (p=0.048; Table 1; Fig. 3). Also, throughout the assay, T+ colonies had less adult honey bees (p=0.011; Table 1; Fig. 3) and a lower Varroa infestation percentage than T- colonies (p< 0.001; Table 1; Fig. 4). No differences on *N. ceranae* abundance and nutritional reserves (pollen and honey cells) between HFCS and SS colonies nor T+ and T- were detected (Table 1; Fig. 5). No significant effect of the protein supplementation during the course of the study on any of the response variables was observed (Table 1). Within T+ colonies, the 2016 mite invasion rates tend to be higher in SS colonies (mean=30.63 ± 6.20 mites/week) than HFCS colonies (20.08 ± 4.03 mites/week; p=0.074, Table 1). During 2017, SS colonies from all groups had significantly more mites drop counts than HFCS colonies (278.27 ± 53.39 and 59.16 ± 12.78 mites/week, respectively; p<0.001) (Table 1). By means of acaricide effect, during 2016 T+ colonies had more mites drop counts (mean=24.80 ± 3.53 mites/week) than T- colonies (mean=15.38 ± 2.46 mites/week; p=0.026) (Table 1).

Effect of acaricide treatment and nutritional management on pathogen levels in different sampling times

— Short term effects on *Varroa destructor.* During May 2016 (after acaricide treatment), T+ colonies had less Varroa infestation level (0.91 ± 0.16) than T- colonies (2.51 ± 0.44; p<0.001) (Table 2). At the same moment, P- colonies had more Varroa infestation level (1.93 ± 0.34) than P+ colonies (1.18 ± 0.20; p=0.05; Table 2). In June, the joint effect of acaricide treatment, protein supplementation and kind of syrup was significant (p=0.027). The highest Varroa infestation level was recorded in SS T-/P+ colonies (3.35 ± 1.32; p=0.011) (Table 2; Fig. 4) while the lowest Varroa infestation level was recorded in HFCS T+P- (0.01 ± 0.003; p=0.05).

— Mid-term effects on *Varroa destructor.* During spring (September 2016), the joint effect of acaricide treatment, protein supplementation and kind of syrup was significant (p=0.016). The highest Varroa infestation level was observed in SS T-/P-colonies (3.25 ± 1.20; 2.58 ± 0.9, respectively) (Table 2; Fig. 4) while the lowest Varroa infestation level was recorded in HFCS T+P- (0.19 ± 0.07; p=0.03).

— Long term effects on *Varroa destructor.* Differences between T+ and T- colonies deepened in November (1.44 ± 0.26; 5.38 ± 0.94, respectively) (p<0.001; Table 2). Similarly, SS colonies from all groups showed higher Varroa infestation level (3.59 ± 0.66) than HFCS colonies (2.15 ± 0.37; p=0.042; Table 2). However, unlike previous sampling times, no interaction effect was found. During February 2017 (before final acaricide treatment) the differences between T+ and T- colonies became more remarkable (6.04 ± 1.05 and 10.6 ± 1.79, respectively; p=0.02; Table 2).

— *Nosema ceranae. N. ceranae* abundance was similar between all groups all over the year (repeated measures, Table 1). However, during February 2017 (before the final acaricide treatment) the effect of the interaction between acaricide treatment and protein supplementation on the *N. ceranae* abundance was significant (p=0.007, Fig. 6). For instance, T-/P- colonies had the highest *N. ceranae*. abundance (2.02 ± 0.67 Log_{10} spores/bee) and T+/P+ colonies had the lowest *N. ceranae* abundance (0.18 ± 0.06 Log_{10} spores/bee). T+/P- colonies had less *N. ceranae* abundance (mean=1.39 ± 0.46 Log_{10} spores/bee) than T-/P+ colonies (1.55 ± 0.5 Log_{10} spores/bee) (Fig. 6).

Effect of acaricide treatment and nutritional management on honey production, brood harvest and colony losses

Honey production was similar between colonies fed with SS and HFCS (p=0.647), between T+ and T- colonies (p=0.991) and between P+ and P- colonies (p=0.709). Furthermore, no double or triple significant interaction were found.

On the contrary, the number of harvested brood frames during October was higher in the HFCS colonies (2.31 ± 0.31) compared to SS colonies (1.49 ± 0.2) (p=0.022) and was higher in the in T-/P+ (2.96 ± 0.56) compared to T+/P+ colonies (1.12 ± 0.21; p=0.012).

From the 38 colonies computed as death at the end of field trial (total losses), 23 were registered between May and September (winter mortality). For total losses, overall comparisons between all groups were non-significant (Chi-square=0.673; p=0.879). Similarly, there were no significant differences in winter mortality when all groups were compared (p=0.725). However, there was a significant effect of the carbohydrate feeding and acaricide treatment on the OI. The highest value was registered in the HFCS/T- colonies (OI=1.16 ± 0.054) and the lowest value was registered in SS/T+ colonies (OI=0.65±0.054; p=0.03).

Discussion

Field studies based on semi-controlled conditions including numerous honey bee colonies are scarce. Here the effect of recommended nutritional and sanitary management on the pathogen dynamics, honey production and colony survival throughout a year was simultaneously evaluated.

Effect of acaricide treatment and nutritional management on colony strength, nutritional reserves and pathogen levels

Feeding the colonies with HFCS during autumn had an effect on population dynamics, particularly on brood cells availability during the year. These results are different from previously reported studies where bees provided with sucrose syrup produce higher numbers of brood cells (Neupane & Thapa, 2005; Sammataro & Weiss, 2013) or no differences were found (Lu *et al*., 2014). However, methodology is not accurately comparable, as for instance Neupane & Thapa (2005) calculated the number of brood cells including cells having egg or larva or pupa and here only sealed brood cells were counted. During autumn 2016 and 2017 mite invasion was lower in the HFCS fed colonies probably because it was proved to be less attractive to bees than SS in field studies (Sammataro & Weiss, 2013) and laboratory studies (Neupane & Thapa, 2005). Varroa mites horizontal transmission might varied as a function of mite invasion (Fries & Camazine, 2001) driven by several factors including foraging behavior mediated by sucrose responsiveness (Kuszewska *et al*., 2019). A reduced invasion pressure by means of robbing (Greatti *et al*., 1992) or drift (Goodwin *et al*., 2006) at late autumn might be essential to avoid winter losses (Frey & Ronsenkranz, 2014). Thus, providing HFCS as carbohydrate supply might decrease the "robbing" behavior among the colonies and consequently the mite invasion pressure.

Table 2. Effect of acaricide treatment and nutritional management on percentage of infestation with *Varroa destructor* at different sampling times by means of generalized linear model (gamma distribution).

	Short term effects				**Mid-term effects**		**Long term effects**			
	May		**June**		**September**		**November**		**February 2017**	
	p	**Mean**	***p***	**Mean**	***p***	**Mean**	***p***	**Mean**	***p***	**Mean**
Kind of syrup (HFCS/SS)	0.91		0.270		0.21		**0.042**	SS 3.59% HFCS 2.15%	0.21	
Acaricide treatment (Yes/No)	**<0.001**	T+ 0.91% T- 2.51%	<0.001		<0.001		**<0.001**	T+ 1.44% T- 5.38%	**0.02**	T+ 6.04% T- 10.60%
Protein supplementation (Yes/No)	**0.05**	P+ 1.18% P- 1.92%	0.023		0.783		0.63		0.41	
Syrup × Acaricide	0.82		0.48		0.07		0.99		0.35	
Syrup × Protein	0.65		0.13		0.003		0.26		0.92	
Acaricide × Protein	0.35		0.011		0.98		0.55		0.75	
Syrup × Acaricide × Protein	0.82		**0.027**	SS T-/P- 2.95% SS T-/P+ 3.35% SS T+/P 0.48% SS T+/P+ 0.65% HFCS T-/P- 3.21% HFCS T- P+ 2.46% HFCS T+/P- 0.01% HFCST+/P+ 0.11%	**0.016**	SS T-/P- 3.25% SS T-/P+ 2.59% SS T+/P 0.71% SS T+/P+ 0.16% HFCS T-/P- 1.24% HFCS T-/P+ 1.33% HFCS T+/P- 0.19% HFCST+/P+ 0.76%	0.33		0.234	

p-values significant (<0.05) are highlighted in bold. The highest and lowest percentage of infestation with *Varroa destructor* for significant triple interaction during June and September are highlighted with black boxes.

High efficacy of the acaricide products applied was expected but opportunely checked. Still, treated colonies showed a reduced colony size during the entire year. The results presented suggested that regardless of the benefits, the same acaricide could shorten the adult honey bee lifespan (Wu *et al*., 2011) especially oxalic acid application (Martin Hernández *et al*., 2007; Schneider *et al*., 2012). Moreover, resistance development (Lodesani and Costa, 2005) and residues of varroacides in bee products (Bogdanov, 2006; Le Conte *et al*., 2010) should also discourage its prolonged use. This result is different from some previous studies that suggested that exposure to miticides does not affect bee populations at colony level (Berry *et al*., 2013; Rangel & Tarpy, 2016).

Pollen patties supplementation had neither global effect nor specific effect at any sampling time on colony strength and nutritional reserves. Similar to reported in DeGrandi-Hoffman *et al*. (2016) nutritional differences not necessarily translate into population sizes differences. Also, Van Dooremalen *et al*. (2013) demonstrated that abundant pollen cannot compensate for damage caused by Varroa parasitism as mite presence might reduce the benefits of nutritional resources (DeGrandi-Hoffman & Chen, 2015). Another even more feasible explanation is that pollen patties should have been heavier and more frequently administered in order to achieve a significant impact at colony level (Branchiccela *et al*., 2019). In addition, even when the percentage of crude protein was within the recommended range of concentra-

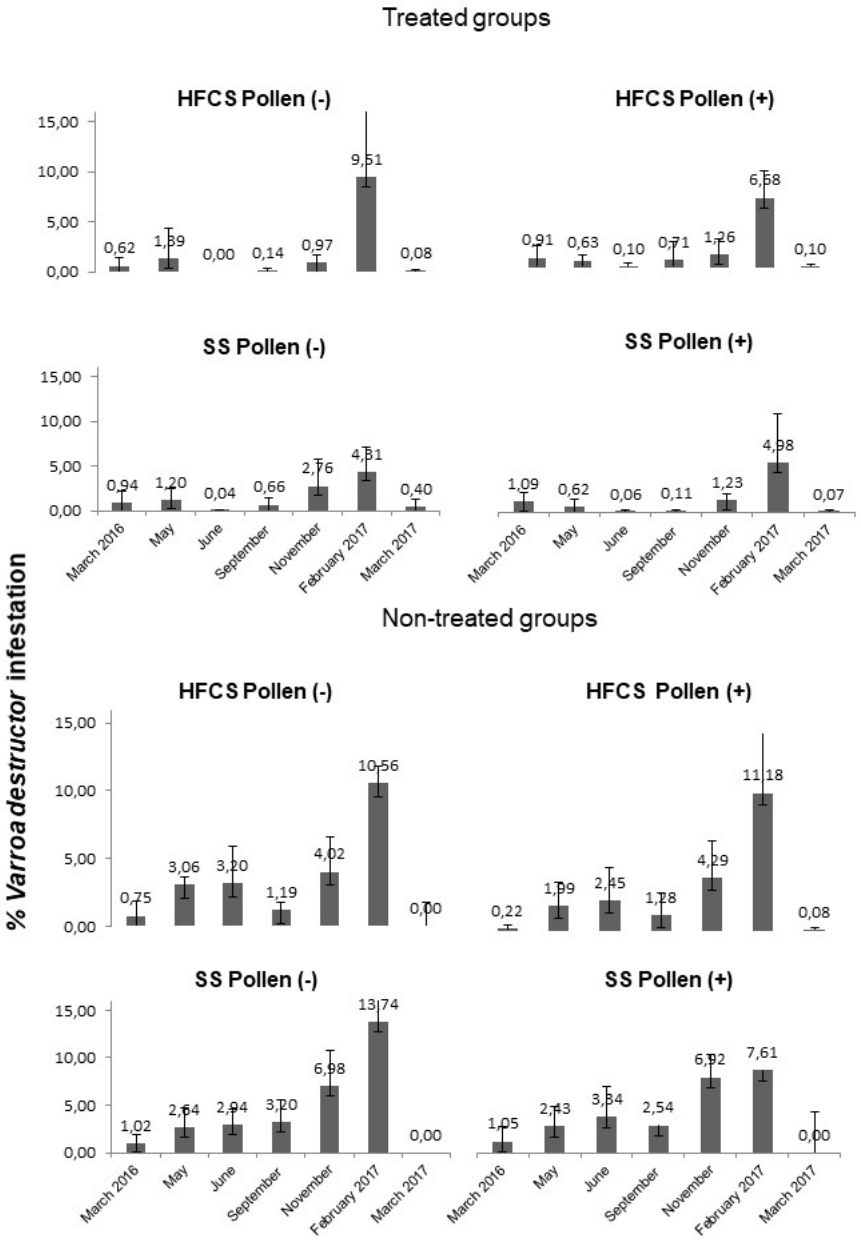

Figure 4. *Varroa destructor* infestation in treated (T+) and non-treated with acaricide (T-) groups for colonies fed with sucrose syrup (SS) and high fructose corn syrup (HFCS) and for colonies fed with (P+) and without (P-) pollen patties

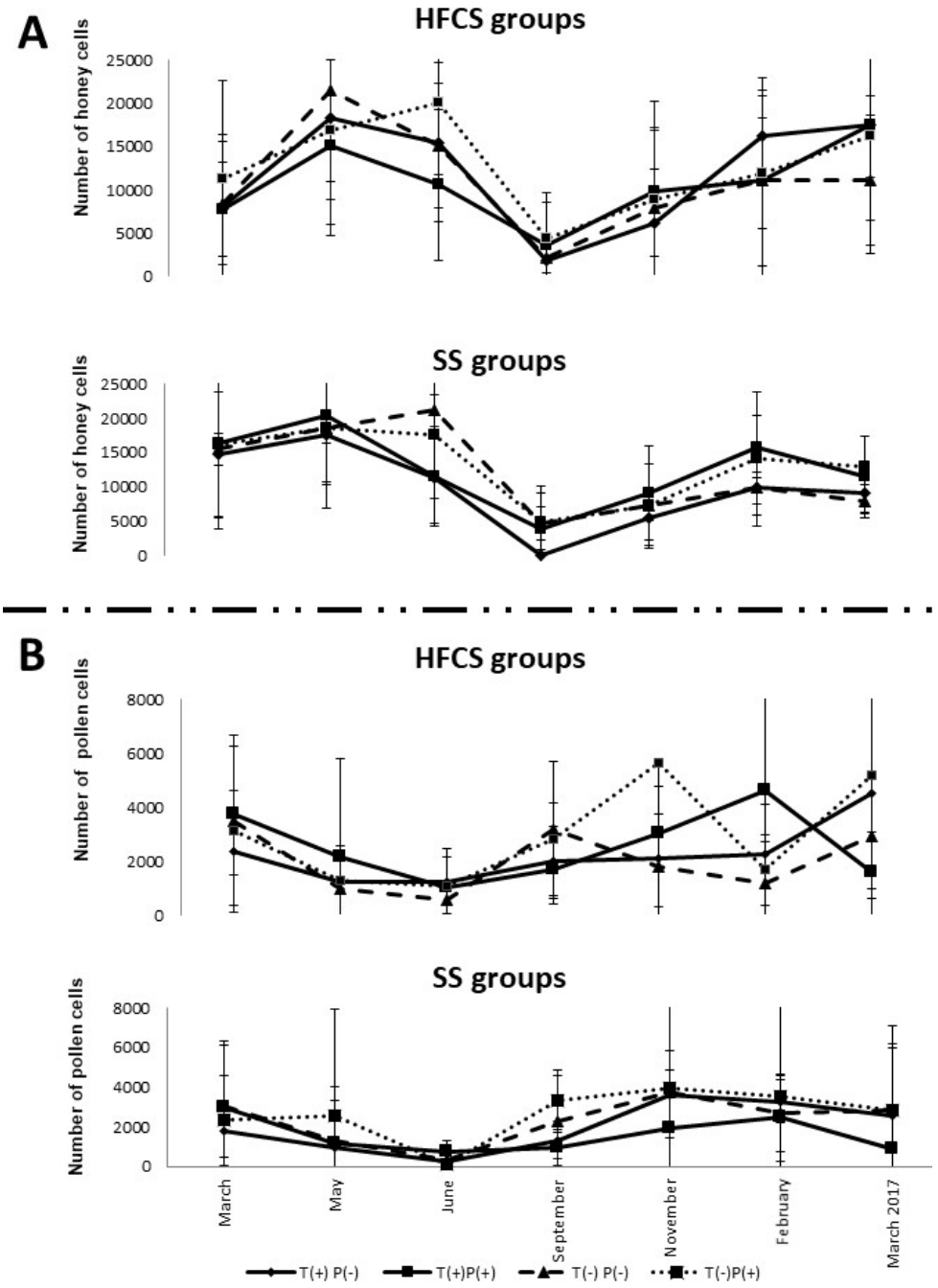

Figure 5. Nutritional reserves (A for honey cells and B for pollen cells) from sucrose syrup (SS) and high fructose corn syrup (HFCS) apiaries for colonies fed with (P+) and without (P-) pollen patties and for colonies treated (T+) and non-treated (T-) with acaricide.

tion (Herbert *et al*., 1977) it might be lower than optimal amount of protein (van der Steen, 2007) or similar to low protein-content pollen (Basualdo *et al*., 2013).

Overall, there was no effect of acaricide treatment and nutritional management on *N. ceranae* abundance and nutritional reserves (pollen and honey cells) probably because they fluctuate seasonally and are strongly influenced by the environment conditions (Alaux *et al*., 2010; Fries, 2010; Di Pasquale *et al*., 2016). However, at the end of the study, it was observed that T- colonies and P- colonies showed the highest *N. ceranae* abundance. T- colonies showed significantly more Varroa than T+ colonies in November and February sampling times what could explain the substantial increase of the *N. ceranae* spores counts by the end of the study. Interactions between *N. ceranae* and *V. destructor* mites (Pacini *et al*., 2016; Giacobino *et al*., 2018) and among both pathogens with chemical treatments were previously reported (Little *et al*., 2016). It seemed that Varroa-controlled colonies and better-nourished honey bees during autumn results in better general sanitary conditions the following honey yield season. This is important since the nutritional status

of the colonies is closely related to a negative impact of *N. ceranae* infection (Branchiccela *et al*., 2019).

Effect of acaricide treatment and nutritional management on pathogen levels in different sampling times

Acaricide treatment and nutritional supplementation impact on short term Varroa loads as infestation level was lower in T+ and P+ colonies during May 2016 and in HFCS T+/P- colonies during June 2016. High efficacy of the acaricide is revealed as T+ colonies begin winter season with significantly less Varroa mites. This is important since lowest winter losses were associated to varrocides use comparing among several Varroa control methods (Haber *et al*., 2019). Additionally, carbohydrate feeding might enhance this positive effect by reducing mite invasion during early autumn as it was observed in HFCS fed colonies. During autumn, mite invasion and reproduction are key topic for beekeepers, especially those who treated their colonies earlier in the season (Frey & Rosenkranz, 2014). A short-term effect of pollen patties was difficult to recognize in the observed pathogen levels, as P+ colonies had less Varroa mites in May but had higher infestation levels during June. Different from expected, pollen supplementation did not enhance chemical treatment effectiveness as T+/P- had the lowest mites. Benefits of pollen diet might be limited by Varroa infestation (DeGrandi-Hoffman & Chen, 2015). Nevertheless, as the weight and frequency of supplementation should be revised, further studies should be conducted in order to elucidate this effect.

Similar to what was observed before winter (June 2016) infestation level was lower in HFCS T+/P- colonies after winter season (September 2016). This is a key moment in the beekeeping cycle as the colonies start to grow and benefit from spring forage, but also because Varroa reproduction starts to speed up as bee brood became available (DeGrandi-Hoffman & Chen, 2015). Beginning honey yield season with a low infestation level almost certainly guarantee a healthy condition in the colonies up until the end of the season avoiding additional intervention (Lodesani *et al*., 2019).

During November 2016 (late spring) Varroa infestation was lower in HFCS fed colonies compared to SS fed colonies. However, as exponential growth occurs in both adult bees and mites (DeGrandi-Hoffman & Chen, 2015) this slightly (but significantly) difference disappeared during summer. On the contrary, T+ colonies went through the honey yield season with a significantly lower number of mites in adult bees (from November 2016 to February 2017). In late summer (February 2017) differences in Varroa infestation level were only explained by the treatment application during autumn 2016. The nutritional management seems to have a short or mid-term impact on the colony dynamics and pathogen loads (Branchiccela *et al*., 2019) whereas the acaricide treatment effect was observed throughout the year, resulting in a long-term positive effect.

Effect of acaricide treatment and nutritional management on honey production, brood harvest and colony losses

Although Varroa infestation levels were remarkable different in November 2016 (late spring) and February 2017 (late summer), honey production was similar between T+ and T- colonies and between SS and HFCS colonies. This was unexpected but consistent with the fact that colony size was similar between all groups from September on and that previous results suggested that colonies fed with Sucrose or HFCS showed similar honey production among these diets (Severson & Erickson, 1984). As discussed previously here, the HFCS colonies showed significantly more brood during the entire study, so the amount of harvested brood during October was higher in this group than in SS colonies. After that, brood cells were similar between groups. Similarly, more frames fully covered with worker brood were gathered from T- colonies consistent with the reported side effect of chemical treatment on brood (Berry *et al*., 2013; Zhu *et al*., 2014; Terpin *et al*., 2019) and with the fact that treated colonies showed reduced bee population during the entire study. Differences registered in the overwintering index between HFCS/T- and SS/T+ colonies were probably a consequence of those HFCS colonies had more brood cells and T- colonies had more bee population during the entire year.

Two possible hypotheses were raised given that, surprisingly, total losses and winter mortality were similar between all groups. The first hypothesis is that concerning treated and non-treated colonies, the time frame of the study was not enough to observe the negative effect of the presence of Varroa and so it is necessary to study the colonies for longer periods. Varroa infestation impact may be undercover as colonies were headed by young queens with a probable higher resilience (Straub *et al*., 2015). In addition, is quite difficult to differentiate the negative effect of Varroa presence from the use of acaricide side effects (Boncristiani *et al*., 2012). Regarding nutritional management, recently a supplemental forage study in overwintering colonies showed that colony losses were not necessarily linked to pollen or nectar scarcity (Carroll *et al*., 2018). There are multiple drivers associated with colony losses including the acaricide treatment timing (Beyer *et al*., 2018) as well as the improvement of the best management practices (El Agrebi *et al*., 2021) and queen replacement (Gray *et al*., 2020). The second and more likely hypothesis is that all colonies were exposed to a climatic stress factor that could had veiled the nutritional and treatment effect as autumn 2016 was unusual rainy. Previously it has been observed that environmental background conditioned the

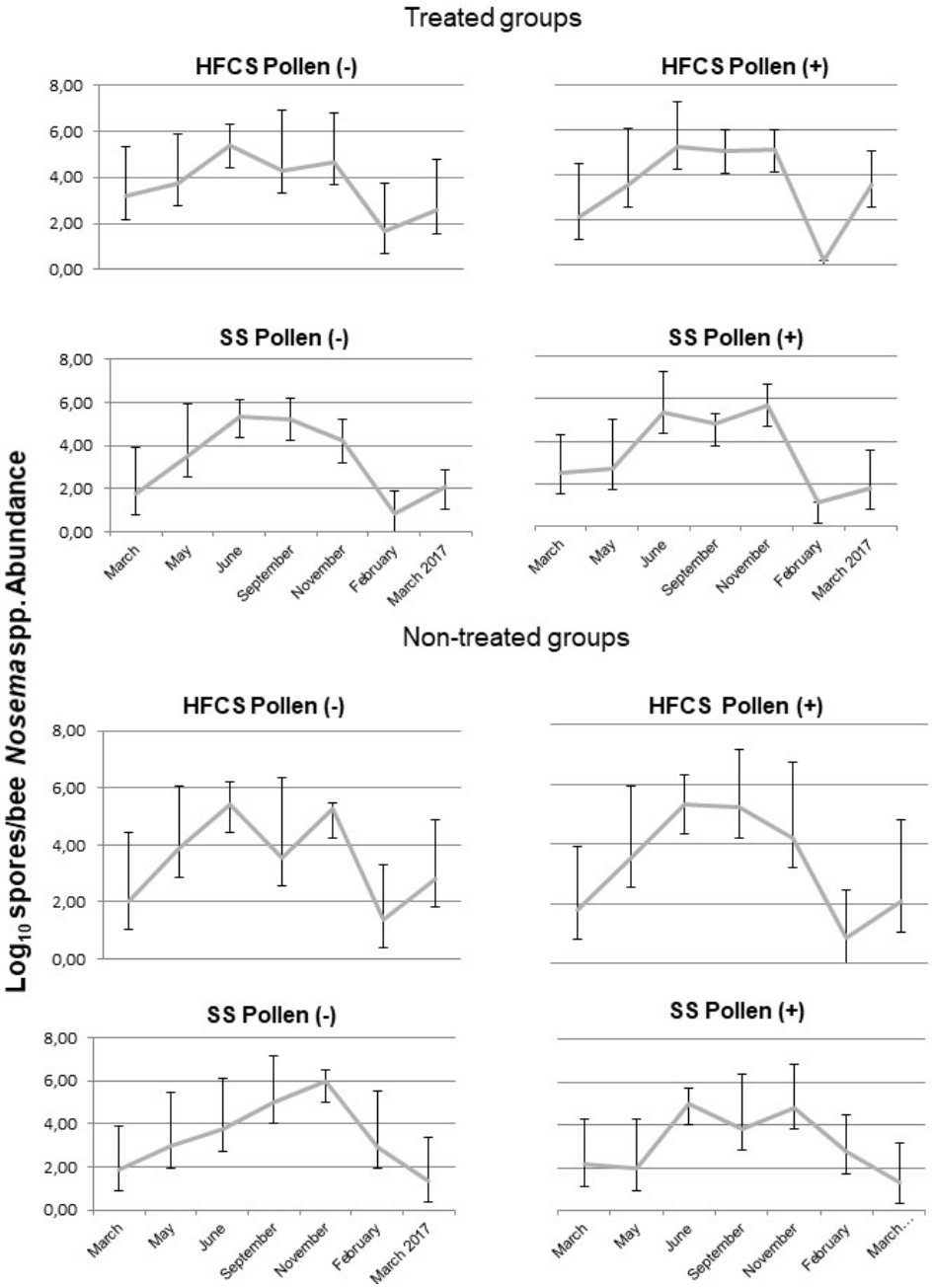

Figure 6. *N. ceranae* abundance in treated (T+) and non-treated with acaricide (T-) groups for colonies fed with sucrose syrup (SS) and high fructose corn syrup (HFCS) and for colonies fed with (P+) and without (P-) pollen patties.

impact of the management practices on the colony losses (Molineri *et al*., 2018). Honey bee colonies are frequently exposed to multiple stressors, so trying isolating the relevant variables is challenging (Boncristiani *et al*., 2012).

Conclusion

Unlike previous studies, colonies fed with HFCS showed higher brood rearing all over the year and reduced mite invasion pressure during autumn compared to colonies fed with SS. From a beekeeping point of view if no harm to colonies is registered, HFCS is a suitable substitute of sucrose syrup since is cheaper and easy handling. Further studies should be conducted in order to elucidate HFCS pros and cons including a biological, ecological and commercial beekeeping perspective.

Chemical control had advantages and disadvantages, but still a large number of beekeepers rely on acaricide treatment for Varroa control. Considering that a reduced frequency of application is desirable, our results suggest that nutrition management could enhance chemical treatment effectiveness since honey bees might profit from improved nutrition. However, further studies should be

conducted in order to improve our knowledge about the feedback between nutrition status and pathogens control under field conditions.

In addition, the mite population dynamics in the colonies varied according with different management strategies including different kind of syrup, the protein supplementation by means of pollen patties, both combined with the application of acaricide treatment during autumn. In particular nutritional supplementation impact on short term Varroa loads (the first three months) whilst the effect of acaricide treatment showed a lengthened effect. The colonies treated during autumn 2016 showed significant reduced number of mites during following summer (eight months later). The impact of nutritional management alternatives combined with autumn acaricide treatment was observed only in the positive effect of the kind of syrup on the amount of brood cells and the negative effect of the chemical treatment on the adult bee population.

The implement of nutritional and mite control management strategies recommended for the overwintering season might not compensate the detrimental effect of bad climatic conditions as an outstandingly high winter mortality was observed during the study. Beekeepers should be aware of that an exhaustive monitoring of the colonies should be performed during poor weather conditions or abnormal climatic situation. A longer period field assay should be conducted in order to elucidate the impact of beekeeping management under several climatic and sanitary conditions.

Authors' contributions

Conceptualization: A. Giacobino, A. Molineri, A. Pacini
Data curation: Not applicable.
Formal analysis: A. Giacobino, M. Signorini
Funding acquisition: Not applicable
Investigation: E. Orellano, N. Bulacio-Cagnolo, J. Merke, M. Gaggiotti
Methodology: A. Giacobino, A. Molineri, A. Pacini
Project administration: A. Giacobino
Resources: Not applicable
Software: Not applicable
Supervision: M. Signorini
Validation: M. Signorini, A. Molineri
Visualization: A. Giacobino, M. Signorini, A. Pacini
Writing – original draft: A. Giacobino
Writing – review & editing: A. Giacobino, A. Molineri, A. Pacini, M. Signorini

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