Journal of Apicultural Research

ISSN: 0021-8839 (Print) 2078-6913 (Online) Journal homepage: https://www.tandfonline.com/loi/tjar20

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To cite this article: Beata Bąk, Jerzy Wilde & Maciej Siuda (2018) The condition of honey bee colonies (*Apis mellifera*) treated for *Varroa destructor* by different methods, Journal of Apicultural Research, 57:5, 674-681, DOI: 10.1080/00218839.2018.1495440

To link to this article: https://doi.org/10.1080/00218839.2018.1495440

Journal of Apicultural Research, 2018
Vol. 57, No. 5, 674–681, https://doi.org/10.1080/00218839.2018.1495440

ORIGINAL RESEARCH ARTICLE

The condition of honey bee colonies (*Apis mellifera*) treated for *Varroa destructor* by different methods

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(Received 16 January 2018; accepted 11 June 2018)

The aim of this study was to examine how different methods of varroa treatment affect the condition of honey bee colonies. There were four groups of bee colonies formed, with 25 colonies per group: group I (MT): chemotherapy only; group II (IT): integrated varroa treatment; group III (NT): natural compounds treatment; and group IV (C): control group, without any varroa treatment. After the first year of assay, the autumn measurements revealed that the colonies in the control group had significantly more combs inhabited by bees than the colonies in the groups treated for varroa with chemotherapy. In the following year, the colonies in the control group weakened so much that eventually their average number of combs inhabited by bees for the third assay year was significantly lower than in the remaining groups (from 13.5 in the NT group to 14.8 in the IT group), with only 10.2 inhabited combs in the C group. The varroa treatment applied in the treated colonies did not reduce the winter debris weight in comparison to the non-treated ones. We did not observe any negative effect of the selected varroa treatment methods on the condition of bee colonies. Failing to apply varroa treatment results in the gradual and systematic decrease in the number of combs inhabited by bees and condition of bee colonies and consequently, in their death.

Estado de las colonias de abejas melíferas (*Apis mellifera*) tratadas por *Varroa destructor* con diferentes métodos

El objetivo de este estudio fue examinar cómo los diferentes métodos de tratamiento de la varroa afectan la condición de las colonias de abejas melíferas. Se formaron cuatro grupos de colonias de abejas, con 25 colonias por grupo: grupo I (MT) -sólo quimioterapia, grupo II (IT) -tratamiento integrado de la varroa, grupo III (NT) -tratamiento con compuestos naturales- y grupo IV (C) -grupo control, sin ningún tratamiento de la varroa. Después del primer año de ensayo, las mediciones de otoño han revelado que las colonias del grupo control tenían un número significativamente mayor de panales habitados por abejas que las colonias de los grupos tratados contra la varroa con quimioterapia. Durante el año siguiente, las colonias del grupo de control se debilitaron tanto que, finalmente, su número medio de panales habitados por abejas en el tercer año de ensayo fue significativamente menor que el número de panales habitados por abejas de las colonias del resto de los grupos (de 13,5 en el grupo NT a 14,8 en el grupo IT), con sólo 10,2 panales habitados en el grupo C. El tratamiento de la varroa aplicado en las colonias tratadas no redujo el peso de los escombros invernales en comparación con los no tratados. No se observó ningún efecto negativo de los métodos de tratamiento de la varroa seleccionados sobre el estado de las colonias de abejas. Si no se aplica el tratamiento contra *Varroa destructor*, se produce una disminución gradual y sistemática del número de panales habitados por abejas y del estado de las colonias de abejas y, en consecuencia, su muerte.

Keywords: varroa control; integrated varroa treatment; condition of honey bee colony; essential oils; organic acids

Introduction

The parasitic varroa mite *Varroa destructor* can cause death of a bee colony within 3–4 years due to its strong pathogenic effect on honey bees (Fries, Camazine, & Sneyd, 1994; Shimanuki, Calderone, & Knox, 1994). The death of honey bees infested with this parasite is determined by many factors. One of them is the formation of entries of infection by the varroa mites for many pathogens living on the body of brood and bees (Gliński & Jarosz, 1988), as well as those transmitted by varroa (Ball & Allen, 1988; Gliński & Jarosz, 1990, 1991; Pohl & Ritter, 1997; Ritter & Koch, 1991; Strick & Madel, 1986). The role of varroa in the spread of diseases such as American foulbrood, chalkbrood (Strick & Madel, 1986) ,and most of all viral diseases (Carreck, Ball, & Martin, 2010; Martin, 1998, 2001; Martin, Ball, & Carreck, 2010; Tentcheva et al., 2004) has been proven. It was also observed that the negative effects of *Nosema ceranae* can reinforce the harmful consequences of varroa infestation of bee colonies (Higes et al., 2008).

In order to prevent such a destructive effect of the mite on bees and brood, and thus on the whole colony, it is recommended to control the parasite regularly every year. Failing to carry out varroa treatment in one season can have a negative effect on the condition of the whole apiary and its productivity in consecutive

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Table 1. Varroa treatment schemes in groups and years.

Assay year	Group	Supportive spring treatment (March–May)	Supportive spring-autumn treatment (May–July)	Main summer treatment (August)	Supportive autumn treatment (October–November)
2010	MT			Bayvarol	
	IT	Api Life Var	Removing drone brood	Bayvarol	Oxalic acid
	NT	Api Life Var		Api Life Var	Oxalic acid
2011	MT			Biovar	
	IT	Api Life Var	Removing drone brood	Biovar	Oxalic acid
	NT	Api Life Var		Api life var	Oxalic acid
2012	MT			Apivarol	
	IT	Api Life Var	Removing drone brood	Formic acid	Oxalic acid
	NT	Api Life Var		Formic acid	Oxalic acid

Group MT: only main treatment with the use of chemotherapy; group IT: integrated *varroa* treatment; group NT: only natural treatment with the use of essential oils and organic acids.

years, even if varroa treatment is resumed (Bąk, Bratkowski, & Wilde, 2002).

However, it should be taken into account that chemical substances used for treating varroa, as well as the bee keeper's activities connected with the treatment, can have a negative effect on the condition of bee colonies (Johnson, Ellis, Mullin, & Frazier, 2010). This is possible especially if we interfere with the homeostasis of the honey bee colony during a year, as it happens in case of the recommended integrated varroa treatment (Bąk, Wilde, & Siuda, 2013; Rice, Winston, & Higo, 2004). Herbert, Witherell, and Shimanuki (1988) tested different active substances of medicines used in varroa treatment, and found that they show toxic effect on bees. Dahlgren et al. (2012) also examined the toxicity of acaricides on worker bees and queen bees. The results indicate that worker bees are several times more vulnerable to most of the tested substances than the queen bees. Amitraz proved to be the only substance displaying the same toxicity on both castes.

In many studies, fluvalinate displayed a relatively low toxicity on bees (Berry, Hood, Pietravalle, Delaplane, 2013; Stoner, Wilson, & Moffett, 1984; Taylor, Waller, & Crowder, 1987). The coumaphos substance is the one exerting an extremely negative effect on queen bees. Queen bees exposed to this acaricide, prior to their introduction to bee colonies, are being accepted unwillingly; besides, coumaphos decreases queen bees' life span and their body mass (Collins, Pettis, Wilbanks, & Feldlaufer, 2004; Haarmann, Spivak, Weaver, Weaver, & Glenn, 2002; Pettis, Collins, Wilbanks, & Feldlaufer, 2004).

Substances described as soft chemicals also have their effect on bees. Formic acid shortens the life span of worker bees and decreases the viability of brood (Fries, 1991; Underwood & Currie, 2003). Strachecka, Paleolog, Borsuk, and Olszewski (2012a) affirmed that formic acid can destabilize the activity of the body surface proteolytic system, lower protein concentration, decrease protease activity in workers and reduce natural protease inhibitor activity in larvae and pupae. The use of thymol in varroa treatment can induce a removal of brood (Floris, Satta, Cabras, Garau, & Angioni, 2004); it also increases the death rate of queen bees (Whittington, Winston, Melathopoulos, & Higo, 2000).

Over time, varroa mites can sometimes develop drug resistance to many chemical substances used for the mite controlling (Elzen et al., 2001; Strachecka, Borsuk, Olszewski, & Paleolog, 2015). Therefore, it is crucial to systematically monitor the effectiveness of varroa treatment (Bąk et al., 2013), and search for new schemes of parasite treatment (Elzen et al., 2001).

Most studies on the effect of substances used in varroa treatment are carried out under laboratory conditions, and they rarely examine the effect the substances have on the condition of bee colonies. Therefore, the aim of this field assay was to find out what treatment of varroa is most favorable for bee colonies, and how varroa treatment methods affect egg-laying. Treatment scheme presented in the study displayed high effectiveness in the treatment of varroa (Bąk et al., 2013). In addition, the group of untreated colonies was introduced to compare their condition and survival with colonies treated against varroa in various ways.

Materials and methods

In September 2009, we bought 100 bee colonies which have not been treated for varroa since April 2009. They were placed in Ostrowska hives (frame size 360 mm $\times$230 mm). Then we replaced the queen bees in all the colonies with new ones. We introduced the naturally mated sister-queens originating from one Carniolan mother-queen of the Kortówka line. After that, the colonies were prepared for wintering. All colonies were wintering on sugar syrup. In October 2009, the colonies were randomly divided into four assay groups (25 colonies in each), depending on the varroa treatment to be applied (compare Table 1): Group I (MT): main treatment with the use of chemotherapy only; Group II (IT): integrated varroa treatment (combination of chemotherapy and natural compounds treatment); Group III (NT): treatment with natural compounds: essential oils and organic acids only; and Group IV (C): control group, without any treatment. We used the control group to compare the condition of bee colonies from groups in which varroa treatment was carried out.

Table 2. Mean number of combs occupied by bees in spring.

	Group								Years overall	
Year	*n*	MT	*n*	IT	*n*	ET	*n*	C	*n*	mean
2010	23	4.3^{Aa}	22	4.4^{Aa}	22	4.9^{ABab}	20	4.3^{Aa}	87	4.5^{A}
2011	23	10.10^{Bd}	24	10.2^{Bd}	21	10.4^{Bd}	23	10.1^{Bd}	91	10.2^{C}
2012	18	8.4^{Bc}	20	8.2^{Bc}	15	7.3^{bc}	2	6.5^{abc}	55	8.0^{B}
Groups overall	65	7.5	67	7.6	58	7.5	42	6.9	232	7.4

Group MT: only main treatment with the use of chemotherapy; group IT: integrated varroa treatment; group NT: only natural treatment with the use of essential oils and organic acids; group C: control group.

Different capital letters denote significant differences between the means at $p < 0.01$; lower case letters at $p < 0.05$, n = number of honey bee colonies.

The experiment was carried out over the years 2009–2012, according to the schedule showed in Table 1. In this assay, we used preparations registered in Poland only, i.e.: Apiwarol: fumigating tablets, 1 tablet containing 12.5 mg of amitraz as an active substance (3 times, one tablet every 7 days in August, every season); Bayvarol: strips to be hang in a hive, one strip containing 3.6 mg of flumethrin; Biowar: strips to be hang in a hive, 1 strip containing 500 mg of amitraz; Api Life Var: A preparation in a form of vermiculite blocks containing essential oils: thymol (76 g/100 g), mint oil (3.8 g/100 g), eucalyptus oil (16.4 g/100 g), and camphor (3.8 g/100 g).

Apart from the above-mentioned acaricides, we also used organic acids: formic and oxalic. All preparations were used in accordance with the manufacturer's instructions. In the colonies treated with formic acid, 200 ml of 85% acid were evaporated with a use of a horizontal Nassenheider dispenser. The colonies treated with oxalic acid were sprayed with its 3.5% solution in 1:1 sugar solution.

The condition of bee colonies was analyzed, i.e., the status of bee colony, which consisted of the strength of bee colonies determined by the number of combs inhabited by bees, fertility of bee mothers, and health status. The overwintering of bee colonies was also monitored by analyzing the weight of winter debris and colony survival after winter. The effect of the particular treatment on the number of combs inhabited by bees was assessed both in spring and autumn. For this purpose, the number of combs inhabited by bees was monitored every week from mid-April to the end of May, as well as from the beginning of August to mid-September.

The number of dead colonies was recorded in each group, every year, after wintering, in order to assess the effect of the varroa treatment on the bees' overwintering. The colonies lost in particular groups were replaced with splits from colonies in a given group. Therefore, the number of colonies in experimental groups was maintained the same as at the beginning of each assay year. Each of the newly created colonies was provided with young egg-laying Carniolan queen bees of the Kortówka line. Such queen bees were also introduced to the existing colonies in case of loss of their queen bee due to accidental causes.

We also examined the winter debris weight and the number of varroa mites in it. After the last varroa treatment, the hive bottoms in all colonies were cleaned. Therefore, we used hygienic bottom boards with drawers. The degree of worker bee infestation with *Nosema* spp. spores was assessed with the Kirkor method in all groups. For this purpose, as well as to avoid sampling of newly emerged, uninfected bees, a sample of 30 bees was taken from the peripheral combs without brood, containing the honey/sugar storages only. The method consists in observing the water solution of homogenized bee abdomens under a microscope (400×), and counting the number of *Nosema* spp. spores in the microscope field of vision.

The number of eggs laid by queen bees in all the colonies was also counted each year, at the end of August, after every varroa treatment had been applied. The number of eggs laid by queens was determined in each colony by measuring the total comb area containing eggs, assuming that 1 dm^2 contains 400 cells/eggs. The procedure was performed each year, at the end of August, after varroa treatment termination. When the procedures were over, we checked the presence of queen bees in colonies, and we examined them for any behavior changes.

The significance of differences between the group means were estimated by the one-way ANOVAs and the Duncan multiple range tests and χ^2 test (only winter losses). Statistical analyses were conducted using the Statistica software package.

Results

The number of combs inhabited by bees

The number of combs inhabited by bees in spring was not determined by the treatment type ($F_{3,\ 231} = 0.16$, $p = 0.923$). We observed that in spring, the bees inhabited from 6.9 combs in group MT to 7.6 combs in group IT, on average. At that time the number of combs inhabited by bees was determined by the season ($F_{2,231} = 71.38$, $p = 0.0000$). In spring (Table 2), the highest number of combs inhabited by bees was observed in 2011, with 10.2 combs inhabited, on average, whereas the colonies were weakest in 2010 (the mean number of inhabited combs spaces was only 4.5). The number of combs inhabited by bees in autumn was significantly

Table 3. Mean number of combs occupied by bees in autumn.

	Group								Years overall	
Year	*n*	MT	*n*	IT	*n*	NT	*n*	C	*n*	mean
2010	25	$13.1^{BC} \pm 2.5$	25	$13.3^{BCDa} \pm 2.2$	25	$13.5^{BCDa} \pm 1.4$	25	$14.9^{Db} \pm 2.4$	100	13.7 ± 2.2
2011	25	$14.1^{BCb} \pm 2.4$	25	$15.0^{Db} \pm 2.1$	25	$13.6^{BCDa} \pm 1.3$	25	$12.5^{Ba} \pm 2.3$	100	13.8 ± 2.2
2012	25	$14.2^{CDab} \pm 2.2$	25	$14.8^{Db} \pm 1.6$	25	$13.5^{BCDa} \pm 1.3$	25	$10.2^{A} \pm 2.4$	100	13.2 ± 2.6
Groups overall	75	$13.8^{\Phi\acute{\alpha}\acute{\varepsilon}} \pm 2.4$	75	$14.3^{\Phi\acute{\varepsilon}} \pm 2.1$	75	$13.5^{\Phi\acute{\alpha}} \pm 1.3$	75	$12.5^{\Lambda} \pm 3.0$	300	13.5 ± 2.4

Group MT: only main treatment with the use of chemotherapy; group IT: integrated varroa treatment; group NT: only natural treatment with the use of essential oils and organic acids; group C: control group. Different capital letters denote significant differences between the means at $p < 0.01$; lowercase letters at $p < 0.05$. Latin letters refer to 12 means; Greek letters refer to overalls in the same line.

Table 4. Mortality of bee colonies during wintering in individual years and groups.

		Number of lost colonies after wintering				
Year	Number of wintering colonies	MT	IT	NT	C	Years overall
2010	100	2	3	3	5	13^{B}
2011	100	2	1	4	2	9^{B}
2012	100	7	5	10	23	45^{A}
Groups overall	300	11^{B}	9^{Bb}	17^{Ba}	30^{A}	67

Group MT: only main treatment with the use of chemotherapy; group IT: integrated varroa treatment; group NT: only natural treatment with the use of essential oils and organic acids; group C: control group. Different capital letters denote significant differences between the means at $p < 0.01$; lowercase letters at $p < 0.05$.

determined by the treatment scheme ($F_{3,299} = 10.28$, $p = 0.0000$), not by season ($F_{2,\ 299} = 2.49$, $p = 0.085$). The Interaction of treatment × season was significant ($F_{6,\ 299} = 12.28$, $p = 0.0000$). The highest autumn number of combs inhabited by bees was observed in group IT in 2011 (15 inhabited combs) and in group MT in 2010 (14.9 inhabited combs). The lowest autumn number of combs inhabited by bees was observed in the control group in 2012, with only 10.2 inhabited combs. In the effect, the mean number of combs inhabited by bees of bee colonies in autumn, for the following three assay years, was significantly lowest in group C, with 12.5 inhabited inter-comb spaces (Table 3).

Winter losses

Total winter losses means differed between groups ($\chi^2_3 = 20.86$; $p = 0.01$). The highest mortality was observed in group C, where 30 colonies died during wintering over the 3 years of the experiment, and it was significantly different from the mortality observed in the remaining groups. In 2012, 45 colonies did not survive winter in the experimental apiary, which was significantly more than in previous years ($\chi^2_2 = 59.10$; $p = 0.01$) (Table 4).

Winter debris weight

The winter debris weight was not determined by the scheme of treatment ($F_{3,299} = 2.58$, $p = 0.053$), but it was determined by season ($F_{2,299} = 5.90$, $p = 0.003$); the significance of interaction between treatment x season was not confirmed ($F_{6,299} = 1.24$, $p = 0.287$).

In 2012, we observed the highest statistical average winter debris weights in group NT (244 g) (Table 5) as compared to previous years. The average weight of the debris each year did not differ in the remaining groups.

Number of dead varroa mites in winter debris

We observed a significant effect of treatment ($F_{4,299} = 41.28$, $p = 0.000$) and season ($F_{2,299} = 14.93$, $p = 0.000$) on the number of the varroa mites in 100 g of winter debris. The Interaction of treatment × season was highly significant ($F_{6,299} = 20.59$, $p = 0.000$). The mean number of mites in 100 g of winter debris found in group C was systematically increasing from 12.3 in 2010 to 114.8 in 2012, whereas in the other groups it remained at a similar level of a dozen or so mites in each assay year (Table 6).

Nosema *spp.*

One of the indicators of the colony health condition is the degree of infestation with *Nosema* spp. spores. The *Nosema* spp. infestation of bees in debris was not determined by the treatment scheme ($F_{3,\ 299} = 2.40$, $p = 0.068$), but it was significantly determined by season ($F_{2,\ 299} = 13.63$, $p = 0.0000$). The Interaction of treatment × season was not significant ($F_{6,\ 299} = 0.59$, $p = 0.738$). The highest average infestation of bee colonies with *Nosema* spp. spores (35.7 and 36.4 spores in the field of vision) was observed in groups MT and C, and it significantly differed from the infestation of colonies in group NT (Table 7).

Queen egg laying

We observed a highly significant effect of treatment ($F_{3,282} = 4.61$, $p = 0.0036$) and year ($F_{2,282} = 8.59$,

Table 5. Winter debris weight (g) in individual groups and years.

	Group								Years overall	
Year	*n*	MT	*n*	IT	*n*	NT	*n*	C	*n*	mean
2010	25	136.6^{A}	25	133.7^{A}	25	136.7^{A}	25	145.3^{A}	100	$138.0^{\acute{\Omega}}$
2011	25	171.1^{ABa}	25	148.3^{A}	25	174.4^{a}	25	171.1^{ABa}	100	$166.2^{\acute{\Omega}\Sigma}$
2012	25	170.1^{ABa}	25	140.8^{A}	25	244.0^{Bb}	25	200.3^{ABab}	100	189.3^{Σ}
Groups overall	75	$159.8^{\acute{\alpha}\acute{\varepsilon}}$	75	$141.2^{\acute{\alpha}}$	75	$186.1^{\acute{\varepsilon}}$	75	$172.2^{\acute{\alpha}\acute{\varepsilon}}$	300	164.4

Group MT: only main treatment with the use of chemotherapy; group IT: integrated varroa treatment; group NT: only natural treatment with the use of essential oils and organic acids; group C: control group. Different capital letters denote significant differences between the means at $p < 0.01$; lowercase letters at $p < 0.05$. Latin letters refer to 12 means; Greek letters refer to overalls in the same column or line.

Table 6. Mean number of mites in 100 g of debris and bracket the number of mites in complete winter debris in individual groups and years.

	Group								Years overall	
Year	*n*	MT	*n*	IT	*n*	NT	*n*	C	*n*	mean
2010	25	15.2^{a} (7–32)	25	13.2^{a} (5–11)	25	14.1^{a} (2–53)	25	12.3^{a} (1–19)	100	13.7^{A} (1–53)
2011	25	12.4^{a} (7–39)	25	15.4^{a} (3–32)	25	13.4^{a} (11–65)	25	43.5^{b} (16–201)	100	20.6^{A} (3–201)
2012	25	14.8^{a} (5–56)	25	4.2^{a} (1–17)	25	12.5^{a} (13–35)	25	114.8^{c} (34–189)	100	36.5^{B} (1–189)
Groups overall	75	14.0^{A} (5–56)	75	11.1^{A} (1–32)	75	13.3^{A} (2–65)	75	56.5^{B} (1–201)	300	23.3 (1–201)

Group MT: only main treatment with the use of chemotherapy; group IT: integrated varroa treatment; group NT: only natural treatment with the use of essential oils and organic acids; group C: control group. Different letters after means indicate significant differences between them ($p < 0.01$). Normal letters refer to twelve means. Capital letters indicate means in the same column or line.

Table 7. *Nosema* spp. infestation of bees in debris in individual groups and years.

	Group								Years overall	
Year	*n*	MT	*n*	IT	*n*	ET	*n*	C	*n*	mean
2010	25	18.1^{a}	25	4.3^{A}	25	6.0	25	11.1^{A}	100	9.9^{A}
2011	25	34.0	25	23.0^{ab}	25	4.0^{A}	25	38.0	100	25.0^{A}
2012	25	51.4^{B}	25	61.3^{Bc}	25	31.0	25	57.0^{Bbc}	100	50.7^{B}
Groups overall	75	35.7^{b}	75	31.0	75	11.1^{a}	75	36.4^{b}	300	29.6

Group MT: only main treatment with the use of chemotherapy; group IT: integrated varroa treatment; group NT: only natural treatment with the use of essential oils and organic acids; group C: control group. Different capital letters denote significant differences between the means at $p < 0.01$; lowercase letters at $p < 0.05$. *n*: number of honey bee colonies.

$p = 0.0002$) on the number of eggs laid by queen bees after treatment. The Interaction of treatment × year was highly significant ($F_{4,\ 282} = 3.80$, $p = 0.0012$). The highest number of eggs, after the treatment had been applied, was found in group NT in 2011 (4.8 thousand). In 2011, we noted 3.7 thousand eggs in experimental colonies, on average, which was significantly more than in the remaining years. The lowest average number of eggs was found in group C, after the treatment had been applied, for the three assay years. It was statistically different from the number noted in the remaining groups (Table 8). The effect of the medicines on the number of eggs laid by the queen bee after the treatment had been applied, was not statistically confirmed (Table 9). The effect of oxalic acid on egg-laying was not determined because of the lack of eggs in honeycombs after its application.

Queen behavior and losses

The observations of queen bee behavior, and the attitude of worker bees towards queen bees did not display anomalies. Yet, we noticed missing queen bees after the treatment with formic acid. Out of 50 colonies, 8 queen bees died in 2 groups (4 in group IT and 4 in group NT).

Discussion

Our colonies were weakest in the spring of 2010, when they inhabited 4.5 inter-comb spaces, on average. The lengthening winter must have contributed to this situation. However, the number of combs inhabited did not depend on *varroa* treatment. Autumn measurements showed that, in the first assay year, colonies in the group C had significantly higher number of combs inhabited by bees than colonies in groups treated for varroa. It could suggest that the use of various medicines and substances controlling the parasite have slightly weakened bee colonies. It evidently shows that the colonies in which varroa treatments were carried out, were in a worse condition than those not treated for varroa. Numerous studies on the effect of medicines on the honey bee prove that their active ingredients can have adversary effect on immunity and the biochemical composition of hemolymph (Collins et al., 2004; Dahlgren et al., 2012; Haarmann et al., 2002; Pettis

Table 8. Mean number of eggs laid by queen bees after completing the treatment (in thousands).

	Group								Years overall	
Year	*n*	MT	*n*	IT	*n*	NT	*n*	C	*n*	mean
2010	24	$3.0^{AB} \pm 0.2$	24	$3.5^{Bb} \pm 0.5$	22	$1.8^{A} \pm 0.2$	23	$2.3^{ABab} \pm 0.3$	93	$2.7^{\Upsilon} \pm 0.2$
2011	25	$3.8^{Bc} \pm 0.4$	25	$3.3^{ABbc} \pm 0.3$	25	$4.8^{Cd} \pm 0.7$	25	$2.8^{AB} \pm 0.4$	100	$3.7^{\Omega} \pm 0.2$
2012	23	$2.8^{AB} \pm 0.3$	24	$2.7^{AB} \pm 0.2$	23	$3.7^{Bc} \pm 0.5$	22	$1.8^{Aa} \pm 0.2$	92	$2.8^{\Upsilon} \pm 0.2$
Groups overall	72	$3.3^{\Omega} \pm 0.2$	73	$3.2^{\Upsilon\Omega\delta} \pm 0.2$	70	$3.5^{\Omega} \pm 0.3$	70	$2.3\Upsilon\gamma \pm 0.2$	285	3.1 ± 0.2

Group MT: only main treatment with the use of chemotherapy; group IT: integrated varroa treatment; group NT: only natural treatment with the use of essential oils and organic acids; group C: control group. Different capital letters denote significant differences between the means at $p < 0.01$; lowercase letters at $p < 0.05$. Latin letters refer to 12 means; Greek letters refer to overalls in the same column or line.

Table 9. Mean number of eggs laid by queen bees after treatment with varroa medicines (in thousands).

Medicine	*n*	Number of eggs % ± SD
Api Life Var	50	$3.4^{b} \pm 2.9$
Apiwarol	25	2.8 ± 1.5
Bayvarol	50	3.3 ± 1.9
Biowar	50	$3.5^{b} \pm 1.8$
Formic acid	50	3.2 ± 2.0
Not-treated colonies	75	$2.4^{a} \pm 1.5$
Total	300	3.1 ± 2.1

Lowercase letters denote significant differences between the means at $p < 0.05$.

et al.2004; Strachecka, Paleolog, Olszewski, & Borsuk, 2012b; Stoner et al., 1984; Taylor et al., 1987).

The use of even light chemicals can negatively affect the individual bee worker organisms (Underwood & Currie, 2003; Strachecka, Paleolog, Borsuk, and Olszewski, 2012a; Paleolog, Olszewski, & Borsuk, 2012b). This, in consequence, leads to the weakening of the whole colony, which was proved by the authors in the first year of the experiment. In the following year, the situation changed dramatically and families from group C weakened so much that in the third year of the experiment their number of combs inhabited by bees was significantly lower than the number of combs inhabited by bees from other groups (Table 2). This was most probably caused by a growing varroa population, which confirms the results achieved by Bąk et al. (2002).

Our analysis of the number of colonies that died out in spring clearly showed that varroa treatment failure leads to the destruction of bee colonies. During the 3 years of the assay, 30 colonies died out in group C, with the highest mortality observed in the last year of the assay (23), which was significantly more than in the remaining groups. The increased mortality was related to a growing population of the parasite (Bąk et al., 2002; Fries et al., 1994; Shimanuki et al., 1994).

We also observed that in 2012, the losses of bee colonies in all groups reached 20% and more. So they were higher than in the other years, which coincided with the highest infestation of bees with *Nosema* spp. spores in this year of the experiment. It is possible then that the parasite itself contributed to high winter losses in 2012.

The highest winter debris weight was noted in group NT in 2012 (Table 5). After the last honey collection, the bees were treated with formic acid, and then in late autumn with oxalic acid. So, unlike the previous years, we did not use Api Life Var, administering only organic acids. The debris weight was similar to the average weight of winter debris noted in group C, and it differed significantly from the winter debris weight in groups MT and IT (Table 5). This may suggest that a use of organic acids in *Varroa* treatment can have a negative effect on the winter hardiness of worker bees, and especially formic acid can shorten the life span of worker bees (Underwood & Currie, 2003).

The large amount of debris observed in group NT in 2012 could have also resulted from the fact that in 2012 as many as 40% of colonies died out in this group. However, a similar amount of debris was observed in group C, in which more than twice as many colonies died out. Furthermore, at that time, the group C was characterized by considerably weaker colonies.

In group C without varroa treatment, year by year, the number of mites in debris in colonies from this group was growing systematically, as compared to the other groups (Table 6). At the same time, in groups MT, IT, and NT, the number of mites remained at the level of a dozen or so mites in 100 g of debris throughout all the years of the assay. This fact indicates that the method for varroa treatment does not influence the number of mites in debris. Liebig, Schlipf, Fremuth, and Ludwig (1984) found that there is a correlation between the number of mites in debris and the population of living mites in the bee colony. Therefore, it should be concluded that the population of the parasite in the examined assay groups was not determined by the method of treatment, and remained at a similar level.

The mean number of eggs laid by queen bees, after the varroa treatment had been applied, was higher in treated colonies than in group C (Table 8) for the three years of the assay duration. The situation regarding the number of combs inhabited by bees in autumn was similar. This proves that systematic varroa treatment keeps colonies in good condition, and has a positive effect on the egg-laying activity of queen bees. At the same time, no effect of particular varroa medicines on the fertility of queen bees was observed. However, we noticed a disturbing influence of formic acid on the survivability of

queen bees. A similar problem was reported by Fries (1991) and Underwood and Currie (2003).

Regardless the method of treatment, the use of procedures for varroa treatment can slightly decrease the number of inhabited combs in bee colonies in comparison to not-treated ones. However, failure to varroa treatment brings catastrophic consequences in the form of colonies weakening and then their die off. Varroa treatment does not reduce the mass of winter debris in bee colonies in comparison to not-treated colonies, and keeps the number of mites in debris at a stable low level. The applied varroa treatment scheme had no effect on the degree of infestation with *Nosema* spp. spores in bees. Queen bees from colonies treated for varroa showed higher fertility than queen bees from not-treated colonies.

Summing up, the health condition of colonies treated for varroa is not determined by the applied parasite management scheme, and the used medicines lower the number of inhabited combs in bee colonies. However, only colonies in which systematic varroa treatment is carried out have a chance to survive and function. The obtained results provide a clear message: no matter what treatment is chosen, to maintain the honey bee colonies, it is crucial to carry out systematic treatment procedures against varroa.

Funding

This work was financially supported by COST ACTION FA0803: "COLOSS: Prevention of Honey Bee Colony Losses" and by State Committee for Scientific Research [grant number 520/N-COST/2009/0].

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