Original Article

CONTROLLED INFESTATION OF HONEYBEE COLONIES WITH *VARROA DESTRUCTOR* FEMALES

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Received: 28 November 2018; accepted: 07 January 2019

Abstract

The development of female *Varroa destructor* mites in the bee colonies was examined in the apiculture season through a developed research system in which bee colonies were experimentally infested with fifty *V. destructor* females. Differences in infestation rates were observed between the control group (C) and the infested group (E). The average number of female mites per colony was determined at 513 in group E and 261.6 in group C. Natural daily mortality reached 0.16 mites in group E and 0.09 mites in group C. In group E, the number of *V. destructors* increased 7.96 to 13.32-fold, subject to colony. The size of *V. destructor* populations increased at a higher rate in group E than in group C (F= 12.39, P= 0.047). At the end of the experiment, the percentage of infested honey bee workers was determined at 0.97% in group E and 0.46% in group C. The results of this study confirmed that *V. destructor* mites continue to proliferate rapidly in honey bee colonies, and that the population growth rate in bee colonies and apiaries has to be closely monitored due to growing levels of resistance to acaricides.

Keywords: *Apis mellifera*, monitoring of varroosis, population development, *Varroa* control

INTRODUCTION

V. destructor is regarded as a leading cause of colony collapse disorder (CCD) (Guzmán-Novoa et al., 2010; Le Conte, Ellis, & Ritter, 2010). In 2007-2008, CCD caused a 35.8% loss in bee colonies in the USA (van Engelsdorp et al., 2008), and in 2003 and 2006 similar losses were noted in Southern and Central Europe (Hendrikx et al., 2009). Untreated bee colonies die in the third or fourth year of *V. destructor* invasion (Korpela et al., 1992; Romaniuk, Sokół, & Witkiewicz, 1992). Mite infestations are controlled in bee colonies with acaricide which exhibit different mechanisms of action. Various control methods are used, including biological methods which rely on pathogenic fungi and biotechnological methods where the drone brood is removed from bee colonies. Integrated management strategies are also applied in late autumn. Alternative control methods include the use of organic acids and thymol formulations. Despite these efforts, *V. destructor* continues to infest bee colonies, which is attributed to its ability to proliferate in wild bee colonies (Madras-Majewska et al., 2016).

The development of *V. destructor* mites is linked with the life cycle of bees. *Varroa* females are more likely to reproduce at the beginning of the spring-summer season when drone cells are not present in the hive, which prevents a detailed assessment of infestation severity (Fuchs, 1990). Females born in spring live for two or three months (De Ruijter, 1987), and their proliferation rate can be influenced by such factors as permanent infertility, laying of unfertilized eggs, and damage to eggs and young mites caused by moving bees inside cells (Donzé & Guerin, 1994; Martin, 1998). During the breeding season, one *V. destructor* female can produce 2.5 fertile females (Martin, 1998), of which 1.3-1.4 survive into adulthood per one worker cell and 2.2-2.6 per one drone cell (Schulz, 1984; Fuchs & Langenbach, 1989). One female mite can undergo up to seven reproductive cycles during one beekeeping season (De Ruijter, 1987), and

twelve generations of bees can be produced in a bee colony within one year (Ben, 1997). Hypothetically, a single *Varroa* female present in a bee colony can contribute to producing up to 1500 offspring by the fourth year of infestation (Martin, 1998).

In Europe, attempts to eliminate *V. destructor* have been made for nearly forty years in various beekeeping systems, but the problem has not been resolved yet. In the fight against such a parasite, its biology and behavior must be monitored to determine whether these actions based on selection did not change the biology and behavior of the parasite. This is important due to the possibility of modifying methods of controlling the development of the parasite population. The aim of this study was to monitor the size and growth rate of *V. destructor* populations during the beekeeping season in bee colonies experimentally infested with female mites.

MATERIAL AND METHODS

In early May 2015, ten bee colonies with a similar number of workers (weight-based measurement - 1.5 kg of bees per colony, average body weight of workers - 112 mg in experimental group - group E, and 110 mg in control group - group C). The average body weight was determined based on one-hundred randomly CO_2 euthanized workers. The number of bees was determined based on the body weights of individual workers. Bees were established in twelve-frame Dadant beehives on four frames with wax foundation sheets from the same batch, complying with PN-R-78894: 1997 standards. Naturally inseminated Carnolian honey-bee queens were introduced to the beehives, and in order to standardize the results, only one-year queens were used. The colonies were established far from other apiaries (r=3 km) near Mierki, Poland (*Degree-Minute-Second*- DMS: 53°35'35.085" N 20°20'34.696" E) and fed 1 L of sugar syrup three times every two days, before *Varroa* mites were introduced. The bee colonies in a group were separated from one another by a distance of 10 m, and group E hives were separated by a distance of around 100 m from group C hives. *V. destructor* mites were controlled with an Apiwarol tablet (active ingredient - amitraz, 12.5 g/tablet), which was placed on the bottom board in the hive at 6 p.m. once every 24h. During Apiwarol administration, the hive entrance was blocked for around thirty minutes. Mites were combated with the presence of a bee queen. The treatment was continued daily for four to five days until dead female mites were no longer found on the mesh screen at the bottom of the hive. Before the experiment, twenty bees from each hive had been tested with standard diagnostic methods for the presence of infectious diseases, laboratory tests found no pathogens present, and the observation of bee behavior did not indicate any ongoing diseases. A colony was regarded as non-infested when female mites were not detected on the mesh screen after two consecutive treatments. After seven days, fifty live *V. destructor* females with evenly colored exoskeletal plates obtained directly from a hatching brood were introduced to five bee colonies (group E) directly onto built combs with open brood cells. The group C comprised five bee colonies without mites. The mites were obtained by uncapping the worker's brood and the drone with a fork to uncork the honeycombs from another apiary. Bees foraged on winter rapeseed, raspberry, linden, weeds and mixed forest vegetation. Colony growth was not stimulated after the introduction of *V. destructor* to observe the natural development (abundance) of mite populations.

The experiment was conducted between 25 May and 21 September throughout one beekeeping season. Mite drops were monitored every fourteen days, and dead mites were removed from the mesh screen and their number recorded. The colonies in each group were provided with frames and wax foundation sheets. On 21 September, bee queens were caged. Mites were removed from all open and capped worker- and drone-brood cells that had been cut out from combs. No combs with drone cells had been placed in the hives, because the bees were building them spontaneously. The mites in every cell was counted, and the bees

from the colonies were euthanized and weighed at the end of the experiment. The bee to mite ratio (infestation rate) was calculated using the formula: number of *V. destructor* mites on workers/final number of workers × 100%. Infestation rate was calculated based on the number of mites on workers.

The research results were tested with the use of the T-Student test for independent samples, and standard deviation in the evaluated population was determined. The results were interpreted through repeated measures one-way ANOVA to determine whether the experimental infestation influenced the population dynamics of *V. destructor*. The results were analyzed statistically in the Statistica 12.5 program with a medical application.

RESULTS

Bee colonies developed normally, as every colony built eight combs on the wax foundation sheets and raised a similar numbers of drones. The measurement was performed three times and the average surface area of drone cells in the season was 2.4 dm^2/colony. On average eleven kilograms of honey were harvested from every colony. No workers or drones were found with developmental anomalies and other bee diseases.

During the experiment, 513 *V. destructor* females, including fifty experimentally introduced females, were detected per bee colony in group E on average. The workers in this group were infested with 333 mites on average (271-402), and brood cells were infested with 180 mites on average (122-260). In group C, workers were infested with 155.6 mites on average (120-184), and brood cells were infested with 106 mites on average (86-124). The mite population in this group, increased from 7.96 to 13.32-fold, subject to colony (*SD*=2.31, *+95% CI*=1.38, *-95% CI*=6.63). Every group C colony was infested with 261.6 mites on average. The average mite drop was determined at 3.8 mites per colony in group E and 2.2 mites per colony in group C. The first mite drops were observed on 29 June in group E and on 24 August in group C. Daily mite mortality reached 0.16 mites in group E and 0.09 mites in group C.

Table 1.

Number of bee workers and *V. destructor* females, and infestation rates in bee colonies in experimental group (E) and control group (C)

Group	Colony	Weight		Calculated number of workers	Weight		Final number of workers	Number of *V. destructor* mites on workers	Infestation rate (%)
		bees in hive (kg)	worker bee (mg)		final weight of bees in hive (kg)	worker bee (mg)			
E	1	1.5	112 (± 2.094)	13393	3.8	116 (± 2.54)	32759	271	0.83
	2				2.9		25000	334	1.34
	3				3.4		29310	362	1.24
	4				3.1		26724	402	1.50
	5				3.9		33621	296	0.88
C	1	1.5	110 (± 2.10)	13636	3.6	114 (± 2.28)	31579	167	0.53
	2				3.5		30702	144	0.47
	3				3.0		26316	120	0.46
	4				3.5		30702	163	0.53
	5				3.3		28947	184	0.64

Key: ± denote to standard deviation

Table 2.

Number of *V. destructor* females in experimental group (E) and control group (C)

Group	Number of *V. destructor* females				
	Introduced to colonies	Mite drops	At the end of the experiment		Total (average)
			In brood cells	On workers	
E (n=5)	50	3.8 (± 0.98)	180* (± 58.38)	333* (± 46.51)	513* (± 92.94)
C (n=5)	-	2.2 (± 0.75)	106* (± 14.12)	155.6* (± 21.88)	261.6* (± 30.89)

Key: ± denote to standard deviation in population σ;
* - statistical differences between groups (P value < 0.05).

During the experiment, the number of workers increased by 220% in group E and 217% in group C, and the observed increase was not statistically significant. The infestation rate increased from 0.33% at the beginning to 0.97% at the end of the experiment in group E, and from 0% to 0.46% in group C; these differences were insignificant. The differences in mite drops in the analyzed groups were significant (t = 4.26, df = 8, p = 0.0054. The *V. destructor* populations grew at a faster rate in group E than in group C (F = 12.39, df = 8, p = 0.047) (Tab. 1 and 2).

DISCUSSION

The increase in the development dynamics of *V. destructor* population among bees, as indicated by Gliński (1994), Hubert et al. (2014) and González-Cabrera et al. (2016) may be affected by its period of presence and control methods. This study documents the development dynamics of the *V. destructor* population after for the first time since they were detected in Poland forty years ago. Population development was assessed through the composing of a monitoring system, based on published scientific observations, to monitor the behavior of *V. destructor* in bee colonies. The reason for this experiment was mainly the progressive *V. destructor* resistance phenomenon on acaricides, confirmed by many researchers (Hubert et al., 2014; González-Cabrera et al., 2016). In the study, the mites were eliminated with an effective short-acting preparation (active ingredient - amitraz) with low accumulation in wax, and the observations were carried out on new generations of workers and drones. The study was deliberately conducted in one beekeeping season because such an approach allowed an objective assessment of the *V. destructor* population threatening overwintering bees. When comparing several seasons, the number of factors that may affect the behavior of the mite population is too high. The conditions for monitoring the *V. destructor* population in each season should be performed in the same research system, i.e. the type of beehive, number of workers, the starting number of combs, young queens of the same species and study site, which allows the same swarm structure to be obtained (Seeley & Smith, 2015).

The size of the *V. destructor* population in a colony is influenced by the species of bees. Carnolian honey bees (*Apis mellifera carnica*), described in many experiments on the building of the largest cells (5.27 mm), are particularly susceptible to the rapid development of mite populations (Piccirillo & De Jong, 2003). Piccirillo & De Jong (2003) found that *V. destructor* invaded a greater number of brood cells by in freshly Carnolian honey bee built combs than in that of other species. We did not compare the development of mite population in different bee species but instead in order to eliminate the impact of these factors on the size of the studied population deliberately created new colonies with the Carnolian queens, the most common species in Poland, which were building new honeycombs. In the future, such a

monitoring system will allow the *V. destructor* population to be assessedly, and its proper functioning will be partially confirmed with very low natural daily mite drop. Akyol et al. (2007) proved that the colony mite infestation increased with the queen's age, so we used young queens at the same age in the study. In addition, new honeycombs eliminated both the possibility of *V. destructor* development being inhibited by acaricides, found in the wax of colonies treated for varroosis, as well as the possibility of its development being stimulated by pheromones, found in the wax of heavily infected colonies (Thrasyvoulou & Pappas, 1988; Slabezki, Gal, & Lensky, 1991; Piccirillo & De Jong, 2004).

The conducted experiment clearly confirmed that *V. destructor* still has a great ability to multiply in bee colonies. According to Gliński (1994), a 10% infestation rate in summer leads to a 30% infestation rate in autumn. In our studies, we showed an increased rate of *V. destructor* population development, as indicated in Tab. 1 and Tab. 2. In group E, the number of introduced fifty females increased over ten-fold. Ritter (1988) and Seeley & Smith (2015) suggested that in field conditions a mite population in bee colonies can be strengthened by parasites from other apiaries, and reinvasions may occur during the robberies from other hives and during bees' wandering. It is also favoured by a lack of mite control in apiaries or poorly conducted anti-varroa therapy (Komeili, 1988). The above facts cannot be excluded in the conducted study. We believe, however, that it is difficult to estimate the number of parasites that entered the tested colonies from the environment and the number of mites that left on the foraging bees flying out for food. The conducted experiment minimized these phenomena through the appropriate setting of beehives in the apiary (material and methods). This arrangement also allowed the eliminate of *V. destructor* drift between colonies resulting from the crowding of colonies in the apiary (Seeley & Smith, 2015; Nolan & Delaplane, 2016).

In conclusion, the size of the female *V. destructor* population is still important for the functioning of the bee colonies. Our research indicates the increased dynamics of population development of this mite than previously reported. With reference to the Polish conditions (Central Europe), despite the use of various preparations and methods of control, *V. destructor* still threatens the development of bee colonies, and our studies shows that it can increase its numbers by nearly 1000% even in colonies previously free of this mite.

ACKNOWLEDGMENTS

The authors would like to offer their thanks to beekeepers for sharing their beehives and the possibility of obtaining *V. destructor* from their apiaries. This research was funded from the resources of the Department of Parasitology and Invasive Diseases, University of Warmia and Mazury in Olsztyn.

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