DOI: 10.2478/v10083-012-0002-4

ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXX (1) SECTIO EE 2012

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The effect of different varroacides on the acidity of winter stores and honey stores

Wpływ rożnych warroacydów na kwasowość zapasów zimowych oraz miodu

Summary. The aim of the study was to assess the effect of selected varroacides on the pH of winter honey stores, spring honey stores, and summer honey stores. With this aim, five groups, each consisting of five colonies, were established. The control group was composed of colonies that were not treated for varrosis. The other groups were treated with oxalic acid, formic acid, Apivarol, and Bee Vital Hive Clean, respectively. The acidity of honey stores was determined with the testo 205 pH-meter. In each colony, pH of centrifuged samples of the winter honey stores, and spring and summer honey was measured in triplicate. Oxalic acid was found to lower the pH of the winter honey stores significantly (control: pH = 3.65, winter honey stores: pH = 3.29) and of the spring honey (control pH = 3.87, spring honey pH = 3.73). Formic acid significantly decreased the pH of the summer honey (control pH = 3.73, summer honey pH = 3.56). Apivarol and Bee Vital Hive Clean did not exhibit any significant impact on the pH of the winter honey stores or honey.

Key words: honey pH, formic acid, oxalic acid, Apivarol, Bee Hive Vital Clean, varroa disease

INTRODUCTION

The *Varroa* mite appeared in Poland approximately 30 years ago; however, its control is becoming increasingly difficult. Up to date, no varroacidal agent which would not produce resistance of the mites has been manufactured. Even pyrethroids (acrinathrin, flumethrin and fluvalinate), which are most efficient *Varroa* mite control drugs characterised by low toxicity to bees [Trouiller 1998, Milani 1995], decreasing their varroacidal efficiency. The ability for the increase of drug-resistance in the *Varroa destructor* population resulted in appearance of resistant mites [Watkins 1997, Bogdanov *et al.*

1998, Wallner 1999], which are capable of reproduction in presence of pyrethroids [Mathieu and Faucon 2000]. In recent years, the *Varroa* mites have become extremely virulent to honeybees, as they are the vectors of viruses [Sumpter and Martin 2004] that cause mortal bee deseases. [Ball and Allen 1988, Brodysgaard *et al*. 2000, Bailey *et al*. 1983). Therefore, *Varroa destructor* mites are regarded as one of the main causes of colony collapse disorder [de Jong 1982, Wilde 2008, 2009].

The rapid increase in resistance to pyrethroids has drawn researchers' attention to organic acids and ether oils [Liebig 1997] as alternative varroacides [Milani 1999]. However, the intensive flavour of ether oils may penetrate honey (Lipiński 2003b, Skubida 2007). Since they are insoluble in lipids, organic acids are believed not to affect the quality of honeybee products, not to demand a waiting period or accumulate in wax [Bogdanov *et al*. 2002]. An additional advantage, e.g. of formic acid, is its ability to permeate through the honeycomb seal and to destroy mites colonizing grubs [Polaczek 2002, Lipiński, 2003a, Polaczek 2004; Polaczek 2005, Liebig 2005, Górczyński 2007]. Organic acids are also approved for use in ecological apiaries instead of perythroids [Skubida *et al*. 2004]. Beside the therapeutic activity, organic acids exhibit a negative impact on honeybees. They can damage the digestive system [Howis *et al*. 2010] and the surface proteolytic barrier [Strachecka *et al*. 2008, 2009] in bees.

Therefore, the aim of the study was to assess the impact of selected varroacides on the acidity of winter honey stores, spring honey, and summer honey.

MATERIALS AND METHODS

Five groups, each composed of five colonies were established:

1. control – the colonies were treated no with varroacides;

2. Apivarol – after July 20, 2008, when the summer honey had been harvested, the colonies were fumigated using one tablet of Apivarol (amitraz 12.5 mg per tablet) three times at 5 day intervals;

3. Bee Vital Hive Clean – after September 20, 2008, each colony was sprayed with 20 ml of "Bee Vital Hive Clean" (Bee Vital Hive Clean composition provided by the manufacturer was: water, saccharose, citric acid, oxalic acid, propolis extract, ether oils);

4. oxalic acid – on September 30, 2008, a 3.2% oxalic acid solution was applied; it was prepared in the following proportions: 30 g oxalic acid, 400 g sugar, and 400 ml water. The solution was applied on the colonies in the volume of approximately 5 ml per each lane;

5. formic acid – on July 20, 2008, after summer honey harvesting, the colonies were treated with three rounds of fumigation using one Apivarol tablet; on June 15, 2009 (10 days before linden blooming), 40 ml of 60% formic acid per colony was applied in evaporators on the top bars of the nest and the treatment was continued for 4 weeks.

Feeding the colonies until the over-wintering was ceased on September 15, 2008; 15 kg of sugar syrup per colony in the ratio 3:2 was used. After the first spring flight (end of March 2009), acidity of winter honey stores was measured. The authors presumed that those agents might have affected the acidity of spring honey (produced from rape and dandelion). Therefore, acidity was determined in the spring honey harvested in mid-June as well as in the summer honey (linden honey) harvested in mid-July and stored by bees during formic acid treatment.

Acidity measurments. Winter honey stores, spring honey and summer honey were sampled (size of the samples 250 g of the five nest places) in triplicate from each colony (9 samples) within each of the group. Each samle was centrifuged and its pH was measured using the testo 205 pH-meter.

Statistics. The results were statistically analysed with the SAS software (SAS Institute 2002-2003 SAS/STAT User's Guide Version 9.13, Cary, NC, Statistical Analysis System Institute) using the one-way ANOVA (a group effect was the experimental factor) and the HSD (honestly significant difference) test.

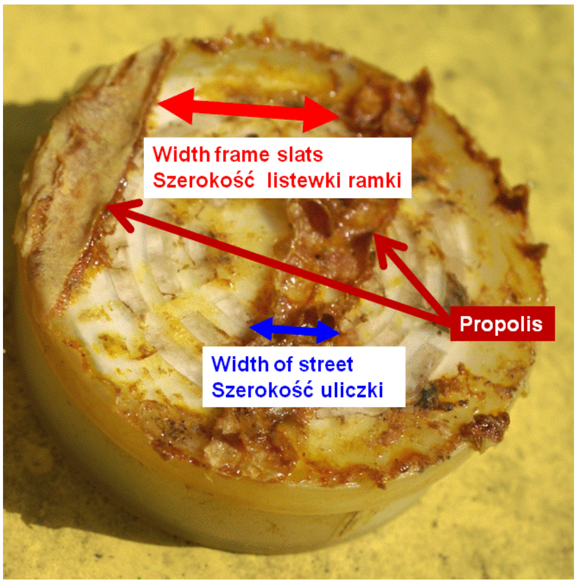
RESULTS AND DISCUSSION

Treatment of the colonies with oxalic acid in autumn significantly decreased pH of the winter honey stores measured in the spring (Tab. 1).

Table 1. The pH of the winter honey stores, spring honey, and summer honey after application of the varroacides
Tabela 1. Kwasowość (pH) zapasu zimowego, miodu wiosennego i letniego po zastosowaniu wybranych warroacydów

Specification Wyszczególnienie		Średnia Mean	SD	Min	Max
Winter honey stores Zapas zimowy	control – kontrola	3.65 [Bb]	0.008	3.64	3.66
	Apivarol – apiwarol	3.56 [Bb]	0.174	3.31	3.74
	Bee Vital Hive Clean	3.66 [Bb]	0.167	3.41	3.80
	kwas szczawiowy – oxalic acid	3.29 [Aa]	0.071	3.20	3.37
Spring honey Miód wiosenny	control – kontrola	3.87 [Bb]	0.065	3.82	3.96
	Apivarol – apiwarol	3.90 [Bb]	0.065	3.81	3.97
	Bee Vital Hive Clean	3.93 [Bb]	0.095	3.85	4.07
	oxalic acid – kwas szczawiowy	3.73 [Aa]	0.007	3.72	3.74
Summer honey Miód letni	control – kontrola	3.73 [ABb]	0.010	3.73	3.75
	Apivarol – apiwarol	3.67 [ABab]	0.083	3.59	3.79
	Bee Vital Hive Clean	3.80 [Bb]	0.038	3.76	3.86
	formic acid – kwas mrówkowy	3.56 [Ba]	0.196	3.34	3.82

A, B – the differences between the pH of the winter honey stores, spring honey, and summer honey after application of varroacides are statistically significant at $p \leq 0.01$.
A, b – the differences between the pH of the winter honey stores, spring honey, and summer honey after application of acaricides are statistically significant at $p \leq 0.05$.
SD – standard deviation.
Min – minimum value of the trait.
Max – maximum value of the trait.
AB – różnice pomiędzy pH produktów: zapasu zimowego, miodu wiosennego i letniego, po zastosowaniu wybranych warroacydów są istotne statystycznie dla $p \leq 0{,}01$.
ab – różnice pomiędzy pH produktów: zapasu zimowego, miodu wiosennego i letniego, po zastosowaniu wybranych akarycydów są istotne statystycznie dla dla $p \leq 0{,}05$.
SD – odchylenie standardowe.
Min – minimalna wartość cechy.
Max – maksymalna wartość cechy.



Phot. 1. Evaporators with formic acid
Fot. 1. Parownik z kwasem mrówkowym

Since oxalic acid is insoluble in lipids [Bogdanov *et al*. 2002], it is not accumulated in wax and, hence, in honeycombs. Therefore, it is difficult to explain the lower pH of the winter honey stores, which might result from the contact of winter honey stores deposited in unsealed honeycomb cells with the solution of oxalic acid present on bees passing honeycomb lanes. Bogdanov *et al*. [2002] claim that a single application of oxalic acid in autumn does not result in a decrease in the pH of spring honey. In our study, however, the single application of oxalic acid in the autumn did cause a rapid decrease in the pH of spring honey (Tab. 1). This may have been the result of permeation of the remaining oxalic acid solution from the honeycomb cell walls to honey.

Formic acid applied during summer honey production caused a decrease in the pH of the honey (Tab. 1). This must have been related to the ability of formic acid vapour to penetrate the honeycomb seal covering cells occupied by larvae [Polaczek 2002, Lipiński 2003a, Polaczek 2004, Polaczek 2005, Liebig 2005, Górczyński 2007]. The seal is porous owing to presence of flower pollen; hence, gas exchange which is vital for larvae is possible. Formic acid is able to permeate through the wax seal that covers honey cells, thanks to the crystal structure of wax. In this study, it was observed that bees were trying to isolate the colonies from the evaporators with formic acid by sealing the gaps in the evaporators with propolis (Phot. 1), thereby trying to eliminate the negative effect of the acid on the colony. Formic acid vapour has been proved to damage the midgut in bees [Howis *et al.* 2010]; lowered pH of the winter honey stores during the six months of winter diapause may have a similar impact. Imdorf *et al*. [2003] found that formic acid was organoleptically perceptible in honey shortly after its application, but hardly detectable after centrifugation of honey. Bogdanov *et al*. [2002] confirmed increased amounts of oxalic and formic acids in honey after they were applied to control *Varroa destructor*.

No significant effect of application of the varroacidal Apivarol and Bee Vital Hive Clean preparations on the acidity of winter honey stores, spring honey stores and summer honey stores was observed (Tab. 1)

CONCLUSION

Until recently, organic acids were considered to be effective in treatment of the varroa disease and, simultaneously, not to affect the quality of honey. However, organic acids have been shown to have a long-term impact on bee colonies because they will decrease the pH of honey obtained up to 7 months after their application.

REFERENCES

Bailey L., Ball B.V., Perry J.N., 1983. Association of viruses with two protozoal pathogens of the honey bee. Ann. Appl. Biol. 103, 13–20.

Ball B.V., Allen M.F., 1988. The prevalence of pathogens in honey bee (*Apis mellifera*) colonies infested with the parasitic mite *Varroa jacobsoni*. Ann. Appl. Biol. 113, 237–244.

Bogdanov S., Charričre j. D. , Imdorf A., Kilchenmann V., Fluri P., 2002. Determination of residues in honey after treatments with formic and oxalic acid under field conditions. Apidologie 33, 399–409.

Bogdanov S., Kilchenmann V., Imdorf A., 1998. Acaricide residues in some bee products, J. Apic. Res. 3, 57–67.

Brodysgaard C.J., Ritter W., Hansen H., Brodsgaard H.F., 2000. Interactions among *Varroa jacobsoni* mites, acute paralysis virus, and *Paenibacillus larvae larvae* and their influence on mortality of larval honeybees in vitro. Apidologie 31, 543–554.

de Jong, D., de Jong, P.H., Goncalves L.S., 1982. Weight loss and other damage to developing worker honey bees from infestation with *Varroa jacobsoni*. J Apic. Res. 21, 165-167.

Górczyński Z., 2007. Moje doświadczenia z kwasem mrówkowym. Pszczelarstwo 7, 8–9.

Howis M, Chorobiński P., Nowakowski P., 2010. Wpływ ekspozycji kwasu mrówkowego na stan fizjologiczny jelita środkowego pszczoły miodnej. XLVII Naukowa Konferencja Pszczelarska, Puławy 10–11 marca, 21.

Imdorf A., Charričre J.D., Kilchenmann V., Bogdanov S., Fluri P., 2003. Alternative strategy in central Europe for the control of Varroa d. in honey bee colonies. Strategie zur alternative Bekampfung von Varroa destructor in Zentraleuropa. Apiacta 38, 258–285.

Liebig G., 1997. Alternative Varroabekämpfung. Mit organischen Säuren aus der Krise? Bienenwelt 39, 289–297.

Liebig G., 2005. Getreidestärkesirup: besser als sein Ruf. Dtsch. Bienen J., 8, 18–19.

Lipiński Z., 2003a. Kwas mrówkowy w zwalczaniu warrozy. Pszczelarstwo, 7, 10–11.

Lipiński Z., 2003b. Olejki eteryczne i ich pochodne w zwalczaniu warrozy pszczół. Pszczelarstwo, 9, 10.

Mathieu L., Faucon J.P., 2000. Changes in the response time for *Varroa jacobsoni* exposed to amitraz. J. Apic. Res., 39(3–4), 155–158.

Milani N., 1995. The resistance of Varroa jacobsoni Oud. to pyrethroids: a laboratory assay, Apidologie, 26, 415–429.

Milani N., 1999. The resistance of Varroa jacobsoni Oudemans to acaricides. Apidologie, 30, 229–234.
Polaczek B., 2002. Chów pszczół bez środków chemicznych. Pszczelarstwo, 6, 11.
Polaczek B., 2004. Zwalczanie warrozy kwasem mrówkowym. Pszczelarstwo, 4, 9–10.
Polaczek B., 2005. Prowadzenie pasieki połączone ze zwalczaniem *Varroa* kwasem mrówkowym. Pszczelarstwo, 5, 10–11.
Skubida P., 2007. Pszczołom trzeba pomóc, same z warrozą sobie nie poradzą. Pszczelarstwo, 5, 8–9.
Skubida P., Skowronek W., Szewczyk A., 2004. Prowadzenie pasiek metodami ekologicznymi OPISiK w Puławach. Krajowe Centrum Rolnictwa Ekologicznego.
Strachecka A., Choroszyńska D., Paździor E., Paleolog J., Olszewski K., 2009. Wpływ akarycydów na powierzchniową aktywność proteolityczną u robotnic *Apis mellifera.* XLVI Naukowa Konferencja Pszczelarska, Puławy 10–11 marca, 69.
Strachecka A., Paleolog J., Grzywnowicz K., 2008. The surface proteolytic activity in *Apis mellifera.* J. Apic. Sci., 52(1), 49–56.
Sumpter, D. J. T., Martin S.J., 2004. The dynamics of virus epidemics in *Varroa* infested honey bee colonies. J Anim. Ecol. 73, 52–63.
Trouiller J., 1998. Monitoring Varroa jacobsoni resistance to pyrethroids in western Europe, Apidologie, 29, 537–546.
Wallner K., 1999. Varroacides and their residues in bee products, Apidologie, 30, 235–248.
Watkins M., 1997. Resistance and its relevance to beekeeping. Bee World., 78(1), 15–22.
Wilde J., 2008. Straty ekonomiczne powodowane przez *Varroa destructor* i sposoby zapobiegania. Pszczelarstwo, 3, 2–4.
Wilde J., 2009. *Varroa destructor* a CCD i zapobieganie masowym ginięciom pszczół. Pszczelarstwo, 3, www.miesiecznik-pszczelarstwo.pl/artykuly/2009_05/artykul_2009_05.html.

Streszczenie. W czterech grupach, każda po 5 rodzin pszczelich, porównano wpływ warroacydów – apiwarolu, Bee Hive Vital Clean, kwasu szczawiowego i kwasu mrówkowego – na pH zapasu zimowego (cukier), zapasów miodu wiosennego oraz zapasów miodu letniego. W piątej (kontrolnej) grupie (n = 5) warroacydów nie stosowano. Kwas szczawiowy obniżył pH zapasu zimowego (kontrola pH = 3,65, zapas zimowy pH = 3,29) i pH zapasów miodu wiosennego (kontrola pH = 3,87, miód wiosenny pH = 3,73). Kwas mrówkowy obniżył tylko pH zapasów miodu letniego (kontrola pH = 3,73, miód letni pH = 3,56). Podanie Apiwarolu i Bee Hive Vital Clean nie miało wpływu na pH zapasu zimowego, pH miodu wiosennego oraz pH zapasów miodu letniego.

Słowa kluczowe: pH miodu, kwas mrówkowy, kwas szczawiowy, apiwarol, Bee Hive Vital Clean, warroza