COMB CONSTRUCTION AND BROOD DEVELOPMENT ON BEESWAX FOUNDATION ADULTERATED WITH PARAFFIN

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S u m m a r y

In 2010 - 2011 the influence of beeswax foundation adulterated with different percentages of paraffin, on comb construction, brood rearing and bee colonies development was evaluated. Beeswax foundation adulterated with different rate of paraffin (10%, 30%, 50%) was prepared. Each year of the study into 7 experimental bee colonies set in Dadant type bee-hives, the three-frames queen insulators were inserted. Every frame in the insulators had a half of the foundation made from pure beeswax (the control - 0% paraffin) and a second half made from wax adulterated with paraffin (experimental). As a result every colony had an insulator with 3 frames with beeswax foundation with different percentages of paraffin (10 and 0%, 30 and 0%, 50 and 0%). The queens were put in the insulators what stimulated bees to prepare a place for laying eggs, thus, to construct the combs on the foundation.

Paraffin used in our experiment had no negative impact on the bee colonies. Bees drew out the foundation properly, and no destruction was observed. The queens laid eggs on the systematically drawn out comb cells. Brood development was normal and its quality was suitable. The worker bees emerged after 21 days. No case of brood or emerging worker bees death was noted.

Because of the different chemical compositions and purities of the paraffin available on the domestic market, it is impossible to exclude the fact that such paraffin may cause harmful effects on bee colonies.

Keywords: *Apis mellifera*, honeybee colony**,** beeswax foundation, adulteration, paraffin, comb construction, brood rearing.

INTRODUCTION

Combs construction is one of the basic life functions of the honey bees determining the development of colonies. They are essential for rearing next bee generations and for food storage. Regular comb replacement is an important part of the good beekeeping practice. During each beekeeping season it is recommended to replace 50 or even 100% of combs in the nest (Wilde, 2008). This technique adjusts the hygienic conditions in the hive, which is important regarding the colonies health (Koenig et al., 1986; Fries, 1988; Piccirillo and De Jong, 2004). The old combs are commonly contaminated with pathogens and can be a source of infections (Shimanuki and Knox, 2000; Flores et al., 2005; Mutinelli, 2011). In addition, such beeswax frequently contains varroacide residues applied in apiculture and the residues of pesticides used in agriculture that can potentially be toxic to bees (Wallner, 1995; Bogdanov, 2003; Johnson et al., 2010).

In managed honeybee colonies combs are build by bees usually on beeswax foundation (thin sheets of beeswax embossed on both sides with proper-size hexagonal cells). The cases of rejection or bad acceptance by bees of the foundation sheets are frequently

observed by beekeepers. This may be due to the poor quality of the commercial beeswax foundation (Wallner, 1997). It has been found that some values of the physicochemical parameters of the beeswax sheets accepted by the bees were within the ranges of guide values for pure beeswax. In most of foundation samples rejected by bees these values were higher or lower (Bernal et al. 2005). Adulterated beeswaxes have high concentrations of even-chain hydrocarbons, free alcohols and short-chain free acids (Jimenez et al., 2007; Waś et al., 2008; Waś et al., 2012).

It is presumed that adulterated beeswax foundation also may have an impact on brood rearing, causing the abnormalities and disorders of brood development, and consequently weakness and increased mortality of honeybee colonies (Wallner, 2005).

Good quality foundation should be produced from first class beeswax according to the Polish Standard for beeswax (PN-72/R-78890, 1996). The hydrocarbon content in beeswax should not exceed 16.5%. Since couple of years now, the Polish Standard is no longer in force, and there is a lack of any other obligatory legal regulations regarding beeswax quality. This has resulted in an increase in the adulteration of this product with cheaper vegetal of industrial waxes (e.g. microcrystalline wax, paraffin, stearin).

Presupposing that the number of bee colonies in Poland oscillates around 1.1 million (Semkiw and Skubida, 2011), and the average wax production from one bee colony equals around 0.3 kg (Skowronek, 1976), it can be estimated that the annual production of beeswax in Poland equals around 330 tons. There is a high demand for this product, but high prices and insufficient scale of production has meant that instances of beeswax adulteration with much cheaper paraffin can be more frequent.

Commercially available paraffin is a mixture of long-chain hydrocarbons containing more than 15 carbon atoms in a molecule. Straight-chain saturated alkanes make up a dominant part of this mixture. However, depending on the kind of paraffin and a purity level of a product from which it was obtained, the paraffin can contain some branched-chain, aromatic and unsaturated hydrocarbons as well as mixed aromatic hydrocarbons with long alkyl ligands. Commercially available paraffin is a group of products differing in their level of refinement and oil content. The classification of paraffin depends on the solidification or melting point. Proprieties and composition of paraffin can vary depending on its intended use (Szpyrka, 1999).

The aim of our study was to evaluate the influence of beeswax foundation adulterated with different percentages of paraffin, on comb construction, brood rearing and bee colonies development.

MATERIAL AND METHODS

Before the field study in the spring of 2010 and 2011 beeswax foundation adulterated with different rate of paraffin (granulated, technical paraffin LTP 56/25, manufactured by the LOTOS company, Poland) was prepared in the following manner:

- pure beeswax foundation sheets (the control - 0% paraffin)
- beeswax foundation sheets with 10% paraffin (experimental)
- beeswax foundation sheets with 30% paraffin (experimental)
- beeswax foundation sheets with 50% paraffin (experimental)

The field study was started on 25 June in 2010 and on 24 June in 2011. Each year, into 7 experimental bee colonies set in Dadant type bee-hives, the three-frames queen insulators were inserted. Every frame in the insulators had half of the foundation made from pure beeswax (the control) and a second half made from beeswax foundation adulterated with paraffin (experimental). As a result, every colony had an insulator with 3 frames with foundation with different percentages of paraffin contents (10 and 0%, 30 and 0%, 50 and 0%) (Fig. 1). The queens were put

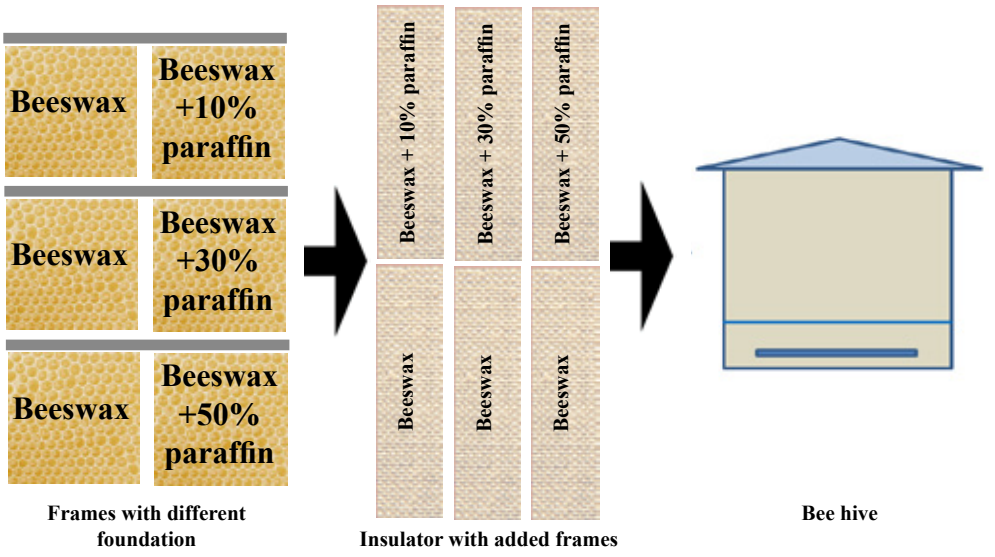


Fig. 1. Diagram of the experiment (example of one bee colony).

in the insulators which stimulated bees to prepare a place for laying eggs, thus, to construct the combs on the foundation. In addition, each year during the studies bee colonies were fed 3 times every 4 days with 2 liters of 1:1 (sugar : water) sugar syrup.

At a starting point of the experiment, every 7 days during 7 weeks, the observations and measurements of each foundation sheet (those adulterated with paraffin, and those made from pure beeswax) were carried out. Observations and measurements involved:

a) dynamics of comb construction on the applied foundation;

b) brood area (total of uncapped and capped brood) and the dynamics of the brood area increase;

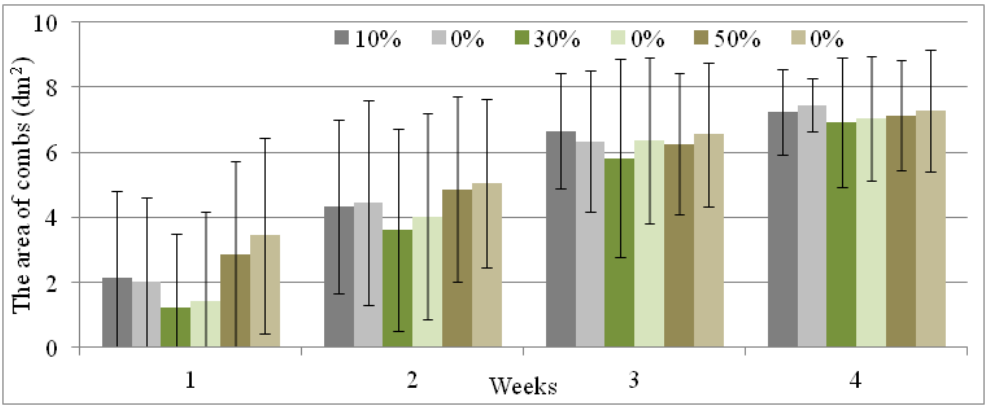
c) brood development and emergence of young bees.

The measurements of the comb construction area and brood area were done each study year during 4-week periods from the experiment starting point (from 25 June 2010 to 22 July 2010, and from 24 June 2011 to 21 July 2011). The queens were then removed from the insulators in order to stop egg laying on the experimental and control combs. From 22 July 2010 to 12 August 2010, and from 21 July 2011 to 11 August 2011 the observations of the emergence of worker bees were conducted.

The results were statistically analysed using STATISTICA 10 software. The characteristics (the area of the constructed combs, brood area, emerged brood area), confirmed with Kolmogorov-Smirnov and Shapiro-Wilk tests, did not have a normal data distribution. For the area of constructed combs, the data distribution was (Kolmogorov-Smirnov $D = 0.24$, $p < 0.01$; Shapiro-Wilk $W = 0.79$, $p = 0.0000$); for brood area (Kolmogorov-Smirnov $D = 0.20$, $p < 0.01$; Shapiro-Wilk $W = 0.79$, $p = 0.0000$); for emerged brood area (Kolmogorov-Smirnov $D = 0.21$, $p < 0.01$; Shapiro-Wilk $W = 0.78$, $p = 0.0000$). Because of this data distribution, the non-parametrical Mann and Witney's U test (significance level $\alpha = 0.05$) was used for comparing the differences between each of the measured parameters.

RESULTS

During both study years, in the first week of the observations, all the bee colonies most willingly constructed combs on the foundation with 50% and 0% of paraffin addition (Fig. 2). The areas of



Mann-Whitney U test for area of combs constructed (dm²) on the foundation with pure beeswax and adulterated with different rate of paraffin (10%, 30% and 50%), P > 0.05. Vertical bars indicate standard deviation.

Fig. 2. The area of combs constructed (dm²) on the foundation with different rate of paraffin adulteration after subsequent weeks - average data for two years.

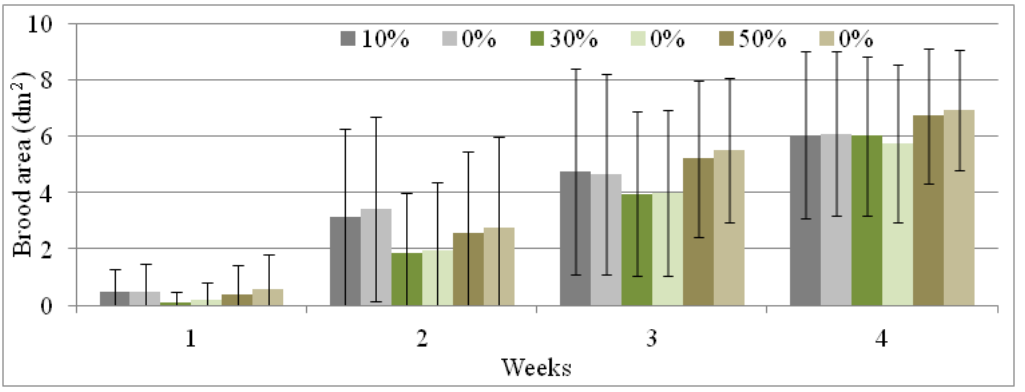
Table 1.

Average (± SD) area of combs (dm²) constructed on the foundation with different rate of paraffin adulteration after subsequent weeks

Year	Number of bee colonies	Rate of paraffin adulteration	Area of combs constructed after subsequent weeks (dm²)			
			1	2	3	4
			Average ± SD	Average ± SD	Average ± SD	Average ± SD
2010	7	10%	1.81 ± 2.66	3.35 ± 2.50	6.14 ± 2.18	7.07 ± 1.49
		0%	1.85 ± 2.50	3.28 ± 2.69	5.66 ± 2.63	7.26 ± 1.01
		30%	0.30 ± 0.63	3.26 ± 3.31	4.74 ± 3.53	6.04 ± 2.62
		0%	0.38 ± 0.62	3.45 ± 3.36	5.13 ± 3.20	6.23 ± 2.53
		50%	3.19 ± 3.26	5.05 ± 2.90	5.64 ± 2.58	6.41 ± 2.25
		0%	4.37 ± 3.28	4.62 ± 2.86	5.99 ± 2.81	6.69 ± 2.62
2011	7	10%	2.50 ± 2.79	5.30 ± 2.63	7.18 ± 1.24	7.40 ± 1.19
		0%	2.24 ± 2.86	5.62 ± 3.29	7.03 ± 1.46	7.63 ± 0.59
		30%	2.14 ± 2.99	3.95 ± 3.12	6.90 ± 2.20	7.79 ± 0.15
		0%	2.45 ± 3.72	4.59 ± 3.11	7.59 ± 0.68	7.85 ± 0.00
		50%	2.57 ± 2.56	4.67 ± 3.01	6.88 ± 1.66	7.85 ± 0.00
		0%	2.52 ± 2.64	5.50 ± 2.43	7.12 ± 1.37	7.85 ± 0.00

Mann–Whitney U test for area of combs constructed (dm²) on the foundation with pure beeswax and adulterated with different rate of paraffin (10%, 30% and 50%), P > 0.05.

the constructed combs were 2.88 dm² and 3.44 dm², respectively. Smaller areas of constructed combs were noted on the foundation with 30% and 0% of paraffin addition; 1.22 dm² and 1.41 dm², respectively. Similar constructing tendency was maintained in the next two weeks. However, in the 4th week, the area of constructed combs on the foundation with a different rate of paraffin adulteration, was comparable. Yet in 2011, combs on both the experimental and the control foundation were constructed much better, and their area was bigger than in 2010 (Tab. 1). The results of the statistical analysis did not confirm the existence of significant differences in constructing the combs on foundation with a different rate of paraffin adulteration both within individual years of the research and between the average values for individual years.



Mann-Whitney U test for brood area (dm²) on the foundation with pure beeswax and adulterated with different rate of paraffin (10%, 30% and 50%), P > 0.05. Vertical bars indicate standard deviation.

Fig. 3. Brood area (dm²) on the foundation with a different rate of paraffin adulteration after subsequent weeks - average data for two years.

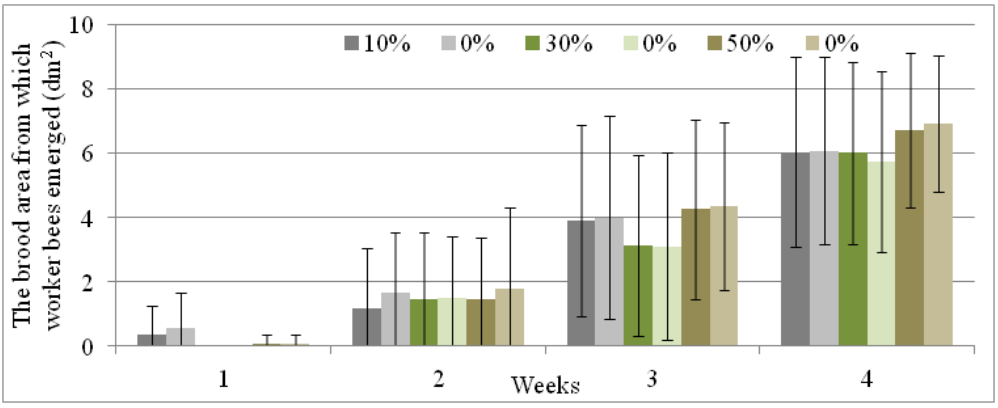
Table 2.

Average (± SD) brood area (dm²) on the foundation with a different rate of paraffin adulteration after the subsequent weeks

Year	Number of bee colonies	Rate of paraffin adulteration	Brood area after subsequent weeks (dm²)			
			1	2	3	4
			Average ± SD	Average ± SD	Average ± SD	Average ± SD
2010	7	10%	0.41 ± 0.81	2.40 ± 3.21	3.73 ± 3.71	5.86 ± 3.08
		0%	0.36 ± 0.68	2.19 ± 2.82	3.43 ± 3.77	5.97 ± 2.98
		30%	0.00	2.34 ± 2.62	3.21 ± 2.85	4.76 ± 3.34
		0%	0.00	1.77 ± 2.29	3.31 ± 2.71	4.59 ± 3.29
		50%	0.23 ± 0.53	2.77 ± 3.00	4.94 ± 3.23	5.59 ± 3.06
		0%	0.64 ± 1.09	2.99 ± 3.72	5.18 ± 2.76	5.98 ± 2.79
2011	7	10%	0.52 ± 0.92	3.85 ± 3.11	5.75 ± 3.60	6.20 ± 3.07
		0%	0.59 ± 1.23	4.63 ± 3.47	5.88 ± 3.14	6.20 ± 3.07
		30%	0.20 ± 0.53	1.33 ± 1.65	4.69 ± 3.02	7.23 ± 1.63
		0%	0.34 ± 0.91	2.14 ± 2.66	4.63 ± 3.20	6.89 ± 1.77
		50%	0.52 ± 1.39	2.33 ± 3.00	5.46 ± 2.45	7.85 ± 0.00
		0%	0.52 ± 1.39	2.55 ± 2.93	5.84 ± 2.54	7.85 ± 0.00

Mann–Whitney U test for brood area (dm²) on the foundation with pure beeswax and adulterated with different rate of paraffin (10%, 30% and 50%), P > 0.05.

The results of brood area (total of uncapped and capped brood) measurements showed that only in the first week of 2010, brood was not observed in beeswax foundation adulterated with 30 and 0% paraffin (Tab. 2). The first week of 2010 and 2011 showed that comparable brood area was noted on combs with 10% and 0% of paraffin addition (0.47 and 0.48 dm², respectively), and with 50% and 0% of paraffin addition (0.38 and 0.58 dm², respectively) (Fig. 3). Smaller area of brood was noted on combs with 30% and 0% of paraffin addition (0.10 and 0.17 dm², respectively). In the next week, the differences in the amount of brood on different combs leveled off, though a bigger brood area could be noted on combs from the groups of: 50 and 0%, and 10 and 0% paraffin addition. In the third and fourth week of the measurements, no bigger differences between the groups were found. Almost all the free surface on the combs (excluding the place for carbohydrate supply and bee bread) was occupied by the brood. In effect, the noted



Mann-Whitney U test for brood area from which worker bees emerged (dm2) on the foundation with pure beeswax and adulterated with different rate of paraffin (10%, 30% and 50%), P > 0.05. Vertical bars indicate standard deviation.

Fig. 4. The brood area (dm²) from which worker bees emerged on the foundation with a different rate of paraffin adulteration after subsequent weeks - average data for two years.

Table 3.

Average (± SD) brood area (dm²) from which worker bees emerged on the foundation with a different rate of paraffin adulteration after subsequent weeks

Year	Number of bee colonies	Rate of paraffin adulteration	Brood area from which worker bees emerged after subsequent weeks (dm²)			
			1	2	3	4
			Average ± SD	Average ± SD	Average ± SD	Average ± SD
2010	7	10%	0.29 ± 0.76	1.13 ± 1.58	3.19 ± 2.54	5.86 ± 3.08
		0%	0.36 ± 0.95	1.64 ± 2.05	3.64 ± 2.93	5.97 ± 2.98
		30%	0.00	1.90 ± 2.41	3.09 ± 2.76	4.76 ± 3.34
		0%	0.00	1.54 ± 1.71	3.12 ± 2.68	4.59 ± 3.29
		50%	0.00	2.25 ± 2.38	4.69 ± 2.78	5.59 ± 3.06
		0%	0.00	1.66 ± 2.38	4.67 ± 2.65	5.98 ± 2.79
2011	7	10%	0.40 ± 1.07	1.20 ± 2.29	4.64 ± 3.39	6.20 ± 3.07
		0%	0.75 ± 1.31	1.66 ± 1.87	4.35 ± 3.56	6.20 ± 3.07
		30%	0.00	1.01 ± 1.71	3.18 ± 3.04	7.23 ± 1.63
		0%	0.00	1.51 ± 2.20	3.08 ± 3.36	6.89 ± 1.77
		50%	0.16 ± 0.43	0.68 ± 0.88	3.81 ± 2.95	7.85 ± 0.00
		0%	0.16 ± 0.43	1.90 ± 2.90	4.03 ± 2.71	7.85 ± 0.00

Mann–Whitney U test for brood area from which worker bees emerged (dm²) on the foundation with pure beeswax and adulterated with different rate of paraffin (10%, 30% and 50%), P > 0.05.

brood area on combs with the 50 and 0% of paraffin addition, reached the mean value of 6.72 and 6.92 dm² for the two-year study period, respectively. Brood area on combs with 10 and 0% of paraffin reached 6.03 and 6.08 dm², respectively and on combs with 30 and 0% paraffin, 6.0 and 5.74 dm², respectively. Statistical analysis confirmed the lack of significant differences in the amount of brood on those combs which had different rate of paraffin adulteration, both within individual years and between the average values for individual years.

The increase in the number of young bees in the subsequent weeks of the observations was proportional to the increase of brood surface in the preceding period. Every week the number of emerged worker bees increased. Bees emerged mostly between the second and third week, and between the

third and fourth week (Fig. 4, Tab. 3). In the last, fourth week of the research the area of the comb from which the bees emerged was measured. This area corresponded with the brood area measured in the preceding weeks. In the fourth week, no brood was found in the experimental (with a different rate of paraffin adulteration) and the control combs. No disturbing symptoms of brood death nor difficulties for emerging worker bees were noted.

DISCUSSION

The results of our research did not confirm the previous report presented by Wallner (2005). Bee colonies constructed combs from the experimental beeswax foundation (adulterated with paraffin) and the control foundation (pure beeswax) at a similar rate. The quality of the combs was normal because they were drawn out evenly. There was no case noted of foundation that was chewed by bees.

No significant differences between the areas of combs constructed on a beeswax foundation with different percentages of paraffin were noted. In the second year of the research higher dynamic of comb construction in relation to the first year was observed. This could be explained by the fact that in 2011 the small-leaved lime supplied the bees with the nectar flow. Despite the fact that during the whole study period, bees were additionally fed with sugar syrup, the flow might have stimulated bees to build combs and prepare a place for the queens to lay eggs.

No notable differences in the increase in the brood area during the study period occurred. The queens laid eggs on the systematically constructed combs and bees brought up the brood. Each year, in the first week, the amount of brood was higher on combs with a 0 and 50%, and 0 and 10% paraffin addition, but it was caused by the fact that those types of foundation were drawn out faster. In the last week the brood areas of different combs were comparable because very often the entire free surface was occupied by the brood.

In the later period young bees started to emerge. The emergence went properly and no irregularities were noted. No case of worker bees deaths was noted in the bee-hives or in the vicinity of the bee-hives. No negative influence from different contents of paraffin in the beeswax foundation was noted on the functioning of the bee colonies. However, adulteration impact on the bee colonies may depend on the kind of paraffin, especially on its purity. Paraffin used in our experiment had a long-chain alkanes, which contained in their molecules from 20 to 40 carbon atoms. The total content of alkanes in paraffin is c.a. 50% (Waś et al., 2012).

Castro et al. (2010) also found that *Apis mellifera* colonies tolerated up to 40% of paraffin in combs and the addition of such a quantity of paraffin to beeswax had no adverse effects on the development of bees.

Based on the results it is difficult to suppose that beeswax adulteration with paraffin is one of the important factors in increased mortality of bee colonies in Poland. Instead, the cause for the losses has to be found in factors described by Topolska et al. (2010) and Pohorecka et al. (2011). However, it need to be stressed that adulterating beeswax with paraffin is inadvisable. Adulteration definitely lowers the product quality and excludes the possibility of using beeswax in other branches of industry.

CONCLUSIONS

1. The paraffin used in our experiment did not cause any difficulties connected with comb reconstruction and worker bees emergence. No instance of brood death was noted.

2. No negative influence from the different rate of foundation adulteration with paraffin on the bee colonies was observed. During the experiment and later, development of bee colonies was undisturbed.

3. It is impossible to exclude the harmful effects of paraffin on bee colonies, because of the different chemical compositions and purities of these substances available on the domestic market.

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ODBUDOWA PLASTRÓW I ROZWÓJ CZERWIU NA WĘZIE Z WOSKU PSZCZELEGO ZAFAŁSZOWANEGO PARAFINĄ

Semkiw P., Skubida P.

S t r e s z c z e n i e

W latach 2010 - 2011 w Oddziale Pszczelnictwa Instytutu Ogrodnictwa w Puławach przeprowadzono badania, których celem była ocena wpływu węzy zafałszowanej parafiną na odbudowę plastrów, wychów czerwiu i rozwój rodzin pszczelich. Na potrzeby doświadczenia przygotowywano we własnym zakresie węzę z wosku pszczelego w różnym stopniu zafałszowaną (10%, 30%, 50%) parafiną techniczną (LTP 56/25) firmy LOTOS S.A.

W każdym roku do 7 doświadczalnych rodzin pszczelich, osadzonych w ulach typu Dadant wstawiano izolatory trzy ramkowe. Poszczególne ramki umieszczone w izolatorze w jednej połowie miały wprawioną węzę wykonaną z wosku pszczelego (kontrola - 0% parafiny), a w drugiej węzę zafałszowaną parafiną (doświadczalna). W rezultacie każda z trzech ramek w izolatorze posiadała węzę o różnej zawartości parafiny (10 i 0%, 30 i 0%, 50 i 0%). W izolatorze umieszczono matkę pszczelą, co stymulowało pszczoły do odbudowywania poddanej węzy i przygotowania miejsca do czerwienia.

Użyta w doświadczeniu parafina nie wpłynęła negatywnie na funkcjonowanie rodzin pszczelich. Niezależnie od stopnia zafałszowania pszczoły odbudowywały węzę prawidłowo, nie odnotowano przypadków jej niszczenia. W systematycznie odbudowywanych plastrach matki składały jaja. Rozwój czerwiu odbywał się normalnie, a jego jakość była właściwa. Ze złożonych jaj po 21 dniowym okresie rozwoju wygryzały się robotnice. Nie stwierdzono przypadków zamierania czerwiu oraz wygryzających się pszczół.

Z uwagi na różny skład chemiczny i czystość dostępnych na krajowym rynku parafin, nie można jednoznacznie wykluczyć szkodliwego oddziaływania tych substancji na rodziny pszczele.

Słowa kluczowe: *Apis mellifera*, rodzina pszczela, węza pszczela, zafałszowanie, parafina, budowa plastrów, wychów czerwiu.