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Content

QUALITY OF HONEYBEE QUEENS COMMERCIALLY AVAILABLE IN SOUTHERN POLAND

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Summary

The quality of 230 honeybee queens was assessed in the years 1998 - 2000. The queens were purchased from 5 queen breeding apiaries in southern Poland. Queen damage and *Nosema apis* infection were checked; 14.8% of the queens were disqualified due to damage or death. *N. apis* spores were found in 7% of the queens and in 8.8% of the workers attending them. Some queens were both damaged and infected. In effect, 20.4% of the queens were of inadequate quality.

Keywords: *Apis mellifera*, *Nosema apis*, queen injuries, workers.

INTRODUCTION

Nosema disease is caused by the microsporidian *Nosema apis*, one of the most widely distributed honeybee parasites (Fries 1988, 1993, 1997, Bailey and Ball 1991). It develops in epithelial cells of the midgut of honeybee workers, queens and drones (Fries 1988, 1993). Infected colonies develop slowly and produce smaller amounts of honey and wax (Fries 1988, 1997, Bailey and Ball 1991). *N. apis* spreads between colonies with drifting workers or drones, during robbing and due to poor sanitary measures. Another source of infection can be queens purchased from commercial queen breeders (Shimanuki et al. 1973). The risk of *N. apis* infection in queen breeding apiaries is relatively high because colonies are manipulated frequently and bees are kept in small mating nuclei and cages (Oertel 1967, Bailey and Ball 1991). Queens become infected within 4 days of the first contact with diseased workers (Shimanuki et al. 1973). In earlier health surveys of commercially available honeybee queens the proportion of infected individuals varied from 7% to 38% (Jay 1967, Loskotova et al. 1980, Liu et al. 1987, Camazine et al. 1998). The presence of infected queen in a colony leads to rapid spread of *N. apis* in the colony because workers ingest the queen's feces (Bailey and Ball 1991, Czekońska 2000). Therefore it is very important to control of *N. apis* in commercially available honeybee queens.

During mass production, queens are subject to injuries. Most of them occur during storage in cages inside the colony and preparation for shipment (Woyke et al. 1956, Jasiński 1987, 1995, Jasiński and Fliszkiewicz 1995, 1998, Woyke 1988). Jasiński (1987) described 26 types of queen injuries and divided them into those impairing and those not impairing their locomotion. Injured queens can have problems with egg laying and are often superseded after introduction. Queen breeders are obliged to eliminate injured individuals, but control often is inadequate. This paper surveys the quality of queens available commercially in Poland. The queens were examined for injuries and *N. apis* spores.

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MATERIALS AND METHODS

The survey was carried out in June and July 1998, 1999 and 2000. Artificially inseminated honeybee queens were purchased from 5 queen breeding apiaries in southern Poland, numbered from I to V here. Among 230 queens examined there were 50 queens of *Apis mellifera caucasica* and 180 queens *of A. m. carnica*, abbreviated Cau and Car respectively. There was no certificate of origin with the queens purchased in 2000 from apiaries II, IV and V. The remaining queens were 9 to 21 days old. The queens were delivered by post in series of 20 - 30 individuals of the same subspecies. Immediately after delivery, the workers attending the queens were counted. Afterwards, feces were collected from the queens. The queens were placed on a piece of aluminum foil and covered with a watch glass 6 cm in diameter. If a queen was not inclined to release feces, the procedure was stopped after 30 minutes of waiting. The feces and aluminum foil were placed in a vial with 0.5 ml distilled water. If the queen did not release feces its rectum was dissected.

Each queen was carefully examined for damage to their legs, antennas and wings. During examination the queens were walking on a glass plate, which helped to reveal leg injuries. Afterwards the queens were anesthetized with CO_2 and their midguts were dissected and homogenized in 0.5 ml distilled water. Five workers were collected from every cage and their midguts were dissected and homogenized in 1 ml distilled water. All samples were frozen for later analysis.

N. apis spores were counted in a hemacytometer in 0.025 mm^3 suspension. If less than 10 spores were found in the sample they were counted in 0.2 mm^3 suspension. The results are expressed as spore counts per individual. For statistical analysis the G-test, Kruskal-Wallis test and Wilcoxon's signed-ranks test were used (Sokal and Rohlf 1981).

RESULTS

Three of 230 queens (1.3%) did not survive transport (tab. 1). It was possible to dissect the rectum from only one of the dead queens. The two others were not verified for the presence of *N apis* because of decay. Among 228 queens examined for the presence of *N. apis*, 17 queens (7.5%) were infected (tab. 2). *N. apis* spores were found in queens from all apiaries (tab. 2). No significant differences were found in the proportion of infected queens, neither between different series from the same apiary (G - test: apiary I - $G = 1.26$, $p = 0.874$, apiary II - $G = 0.47$, $p = 0.971$) nor between different apiaries ($G = 5.54$, $p = 0.243$). Midgut analysis revealed significantly more infected individuals (16 queens - 7.0%) than feces analysis (6 queens - 2.6%) (Wilcoxon signed-ranks test: $T = 4$, $n = 218$, $p = 0.001$). Significantly more *N. apis* spores were found in midgut than in feces (Wilcoxon signed-ranks test: $T = 4$, $n = 16$, $p = 0.001$; tab. 2). No significant differences were found in the proportion of infected queens between subspecies (G - test: $G = 3.31$, $p = 0.069$).

Among queens, which survived transport, 31 (13.5%) were injured (tab. 1). The most common were leg injuries (27 queens - 11.7%): missing tarsus, destroyed tarsal pads, missing claws or missing whole leg. Four queens (1.7%) had injured antennas. No wing injuries were observed. No significant differences were found in the proportion of injured queens, neither between different series from the same apiary (G - test: apiary I - $G = 6.49$, $p = 0.170$; apiary II - $G = 5.25$, $p = 0.297$) nor between different apiaries ($G = 5.83$, $p = 0.218$). Three queens were both injured and infected, so the total

number of queens disqualified was 47 (20.4%).

The number of workers attending queens varied from 3 to 19 and differed significantly between apiaries (Kruskal-Wallis test: H = 77.51, n = 230, p = 0.000; tab. 3). Up to 7 dead workers were found in a cage. There were no dead workers in deliveries from apiaries III and IV. The number of dead workers found in cages differed significantly between apiaries (Kruskal-Wallis test: H = 43.62, n = 230, p = 0.000; tab. 3).

Among 1150 verified workers there were 101 (8.8%) infected individuals. Significant differences between the proportion of infected workers from different series were found in deliveries from apiary I (G - test: - G = 41.57, p = 0.000; tab. 4) but not in deliveries from apiary II (G = 6.55, p = 0.165). The proportion of infected workers was greater among *A. m. caucasica* than among *A. m. carnica* (G - test: G = 16.35, p = 0.000).

Up to 88.8 x 10^6 spores per worker was found. Significant differences between the number of spores in workers from different series were found in deliveries from apiary I (Kruskal-Wallis test: H = 17.59, n = 48, p = 0.000; tab. 4) but not in deliveries from apiary II (H = 3.81, n = 27, p = 0.149). The intensity of infection did not differ significantly between subspecies (Kruskal-Wallis test: H = 1.48, n = 12, p = 0.220).

Table 1

Number of dead and injured queens purchased from 5 queen breeding apiaries.
Liczba martwych i uszkodzonych matek pszczelich zakupionych w 5 pasiekach hodowlanych.

Apiary Pasieka	Queen series Seria matek	No. of queens - Liczba matek				
		N	Healthy Bez wad	Disqualified - Zdyskwalifikowanych		
				Total Ogółem	Dead Martwych	Injured Uszkodzonych
I	Car-98	60	56	4 a*	1	3
	Car-99	30	22	8 a	0	8
	Cau-99	30	26	4 a	0	4
	Subtotal - Razem:	**120**	**104**	**16 A**	**1**	**15**
II	Cau-99	20	18	2 a	0	2
	Car-99	20	12	8 a	2	6
	Car-00	20	16	4 a	0	4
	Subtotal - Razem:	**60**	**46**	**14 A**	2	12
III	Car-00	**20**	**18**	**2 A**	0	2
IV	Car-00	**20**	**20**	**0 A**	0	0
V	Car-00	**10**	**8**	**2 A**	0	2
Total - Razem:		**230**	**196**	**34**	**3**	**31**
Total % - Razem %		**100**	**85.2**	**14.8**	**1.3**	**13.5**

* Same small letters indicate lack of significant differences (p > 0.05) between series from the same apiary. Same capital letters indicate lack of significant differences (p > 0.05) between apiaries.
* Te same małe litery przy średnich oznaczają brak różnic istotnych (p > 0,05) pomiędzy seriami matek z tej samej pasieki. Te same duże litery oznaczają brak różnic istotnych (p > 0,05) pomiędzy pasiekami.

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Table 2

N. apis infection of queens purchased from 5 queen breeding apiaries, determined by feces and midgut examination. - Poziom zarażenia sporami *N. apis* matek pszczelich zakupionych w 5 pasiekach hodowlanych ocenianych na podstawie badania kału i jelita

Apiary Pasieka	Queen series Seria matek	N	No. of infected queens Liczba zarażonych matek			Average no. spores x 10^3 in Średnia liczba spor x 10^3 w	
			Total Ogółem	Feces Kał	Midgut J. środkowe	Feces Kale	Midgut J. środkowym
I	Car-98	59	5 a	3	5	48.4	338.0
	Car-99	30	3 a	1	3	5.0	1.3
	Cau-99	30	1 a	0	1	0	5.0
	Subtotal: Razem:	**119**	**9 A**	**4**	**9**	-	-
II	Cau-99	20	0 a	0	0	0	0
	Car-99	19	1[1] a	1[1]	0	12.5[1]	-
	Car-00	20	1 a	0	1	0	2.5
	Subtotal: Razem:	**59**	**2 A**	**1[1]**	**1**	-	-
III	Car-00	**20**	**2 A**	**0**	**2**	**0**	**2.5**
IV	Car-00	**20**	**3 A**	**0**	**3**	**0**	**5.0**
V	Car-00	**10**	**1 A**	**1**	**1**	**5.0**	**67.5**
Total - Razem:		**228**	**17**	**6**	**16**	-	**-**
Total % - Razem %		**100**	**7.5**	**2.6**	**7.0**		

1 - The result of rectum examination of one dead queens.
1 - Wynik uzyskany na podstawie badania jelita prostego jednej z martwych matek.

Table 3

Number of workers attending queens purchased from 5 queen breeding apiaries. - Liczba robotnice towarzyszących w klateczkach wysyłkowych matkom pszczelim, zakupionym w 5 pasiekach hodowlanych.

Apiary Pasieka	Queen series Seria matek	No. of cages Liczba klateczek	Average no. workers Średnia liczba robotnic	
			in cage w klateczce	dead martwych
I	Car-98	60	12.5 a	0.75 a
	Car-99	30	12.9 a	0.66 a
	Cau-99	30	13.2 a	1.33 a
	Total - Razem:	**120**	**12.8 A**	**0.87 A**
II	Cau-99	20	10.0 a	0.30 a
	Car-99	20	11.6 a	0.35 a
	Car-00	20	11.7 a	0.10 a
	Total - Razem:	**60**	**11.1 B**	**0.25 B**
III	Car-00	**20**	**7.5 C**	**0 C**
IV	Car-00	**20**	**12.9 A**	**0 C**
V	Car-00	**10**	**7.4 C**	**0.40 B**

Table 4

N. apis infection of workers attending honeybee queens in cages purchased from 5 queen breeding apiaries. - Stopień porażenia sporami *N. apis* robotnic towarzyszących matkom w klateczkach wysyłkowych pochodzących z 5 pasiek hodowlanych.

Apiary Pasieka	Queen series Seria matek	No. of workers Liczba robotnic		Average no. spores in infected worker (x10^6 ±SE) Średnia liczba spor u chorych robotnic (x10^6 ±SE)
		Examined Badanych	Infected Zarażonych	
I	Car-98	300	12 a	15.58 ±6.97 a
	Car-99	150	4 a	11.02 ±2.99 a
	Cau-99	150	32 b	2.02 ±0.97 b
	Total - Razem:	**600**	**48**	**6.16 ±2.02**
II	Cau-99	100	7 a	3.13 ±1.53 a
	Car-99	100	5 a	17.28 ±14.09 a
	Car-00	100	15 a	4.09 ±1.96 a
	Total - Razem:	**300**	**27**	**6.28 ±2.83**
III	Car-00	**100**	**9**	**5.71 ±1.74**
IV	Car-00	**100**	**12**	**0.86 ±0.45**
V	Car-00	**50**	**5**	**0.50 ±0.26**

DISCUSSION

Nosema disease is widespread all over the world, particularly in temperate climates (Bailey and Ball 1991, Fries 1997). The proportion of infected queens found in southern Poland was lower than that found elsewhere. However, the presence of infected queens suggests that health control of breeding apiaries should be better. Feeding with fumagillin reduces *N. apis* infection but cannot eliminate it completely (Oertel 1967, Shimanuki et al. 1973). It is suggested that *A. m. caucasica* is more susceptible to *N. apis* infection than *A. m. carnica* or *A. m. mellifera* (Gromisz and Bobrzecki 1984). I did not find differences in the proportion of infected queens of different subspecies. However, workers of *A. m. caucasica* were more infected than those of *A. m. carnica*. The differences may have been related to the time of purchase. It is known that the intensity of nosema disease changes during the year (Taber and Lee 1973, Pickard and El-Shelmy 1989).

It is very important to know whether a queen is infected with *N. apis* spores. The standard way of diagnosing is to count *N. apis* spores in homogenized abdomen or midgut. The queen has to be killed before analysis. L'Arrivee and Hrytsak (1964) suggested an alternative method allowing diagnosis of the live queen, involving feces analysis. I used a similar method. The results suggest that analysis of feces is not as precise as the analysis of midguts. Possibly the queens were too young, the parasite did not finish its life cycle, and was not present yet in the feces. Feces analysis in older queens is much more effective (Bobrzecki 1975).

Most queen injuries occur during the last stage of the breeding process, when they are stored in colonies and prepared for shipment (Woyke et al. 1956, Jay 1967, Jasiński 1987, 1995, Woyke 1988, Jasiński and Fliszkiewicz 1995, 1998). Although breeders are obliged to control the quality of queens produced, it has been found that over 13% of the queens are

injured when they arrive at the customer. This can be caused either by inadequate quality control before shipment or by injury during transport. Legs are more often injured than antennas or wings (Jasiński 1987, 1995). This was confirmed in the present study.

Injured queens or queens infected with *N. apis* are often superseded (Furgala 1962, Jay 1967, Loskotova et al. 1980, Woyke 1988). Usually this happens immediately after introduction (Furgala 1962, Jay 1967). It is suggested to store queens in cages outside the colony in order to minimize the risk of injury (Jasiński 1995), but this makes the infection with *N. apis* more likely (Bailey and Ball 1991). To minimize the risk of infection the queens should be attended by workers that have emerged in an incubator (Gregorc et al. 1992, Loskotova et al. 1980). Because there is no effective drug for nosema disease, hygiene in the apiary is very important. The distribution of queens infected with *N. apis* can be an important way of spreading this parasite, so the health of queen breeding apiaries should be monitored regularly.

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JAKOŚĆ MATEK PSZCZELICH (*Apis mellifera* L.) DOSTĘPNYCH W OBROCIE HANDLOWYM, W POŁUDNIOWEJ POLSCE

K. Czekońska

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

W latach 1998 - 2000, 230 matek pszczelich rasy *Apis mellifera caucasica* i *A. m. carnica*, zakupionych w 5 uznanych pasiekach hodowlanych poddano ocenie jakościowej. Matki badano pod względem występowania uszkodzeń upośledzających ich zdolność ruchową oraz oceniano stopień porażenia sporami *Nosema apis*. Diagnozowanie choroby sporowcowej u matek prowadzono metodą badania kału żywych osobników oraz tradycyjną metodą badania zawiesiny powstałej po roztarciu jelita środkowego pobranego z martwego osobnika. Stopień porażenia robotnic sporami *N. apis* oceniano metodą tradycyjną na podstawie badania jelita środkowego.

Zdyskwalifikowano, z uwagi na uszkodzenia i obecność martwych osobników, 34 matki (14,8%). Uszkodzenia występowały głównie na odnóżach (27 matek - 11,7%), rzadziej czułkach (4 matki - 1,7%). Nie stwierdzono uszkodzeń skrzydeł. Spory *N. apis* stwierdzono u 17 matek (7,4%) i 101 robotnic (8,8%). Posługując się metodą badania kału młodych matek stwierdzono istotnie mniej zarażonych osobników w porównaniu do liczby zarażonych matek badanych metodą tradycyjną. W sumie z powodu śmiertelności, uszkodzeń i porażenia sporami *N. apis* 47 matek (20,4%) nie nadawało się do dalszego użytkowania.

Słowa kluczowe: *Apis mellifera*, *Nosema apis,* uszkodzenia matki, robotnice.

AN ATTEMPT AT OVERWINTERING STING - CLIPPED QUEENS IN MULTIPLE-QUEEN COLONIES

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S u m m a r y

Groups of 2 to 6 queens with clipped sting tips were introduced to queenless colonies at the beginning of September or in October. Thus elimination of the queens was dependent on the workers behaviour. Control single-queen colonies were maintained. Dadant hives with 6 to 8 frames and movable bottom boards were used to control dead bees and queens. Until March and April respectively 33% and 25% of the multiple-queen colonies were maintained successfully. The most promising results of the multiple-queen overwintering were achieved when 3 queens per colony were introduced in early October at the end of the feeding period. The method used did not cause queenlessness or colony losses. 25% to 11% of the surplus queens survived until early March and April, respectively (31.1% - 13.8% of the successfully introduced queens). Significantly more workers died in the multiple-queen colonies. In these colonies problems with the formation of the winter clusters were also observed. The highest losses of surplus queens occurred during the feeding period, winter cluster formation, spring flights and colony spring build-up, - that are the periods of increased colony activity. If the queens were „of a very equal status" non-of them were eliminated even under unfavourable conditions.

Keywords: multiple-queens, overwintering, bee behaviour, queen, clipped sting.

INTRODUCTION

Queen losses are one of the problems of overwintering in honeybees and beekeepers often lack a few extra queens in early spring. Thus studies were performed on overwintering of single (or multiple) queens in different types of small units or queen banks, but usually not under natural conditions (Dietz at al. 1983, Gary at al. 1967, Wborn 1990). All these methods, however, require extra equipment, extra work and also cause some health problems of the bees. Based on our former works (Paleolog 1990) on the behaviour of multiple queens, being kept together in cage tests and in multiple-queen colonies, we decided to study the possibilities of overwintering of the multiple queens, this time in normal colonies under natural conditions. The result of these studies could also widen our knowledge about relations between queens and workers, which could be also important for the commercial queen rearing (Laidlaw 1981, Woyke 1988).

METHODS

Five experiments were carried out in 1994-97. In each of the experiments young, egg-laying queens with clipped tips of stings, which had emerged in the same season, were used. Usage of insemination equipment allowed only blunting (less than 1 mm) the stings. To establish multiple-queen colonies successfully, groups of queens „at the similar status" (Szabo 1977) should be created. Therefore only queens with no visible damages were selected. These queens were also weighed and sorted to obtain groups of queens of a similar

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Table 1

Main assumptions of all experiments. - Główne założenia wszystkich eksperymentów.

	Colonies and queens number Liczba rodzin i matek	Start	Relations between queens Stosunki między matkami
Exp. 1	16 colonies with 2 queens a col. 16 rodzin po 2 matki w każdej	2-08.09.94	Habituated before studies Zaprzyjaźnione przed dośw.
Exp. 2	3 colonies with 4, 5, 6 queens 3 rodziny z 4, 5, 6 matek	5-15.10.95	Habituated before studies Zaprzyjaźnione przed dośw.
Exp. 3	2 colonies with 6 queens a col. 2 rodziny z 6 matkami w każdej	5-15.10.96	Habituated before studies Zaprzyjaźnione przed dośw.
Exp. 4	4 colonies with 3 queens a col. 4 rodziny z 3 matkami w każdej	2-10.10.97	Not habituated Nie zaprzyjaźnione
Exp. 5	3 colonies with 3 queens a col. 3 rodziny po 3 matki w każdej	5-15.10.97	Not habituated Nie zaprzyjaźnione

weight since workers usually prefer queens which are heavier (Hoog 1983a, b, Paleolog 1990). The Dadant hives with movable bottom boards containing synthetic colonies of which occupied 6 - 8 frames were used. Dead bees were counted every two weeks from November to March and dead queens were looked for. The hive entrances were covered with queen excluders, but colonies were opened when necessary to allow bees to fly. To establish a multiple-queen colony each of the multiple-queen groups (all queens together) was introduced into a queenless colony under an isolator made of screen (15 cm x 15 cm), which was pressed into a comb with emerging brood and honey (Laidlaw 1981). Next, the queens were kept together with young, just emerging bees under the isolator for 8 days to get a colony odour and thus to be more acceptable for workers. After that the isolator was removed.

Multiple-queens colonies (table 1) with 2 queens were set up in experiment 1 at the first decade of September, before winter feeding period. In return, in experiments 2 and 3 the multiple queen-colonies implicated 4 to 6 queens were established, but this time in October. Before being introduced each multiple-queen group in experiments 1, 2, 3 was kept together in the separate cage, thus the queens were habituated to each other already while the multiple-queen colonies were created. It means that they did not show any aggressive behaviour, did not avoid physical contacts and even clustered. In experiments 4 and 5 multiple-queen colonies were also created in October, but each of the groups of queens being introduced consisted of only 3 queens. The queens, unlike those used in experiments 1, 2, 3 originated from single-queen mating nucs because this time it was decided to habituate them to each other during the introduction period, when they were kept under isolators. Such procedure should make the multiple-queens introduction easy and less time-consuming. Thus, in experiments 2, 3 and experiments 4, 5 different numbers of queens per colony and different methods of their introduction were applied.

RESULTS

Results of the overwintering of the multiple-queens in all experiments are shown in table 2. Because of different time of the beginning of experiment 1 as opposed to all remaining experiments the results of this experiment will be analysed separately.

Table 2

Results of the overvintering of the multiple-queen colonies in experiments 1, 2, 3, 4, 5. - Wyniki zimowli rodzin wielomatecznych w eksperymentach 1, 2, 3, 4, 5.

	Autumn - Jesień		March (to 10-th \ to 30-th) - Marzec (do 10 \ do 30)	
	MQ	SuQ	MQ	SuQ
Exp. 1	16(12)	16 (12)	1 \ 1	1 \ 1
Exp. 2	3 (3)	12 (9)	1 \ 0	2 \ 0
Exp. 3	2 (2)	10 (9)	1 \ 1	3 \ 1
Exp. 4	4 (4)	8 (6)	1 \ 1	2 \ 1
Exp. 5	3 (3)	6 (5)	1 \ 1	2 \ 2

() - number of the multiple-queen colonies and number of the surplus queens 8 days after the queens introduction was shown in brackets; **MQ** - number of the multiple queen colonies; **SuQ** - number of the surplus queens.
() - liczbę rodzin wielomatecznych i liczbę dodatkowych matek w osiem dni po poddaniu matek podano w nawiasach; **MQ** - liczba rodzin wielomatecznych; **SuQ** - liczba dodatkowych matek.

Table 3

The queen loses in experiments 2, 3 and 4, 5 expressed as numbers (n), as percentage of the introduced queens (pi) and as percentage of the queens accepted 8 days after their introduction (pa). - Straty matek w eksperymentach 2, 3 i 4, 5 wyrażone w liczbach (n), jako procent poddanych matek (pi) i jako procent matek przyjętych w 8 dni po ich poddaniu (pa).

		Losses during introduction Straty podczas poddawania				Losses during overwintering Straty podczas zimowli			
		Introduced Poddane		Accepted Przyjęte		March Marzec		April Kwiecień	
		All	Surp.	All	Surp.	All	Surp.	All	Surp.
Exp. 2 + 3	N	27	22	23	18	10	5	6	1
	Pi	100	100	85.2	81.8	37.0	18.5	22.2	4.5
	Pa					43.5	21.7	27.3	5.5
Exp. 3 + 4	N	21	14	18	11	11	4	10	3
	Pi	100	100	85.7	78.6	52.4	28.6	47.6	21.4
	Pa					61.1	36.4	55.7	27.3
Total	N	48	36	41	29	21	9	16	4
	Pi	100	100	85.4	80.6	43.8	25.0	33.3	11.1
	Pa					51.2	31.1	39.0	13.8

All - All introduced queens (Wszystkie poddane matki); **Surp.** - The surplus queens (Dodatkowe matki)

In experiment 1, 75% of double-queen colonies were still functioning 8 days after the queen introduction but in the remaining 25% worker bees eliminated one queen of the pair. Thus during the introduction period 25% of the surplus queens (12.5% of the total number) were lost. At the time of the first spring flight (first days of March)

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Table 4a

Number of the double-queen colonies (DQ) and number of eliminated queens (EQ) in Experiment 1. - Liczba rodzin dwumatecznych (DQ) i liczba wyeliminowanych matek (EQ) w Eksperymencie 1.

	2-8 Sept. * 2-8. 09. *	8-days	10-15 Dec. 10-15. 12.	14 Feb. 14. 02.	7 Mar. 7. 02.	20 Apr. 20. 04.
DQ	16	12	2	2	1	1
EQ	-	4	14	14	15	15

* - Introduction of the queens (poddanie matek);
8-days - 8 days after the queens introduction (8 dni po poddaniu matek).

Table 4b

Number of queens in each of the colonies (see also tab. 1). Liczba matek w każdej z rodzin (patrz też tab. 1).

		To-15 Oct.* Do 15.10	8-days	20-th Nov. 20.11.	15-th Jan. 15.01.	15-th Feb. 15.02.	5-12 Mar. 5-12.03.	20-th Apr. 20.04.
Exp. 2	QpC	5/6/4	5/4/3	4/3/1	4/3/1	4/3/1	3/1/1	1/1/1
	EQ	-	3	4	0	0	3	2
Exp. 3	QpC	6/6	6/5	5/1	5/1	5/1	4/1	2/1
	EQ	-	1	5	0	0	1	2
Exp. 4	QpC	3/3/3/3	3/3/2/2	3/1/2/1	3/1/2/1	3/1/2/1	3/1/1/1	2/1/1/1
	EQ	-	2	3	0	0	1	1
Exp. 5	QpC	3/3/3	3/3/2	3/2/1	3/2/1	3/1/1	3/1/1	3/1/1
	EQ	-	1	2	0	1	0	0
Total EQ		-	7	14	0	1	5	5
Total MQ		12	12	7	7	6	4	3

*, **8-days, EQ** - see explanations table 4a (patrz objaśnienia tabela 4a); **QpC**- number of queens per colony (liczba matek w rodzinie); **Total MQ** - total number of the multiple-queen colonies (łączna liczba rodzin wielomatecznych); **Total EQ** - total number of the eliminated queens (łączna liczba wyeliminowanych matek).

only one double-queen colony was still functioning. Then in April one of the queens from the colony was introduced to a queenless nucleus to set up a normal colony. No queenless colonies were obtained in this experiment. Because 15 out of 16 surplus queens were lost in experiment 1 in the subsequent experiments we decided to introduce more than 2 queens into a colony and not in September but in October, after the feeding period (see Discussion).

In experiments 2, 3, 4 and 5 (table 3 and also table 2) 85.4% of the 48 totally used queens was successfully introduced and 19.4% of the surplus queens were lost during the introduction period (8 days), but 100% of the multiple-queen colonies were maintained. In experiments 2, 3 18.2 % and in experiments 4, 5 21.4% of the surplus

queens were lost during the introduction period. Thus, different methods of habituation and introduction of the multiple-queen groups applied in experiments 2, 3 and 4, 5 gave almost the same results (χ^2=0.0164; χ^2_1=0.0001 and χ^2_2=3.841 for P>0.05 and P<0.95). After one month, (see also Table 4b), seven multiple-queen colonies remained and successfully formed winter clusters out of the twelve (58.3%) originally established in experiments 2, 3, 4 and 5. These results were more promising than those obtained in experiment 1.

At the beginning of March (table 2, 3) 4 multiple-queen colonies out of 12 were still functioning with 9 surplus queens out of the 29 (31.1%) accepted and 36 introduced (25%) in experiments 2, 3, 4 and 5. Altogether 43.8% of the introduced and 51.2% of the accepted queens survived until March. Until April 3 multiple-queen colonies were maintained (25%). The colonies showed very good spring build up. From these colonies 4 surplus queens (13.8% of the accepted and 11.1% of the introduced) were successfully introduced into new queenless colonies. It is worth noticing that in some cells of the combs more than one egg a cell was observed and that no queenless colonies were obtained.

An important question is when most of the surplus queens were eliminated and how multiple-queens affected workers. In experiment 1 (table 4a) most of the surplus queens were eliminated in early autumn and when feeding was over only two double-queen colonies could be found. No queens were lost during the winter but worker bees eliminated one of the surplus queens in early spring during the first flights. In experiments 2, 3, 4 and 5 (tables 3, 4b) 48% of the surplus queens was eliminated in autumn and during winter cluster formation. About 58% of the multiple-queen colonies successfully formed winter clusters in November. The results were better (χ^2= 6.642; χ^2_1=6.635 for P<0.01) than those obtained in experiment 1. Only one surplus queen was lost during winter but at the time of the first spring flights 17.3% of the successfully introduced surplus queens were lost. Subsequently, during the spring colony build-up 17.3% of the surplus queens were eliminated. More queens were lost in the colonies with 4 - 6 queens than in the colonies with 3 queens (table 3). This finding was not confirmed statistically but it seems that

Table 5

Mean number of dead bees in the multiple-queen (MQ) and single-queen (SQ) colonies during overwintering. - Średnia liczba osypanych podczas zimowli pszczół w rodzinach wielomatecznych (MQ) i jednomatecznych (SQ).

Periods of Overwintering Okresy zimowli	Exp. 3			Exp. 4			Exp. 5		
	MQ		SQ	MQ		SQ	MQ		SQ
15 Oct. - 5 Jan.	508	>	295	447	>	191	449	=	375
5 Jan. - 15 Feb.	403	=	316	296	=	302	580	>	218
15 Feb. - 25 Mar.	199	=	124	178	>	108	98	=	103
Total / Razem	1110	>	735	953	>	601	1127	>	696

The difference is statistically significant (>) or insignificant (=) for P>0,01. Strength of the colonies was 5-7 Dadant frames clustered by bees.
Różnica jest statystycznie istotna (>) albo nieistotna (=) dla P>0,01. Siła rodzin wynosiła 5-7 ramek obsiadanych przez pszczoły.

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it was only because the data basis was too small.

In experiments 3, 4, 5 control groups were maintained, each consisting of five single-queen colonies. Each colony which was able to form a winter cluster including more than one queen was treated as the multiple-queen colony. More worker bees (table 5) died in the multiple-queen colonies during winter. None of the three periods of the overwintering appeared particularly unfavourable either for the single-queen or for the multiple-queen colonies. When colonies were opened (even for a very short period of time) in October and November one could observe that the multiple-queen colonies had more troubles with the winter cluster formation than the control single-queen ones. This phenomenon was particularly visible in the colonies with more than 3 queens (experiment 2, 3) where also more surplus queens were eliminated.

DISCUSSION

Results of the introduction of the multiple-queens into queenless colonies were quite good and queen losses were only a little higher than during the single queen introduction (Chuda-Mickiewicz 1998). Szabo (1977) was able to introduce successfully 21% to 93% of the multiple queens in different experiments but he examined the multiple-queens colonies 24-h after the queen introduction, whereas in our experiments colonies were examined 8 days after the introduction. He also did not clip the stings but introduced 16 individually caged queens per colony. In our experiments (excluding experiment 1) it was possible to maintain respectively until early March and April 33% and 25% of the multiple-queen colonies with 31% and 13.8% of the successfully introduced surplus queens. The only problem was that queens designed for the multiple-queen introduction should be kept in the mating nucs until late autumn. In other experiments performed in small units, queen banks or confined systems 20% to 60% of the queens survived (Dietz at al 1983, Ried 1975, Wborn 1990). Results of experiments 4 and 5 showed that in order to establish multiple queen-colonies using groups of queens which were kept together their introduction (and therefore habituated each other) is not necessary. Those queens can be habituated to each other during the period after their introduction, when they are kept together under isolator. In our experiments elimination of the queens was solely dependent on the behaviour of the workers, not on queen fighting one another (clipped stings). The main problem in maintaining the multiple-queen colonies (or queen banks) is just the acceptance of queens by the workers (Yadava and Smith 1971, Woyke 1988). Aggression of the workers towards the queen may be related to their defence mechanism (Laidlaw 1981, Yadava and Smith 1971). During the feeding period worker bees show the disposition to rob and the open brood is in a hive. It could shift the colony needs in the direction of defence and cause difficulties in the acceptance of the queens (Nelson and Gary 1983, Szabo 1977). This phenomenon probably caused the high queen loses in experiment 1. Therefore, in experiments 2, 3, 4, 5 it was decided to establish multiple-queen colonies in October after feeding termination when queens stopped egg laying and there was only a little sealed brood in the colonies. One can also presumed that when one of the two queens stopped its egg laying earlier, or simply did not fulfilled the colony needs promptly (Hoog 1983b, Szabo 1977, Yadava and Smith 1971), it was eliminated by workers. Therefore, it was also decided to introduce more than two queens into a colony. We hoped that it should be more difficult for worker bees to choose „the best queen" in the colony containing several queens. Results of experi-

ments 2, 3, 4, and 5 showed that the risk to loose supplemental queens increases with the activity of the colony (winter cluster formation, beginning of the spring egg laying, first spring flights, spring build-up). Egg laying is one of the most important components of „a queen status" (Laidlaw 1981, Nelson and Gary 1983, Szabo 1977). Therefore beginning of egg laying in spring could make a difference in the status of the overwintering queens and in their acceptance by bees. In consequence worker bees (which increase their activity and begin a nursing behaviour) could be stimulated to eliminate some of the surplus queens. As the flight activity increases additional queen loses may be initiated (Paleolog 1990). Worker bees can detect differences between queens (queen status) better if circumstances are unfavourable (Chuda-Mickiewicz 1998, Nelson and Gary 1983, Yadava and Smith 1971), but our experiments showed that if queens were of a very equal status almost non of them would be eliminated under unfavourable conditions.

Other researchers found out that multiple queens were usually functioning „in the different corners of the hive" (Hoog 1983a, b) which could be based on a footprint pheromone signal. In spite of these findings in the present experiments a group of queens was frequently observed together on one comb when colonies were opened in September, October, March and April. The level of infestation by Nosema apis and Varroa jacobsoni was low. No differences in the health status of the multiple- and single-queens colonies were observed. No infections or reactions of queens due to the clipping of stings were noticed. Thus these factors should not have significantly influenced the queen status (Nelson and Gary 1983) or the multiple-queens overvintering (Muszyńska 1989).

In multiple-queen colonies overvintering was probably more difficult for worker bees and probably for that, they reduced the number of queens to 3 per colony. Therefore, in case of winter losses of workers and queens establishing multiple-queen colonies with not more than 3 queens should be recommended. The higher worker losses in the multiple-queen colonies did not disturb overwintering and spring build up in the colonies. Coasts of producing so many surplus queens do not allow applying our method commercially. On the other hand, beekeepers sometimes have got some extra queens at the end of the season and therefore it is possible to use them to establish a few multiple-queen colonies without additional hazards.

CONCLUSIONS

1. It was possible overwinter surplus queens successfully in the multiple-queen colonies but the queen losses were high.
2. The highest loses of surplus queens occurred during the periods of the increased colonies activity.

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PRÓBA PRZEZIMOWANIA MATEK Z PRZYCIĘTYMI ŻĄDŁAMI W RODZINACH WIELOMATECZNYCH

J. Paleolog

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

W 5 eksperymentach badano możliwości przezimowania rodzin wielomatecznych. W pierwszej dekadzie września albo w październiku grupy 2 - 6 matek z przyciętymi końcówkami żądeł poddawano do bezmatków. Eliminowanie matek zależało więc od zachowań robotnic. W 3 eksperymentach utrzymywano kontrolne rodziny jednomateczne aby porównać wielkość osypu i zachowanie się pszczół w rodzinach jedno- i wielomatecznych. Roje zimowano w ulach Dadanta z ruchomą wkładką dennicową na 6-8 ramkach. Osyp badano (wraz z szukaniem spadłych matek) co dwa tygodnie. Poddając matki w grupach wielomatecznych uzyskano 12,5% do 14,6% strat. Do marca i kwietnia udało się utrzymać odpowiednio 33% i 25% rodzin wielomatecznych zawierających 31% i 13,8% skutecznie wprowadzonych w jesieni dodatkowych matek. Najlepsze rezultaty uzyskano tworząc rodziny wielomateczne późną jesienią (w październiku a nie wrześniu) pod koniec okresu karmienia i poddając 3 - 4 matki (nie 2 ani 6). Zimowanie rodzin wielomatecznych nie powodowało bezmateczności ani strat rodzin. Utrudnieniem była konieczność przetrzymywania matek w ulikach do późnej jesieni. Ryzyko utraty matek wzrastało w okresach zwiększonej aktywności rodzin (podkarmianie, formowanie kłębu zimowego, wiosenne obloty i rozwój wiosenny), co wynikać mogło zarówno ze wzrostu aktywności robotnic jak i zmiany statusu matek. Pomimo, że robotnice lepiej wykrywają różnice w statusie matek w warunkach niesprzyjających, gdy zadbano aby status matek był podobny niewiele z nich nie zostało straconych nawet w złych warunkach. W porównaniu do kontroli więcej robotnic (31% - 39%) osypało się w rodzinach wielomatecznych, gdzie również obserwowano kłopoty z formowaniem się kłębu zimowego. Porażenie rodzin wielo- i jedno-matecznych przez *Nosema apis* i *Varroa jacobsoni* było podobne i nieznaczne.

Słowa kluczowe: zimowla, zachowanie pszczół, matki, przycięte żądła, wielomateczność.

ATTEMPT TO DEVELOP AN ASSORTMENT OF HERBACEOUS HONEY-PRODUCING PLANTS TO BE USED FOR THE IMPROVEMENT OF BEE PASTURES ON IDLE LANDS

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S u m m a r y

An attempt was made to choose from herbaceous honey plants those which after being sown or planted on a pre-plant cultivated, poor sandy soil are able to persist untended for several years and to provide nectar and pollen forage for bees. Out of 20 tested perennial species 12 were fairly tolerant of competition from weeds: *Cichorium intybus*, *Hypericum perforatum*, *Anchusa officinalis*, *Lathyrus silvester*, *Nepeta nuda*, *Lotus corniculatus*, *Eryngium planum*, *Solidago serotina*, *Echinops commutatus*, *Salvia verticillata*, *Sanquisorba minor*, *Asclepias syriaca*. Of 10 biennial plants 4 were able to compete with weeds: *Centaurea rhenana*, *Cynoglossum officinale*, *Echinops sphaerocephalus*, *Dipsacus silvester*. Of 5 annual species only one, *Impatiens glandulifera*, showed some ability to renew itself from self-seeding on uncultivated soil.

The best sowing season is early spring or late autumn, and mixtures are the best seeding practice. The sowing rates should ensure at least several dozen plants per 1 m^2 with the assumption that every tenth seed produces a plant. On average, untended plants can produce 10-30 (50%) of the flow produced by dense stands of cultivated plants.

Keywords: honey plants, bee forage, improvement of bee forage.

INTRODUCTION

The staff of the Apiculture Division is approached by people from different parts of Poland who seek advice on which honey plants can be sown or planted on different free patches of land, mostly light, on which farming has recently become unprofitable. The beekeepers insist that the plants should be high honey yielders and that, once sown, they should persist on a given site without any special care, which means that they should effectively compete with local non-honey yielding vegetation.

This study is an attempt to screen good honey yielding herbaceous plants (annuals, biennials and perennials) for species that could be successfully grown on non-cultivable lands to enrich honey flows and to improve a forage flow for wild bee-like pollinator insects.

MATERIAL AND METHODS

The experiments were set up in a mid-forest field on a light sandy soil rated as class V and VI. Previously, the field had been ploughed every year and sown to *Phacelia tanacaetifolia*, *Sinapis alba* or *Fagopyrum esculentum*, mainly as forage for bees and also to produce some seed. The field had been pre-plant fertilized with minimal rates of an NPK fertilizer and the mustard crop was topdressed with small rates of nitrate. Weeds had not been managed so the field had been infested to a substantial

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degree with *Agropyron repens* L., *Echinochloa crus-galli* (L.) P.B., *Setaria glauca* (L.) P.B. and *Setaria viridis* (L.) P.B. Weeds of minor importance included *Erigeron canadensis* L., *Stellaria media* Vill., *Spergula arvensis* L., *Raphanus raphanistrum* L., *Papaver rhoeas* L., *Centaurea cyanus* L. and others. Immediately before the trial the field was sown to phacelia. After harvest the crop residues were turned under in August and deep plowing was done in autumn. Before the plowing the field was broadcast-fertilized with 80-100 kg/ha of an NPK fertilizer.

Included in the study were 35 plant species which had a very good, good or average record of beekeeping value and which could be reasonably expected to meet the requirements as stated above. They comprised 5 annuals, 10 biennials and 20 perennials listed in Table 1.

The plants were sown as monocultures on plots 10 m^2 in size and as mixtures (with a roughly equal proportion of component seeds) on plots of 30 m^2. The sowing was done over 3 years (1995-1997) in early spring (as early as practicable) and in late autumn (before the ground became frozen). The seeds were sown broadcast by hand and turned in with a light harrow or a rake with widely spaced tines. The seeding rates were applied at a guess so that a good vegetation cover could be obtained with only a few percent of seeds growing into plants.

Due to unforeseen circumstances the spring seeding was done four times (in the years 1995, 1996, 1998, 2000) and the autumn seeding only twice (in 1995 and 1999). In addition, not all tested species could be sown on every date because of the scarcity of seeds. However, what could be accomplished should be enough to make at least a preliminary survey of the problem.

As suggested by the beekeepers, once the plots were sown no cultural practices were performed on them to find out how the plants would cope with the aggression from weeds and how long they would persist and provide bee forage. Records were taken of plant emergence, degree of weed infestation, competition of emerged plants with local weeds, population density of flowering plants on the plots, presence of bees on the flowers. The data were in part listed in tables and in part discussed in the text only as some of them were difficult to be tabulated.

WEATHER CONDITIONS DURING THE STUDY

The emergence of plants from the seeds sown either in early spring or in late autumn is much dependent on soil moisture in April and in May and soil moisture obviously depends on rainfall. The total rainfall in April of 1995 was 50 mm, in May 36 mm and in 1996 - 12 mm and 108 mm, respectively. In 1997 the respective values were 37mm and 59 mm, 77 mm and 66 mm in 1998, 99 mm and 37 mm in 1999, and 69 mm and 40 mm in 2000. In 2000, scant rainfall in April immediately after sowing followed by virtually no rains in the 1st and the 2nd decade of May were not conducive to good plant emergence on light sandy soils. In the preceding years, the rainfall conditions were medium-good with respect to plant emergence.

RESULTS

Generally, the emergence of the large majority of species was good or very good both from early spring and from late autumn seeding (Table 1). It is only in 2000 that the drought caused not only the failure to emerge of the plants from the early spring sowing but also the death of seedlings from the sowing in late autumn of 1999. In the wake of rains that came only in the third decade of May there was a prolific emergence of weeds, especially of monocots (*Echinochloa* and *Stipa sp.*)

which quickly covered the ground and gave no chance for the test plants to grow. Left to be analyzed were the plots seeded in the spring of 1995, 1996, and 1999 and those seeded in the autumn of 1995.

Of the annuals included in the trial four species (*Centaurea cyanus*, *Phacelia tanacaetifolia*, *Melilotus albus* and *Echium creticum*) flowered very well in the first year and were abundantly visited by honeybees, bumblebees and various solitary bees. In the subsequent years it is actually only weeds that thrived on the plots whereas the experiment plants were missing save for single individuals even in the case of *Centaurea cyanus*. The annual form of *Melilotus albus* emerged very poorly in the first place both when seeded in the spring and in the autumn. It is only in the first year that some flowering individuals of melilot occurred only to disappear completely and to be replaced by local weeds in the subsequent years. *Impatiens glandulifera* was found to be superior to *Melilotus albus* in this respect. The plants of that species grew to small size but persisted, albeit not very abundantly, on the plots in the subsequent years which indicates that they perpetuated themselves by self-seeding. Probably, when grown on sites with adequate moisture, the species was to some extent capable of withstanding the competition from weeds.

In the subsequent years on all fields planted to annuals there was a massive appearance of *Oenothera biennis*, which had spread from an adjacent non-cultivated strip of land. Alongside with *Oenothera biennis*, *Solidago serotina* also started to appear on experiment plots.

Of the biennial plants *Centaurea rhenana*, *Echium vulgare*, *Cynoglossum officinale*, *Echinops sphaerocephalus*, *Verbascum tapsiforme*, and *Dipsacus silvester* performed well or fairly well in the second year. On the plots planted to the remaining four species (*Melilotus albus*, *Reseda luteola*, *Leonurus sibiricus* and *Malva silvestris*) the populations declined to only few flowering individuals. In the third year, flowering plants were rare. Sporadic individuals found in the fourth and the fifth year probably originated from self-seeding. In later years the plots planted to *Centaurea rhenana*, *Echinops sphaerocephalus* and to *Cynoglossum officinale* and, to a lesser extent, those planted to *Dipsacus silvester* and to *Echium vulgare* contained the most abundant populations of flowering individuals. All plots became dominated by *Oenothera biennis* and by *Erigeron acer* as well as by *Agropyron repens*. There also appeared self-seeded plants of *Solidago serotina*, which had spread from the adjacent wood's edge.

Of 20 perennials tested 12 persisted on the lots fairly well. They included *Cichorium intybus*, *Hypericum perforatum*, *Anchusa officinalis*, *Lathyrus silvester*, *Nepeta nuda*, *Lotus corniculatus*, *Sanquisorba minor*, *Eryngium planum*, *Solidago serotina*, *Echinops commutatus*, *Salvia verticillata* and *Asclepias syriaca*. Actually, *Asclepias syriaca* produced the first flowering shoots only in the fourth year after planting but it is likely to establish itself and spread in the years to come. The remaining species (*Agastache anethiodora*, *Agastache rugosa*, *Trifolium hybridum*, *Gypsophila paniculata*, *Reseda lutea*, *Leonurus cardiaca*, *Sisymbrium polymorphum*, and *Lavatera thuringiaca*) produced from a dozen to several dozen flowering plants on a plot. *Trifolium hybridum* and *Sisymbrium polymorphum* turned out to be little persistent as flowering plants were difficult to find among the local weeds as early as in the third year. Much like plots planted to annuals and biennials perennial plots were also invaded by *Oenothera biennis* and the two species of *Erigeron*. Obviously, those weeds were more abundant on plots planted to more vulnerable species.

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Table 1

The results of germination of plants sown on two dates: spring and autumn of 1995-1999. - Wyniki oceny wschodów roślin wysiewanych w dwóch terminach: wiosennym i jesiennym, w latach 1995-1999.

Species of plant Gatunek rośliny	Spring sowing Siewy wiosenne			Autumn sowing Siewy jesienne	
	1995	1996	1998	1995	1999
Annual plants - Rośliny roczne					
Centaurea cyanus L. Chaber bławatek	v.good	poor	av.	v.good	poor
Phacelia tanacaetifolia Benth. Facelia błękitna	v.good	v.good	v.good	good	av.
Impatiens glandulifera Royle Niecierpek Roylego	-	-	poor	good	poor
Melilotus albus Med. Nostrzyk biały	poor	-	poor	-	good
Echium creticum S.S. Żmijowiec grecki	-	v.good	-	-	-
Biennial plants - Rośliny dwuletnie					
Centaurea rhenana Bor. Chaber nadreński	v.good	v.good	v.good	v.good	good
Verbascum tapsiforme Schrad. Dziewanna wielkokwiatowa	-	-	av.	-	-
Melilotus albus Med. Nostrzyk biały	-	-	poor	-	av.
Cynoglossum officinale L. Ostrzeń pospolity	good	v.good	v.good	v.good	good
Echinops sphaerocephalus L. Przegorzan kulisty	v.good	v.good	good	v.good	-
Reseda luteola L. Rezeda żółtawa	poor	poor	poor	av.	-
Leonurus sibiricus L. Serdecznik syberyjski	-	-	av.	-	good
Dipsacus silvester Huds. Szczeć leśna	v.good	v.good	good	v.good	-
Malva silvestris L. Ślaz dziki	poor	v.good	av.	poor	poor
Echium vulgare L. Żmijowiec zwyczajny	v.good	good	good	v.good	good
Perennials - Byliny					
Cichorium intybus L. Cykoria podróżnik	-	-	good	-	v.good
Hypericum perforatum L. Dziurawiec zwyczajny	good	-	good	v.good	-
Anchusa officinalis L. Farbownik lekarski	v.good	v.good	v.good	v.good	v.good

Species of plant Gatunek rośliny	Spring sowing Siewy wiosenne			Autumn sowing Siewy jesienne	
	1995	1996	1998	1995	1999
Perennials - Byliny					
Lathyrus silvester L. Groszek leśny	poor	poor	poor	poor	poor
Agastache anethiodora (Purch.) Kuntze Kłosowiec fenkułowy	-	-	av.	-	good
Agastache rugosa (Fisch.) Kuntze Kłosowiec pomarszczony	-	-	av.	-	good
Nepeta nuda L. Kocimiętka naga	v.good	poor	av.	v.good	good
Lotus corniculatus L. Komonica zwyczajna	-	poor	poor	av.	-
Trifolium hybridum L. Koniczyna szwedzka	v.good	-	av.	-	av.
Sanquisorba minor Scop. Krwiściąg mniejszy	good	v.good	-	v.good	-
Gypsophila paniculata Fisch. Łyszczec wiechowaty	poor	av.	av.	v.good	poor
Eryngium planum L. Mikołajek płaskolistny	v.good	good	good	v.good	good
Solidago serotina Ait. Nawłoć późna	-	-	good	-	-
Echinops commutatus Juratz. Przegorzan węgierski	good	good	good	good	av.
Reseda lutea L. Rezeda żółta	av.	av.	poor	av.	poor
Leonurus cardiaca L. Serdecznik pospolity	-	-	av.	-	good
Sisymbrium polymorphum (Murr.) Roth. Stulisz miotłowy	poor	-	poor	good	-
Salvia verticillata L. Szałwia okręgowa	v.good	v.good	good	v.good	good
Lavatera thuringiaca L. Ślazówka turyngska	av.	v.good	poor	poor	poor
Asclepias syriaca L. Trojeść amerykańska	poor	poor	av.	good	poor

poor — weak germination - słabe wschody
av. — average germination - średnio dobre
good — good germination - dobre
v.good — very good germination - bardzo dobre

Honeybees, solitary bees and bumblebees abundantly visited all plant species under scrutiny.

DISCUSSION

The advocates of enrichments to bee forage (Demianowicz 1936, Lipiński 1982 and also Jabłoński 2000) have suggested planting of different nectar- and pol-

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len-producing species but the effects to be expected are unknown because of the scarcity of data. In principle, given the proper amount of care any, even the most discriminating, plant can be nurtured but the beekeepers insist on having some benefits with minimum inputs. They look for plants, which once sown or planted on minimum-tilled soil will, without any further cultivation, keep growing and providing forage to bees over several years. The data from this study throw some light on the subject. First of all, they furnished evidence that annuals, biennials and perennials alike vary in their ability to tolerate competition from non-honey producing weeds. They also showed which are tougher in this respect.

Among biennial plants, large-seed plants such as *Cynoglossum officinale* or *Echinops sphaerocephalus* tolerate competition from weeds better. However, the rule is not without exceptions as shown by *Centaurea rhenana* and *Oenothera biennis*. The latter species was not included in the tests. However, it spread vigorously to experimental plots from the strip of idle land between the wood and the experiment field and suppressed the experiment plants. Since *Oenothera biennis* is a source of nectar and pollen to insects (Czubacki 1998) it is worth considering as a herbaceous honey plant to be grown on sandy idle lands but it must not be ignored that once established the plant may soon dominate the field.

The amount of pollen and nectar flow from uncultivated experiment plants was not investigated. The good visitation by bees shows that the plants produced nectar and pollen abundantly but they were less numerous per unit area than when grown as a cultivated stand. Compared to dense stands of cultivated plantings the output from uncultivated stands could be estimated at 10-30(50)%.

The observations showed that on light sandy soils herbaceous honey-producing plants should be sown in the spring as early as possible. Late autumn seeding done before the freezes is just as good. If required, a single species flowering at some specified desired time can be sown but mixed plantings will certainly be more reliable. Broadcast sowing by hand is the preferred method especially when mixtures are seeded.

CONCLUSIONS

Of 20 nectar- and pollen-producing perennial plant species (planted on a pre-plant prepared poor sandy soil and left untended afterwards) only 12 - *Cichorium intybus*, *Hypericum perforatum*, *Anchusa officinalis*, *Lathyrus silvester*, *Nepeta nuda*, *Lotus corniculatus*, *Sanquisorba minor*, *Eryngium planum*, *Solidago serotina*, *Echinops commutatus*, *Salvia verticillata*, *Asclepias syriaca* - tolerated competition from weeds fairly well.

Of 10 biennial species 4 showed fairly good renewal from self-seeding on an uncultivated field: *Centaurea rhenana*, *Cynoglossum officinale*, *Echinops sphaerocephalus*, *Dipsacus silvester*.

Annual species (*Phacelia tanacaetifolia*, *Melilotus albus*, *Echium creticum* and even *Centaurea cyanus*) are unable to compete efficiently with weeds on uncultivated land. *Impatiens glandulifera* is some exception, it has fairly large seeds, emerges early in the spring and makes a fast growth.

If required, a single species flowering at some specified desired time can be sown but mixtures of different proportions will certainly be more reliable. The sowing rates should ensure at least several dozen plants per 1 m^2 with the assumption that every tenth seed produces a plant. The best sowing time is early spring or late autumn. Broadcast sowing by hand is the preferred method especially when mixtures are seeded.

On average, untended plantings can produce 10-30(50)% of the honey flow produced by dense stands of cultivated plants.

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PRÓBA OPRACOWANIA DOBORU ZIELNYCH ROŚLIN MIODODAJNYCH DO POPRAWY PASTWISK PSZCZELICH NA NIEUŻYTKACH

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

Spośród miododajnych roślin zielnych próbowano wybrać gatunki, które raz wysiane (lub wysadzone) na uprawionej, słabej, piaszczystej glebie potrafią utrzymywać się bez pielęgnacji przez szereg lat i dostarczać owadom pszczołowatym pożytku nektarowego i pyłkowego. Okazało się, że z 20 badanych gatunków bylin dość dobrze konkurencję z chwastami wytrzymywało 12: cykoria podróżnik, dziurawiec zwyczajny, farbownik lekarski, groszek leśny, kocimiętka naga, komonica zwyczajna, mikołajek płaskolistny, nawłoć późna, przegorzan węgierski, szałwia okręgowa, krwiściąg mniejszy i trojeść amerykańska. Z 10 roślin dwuletnich bardziej zdolne konkurować z chwastami były 4: chaber nadreński, ostrzeń pospolity, przegorzan kulisty i szczeć leśna. Z 5 badanych gatunków rocznych tylko jeden (niecierpek Roylego) wykazywał pewne zdolności odnawiania się z samosiewu na nie uprawianej glebie.

Najlepszą porą siewu jest wczesna wiosna lub późna jesień, a formą uprawy - mieszanki. Ilość wysiewu powinna zapewnić przynajmniej kilkadziesiąt roślin na 1 m^2, przy założeniu, że najwyżej co 10 nasienie wyda roślinę. Średnio udane rośliny nie pielęgnowane po zasiewie mogą zapewnić 10-30(50)% tej wielkości pożytku, jakiej dostarczają ich zwarte łany pielęgnowane.

Słowa kluczowe: rośliny miododajne, pożytek pszczeli, poprawa pożytków.

NECTAR SECRETION AND HONEY POTENTIAL OF HONEY-PLANTS GROWING UNDER POLAND'S CONDITIONS

Part XII

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S u m m a r y

Flowering, nectar production and attractiveness to bees of 10 herbaceous plant species was investigated in Puławy in 1991-2000. The plants grew in a honey plant garden on a light podsolic soil. Routine screening methods were used.

The amount of sugars in nectar from 10 flowers was 10-12 mg in *Malva silvestris*, *Salvia pratensis* and in *Anchusa azurea*, 4-5 mg in *Archangelica officinalis*, *Agastache anetiodora* and in *Agastache rugosa*, 3.5 mg in *Agastache nepetoides* and in *Polygonum cuspidatum*, 1 mg in *Malva crispa*, 0.5 mg in *Cichorium intybus*.

Sugar yield from 1 ha of a closed stand was measured at 500-600 kg for *Agastache sp.*, 200 kg for *Polygonum cuspidatum*, 140 (150) kg for *Archangelica officinalis*, *Anchusa azurea*, *Malva silvestris* and *Salvia pratensis*, 30 kg for *Cichorium intybus* and 10 kg for *Malva crispa*.

Malva crispa was least attractive to bees with an average density of less than 1 foraging bee per 1 m^2. The most attractive forage were *Agastache sp*. and *Polygonum cuspidatum* (50-60 foraging bees per 1 m^2). Bees did not collect pollen from *Malva silvestris, M. crispa* and from *Polygonum cuspidatum*.

Keywords: honey-producing plants, blooming, nectar production, attractiveness to bees.

INTRODUCTION

The present paper is the twelfth part in a succession of studies started in the 1950's by Professor Zofia Demianowicz (1960). The studies are aimed at gaining a better knowledge of the abundance of blooming, nectar production and attractiveness to bees of the major honey-producing plants of Poland.

MATERIAL AND METHODS

The paper covered 10 herbaceous species the majority of which have not been investigated. Seven of them (*Cichorium intybus*, *Anchusa azurea*, *Agastache anetiodora*, *A. rugosa*, *A. nepetoides*, *Polygonum cuspidatum*, *Salvia pratensis*) are perennials, and three (*Archangelica officinalis*, *Malva crispa* and *M. silvestris*,) are biennials. In Poland, *Archangelica officinalis*, *Cichorium intybus*, *Malva crispa*, *M. silvestris* and *Salvia pratensis* occur in nature and are also grown as medicinal plants, *Cichorium intybus* is also grown for food industry. *Polygonum cuspidatum* (an ornamental) and *Malva crispa* (a fodder crop) are native to East Asia and frequently occur in Poland as escapees from cultivation. *Anchusa azurea*, a species native to southern Europe, is an ornamental rarely grown in Poland.

Agastache come from North America (*Agastache anetiodora* and *A. nepetoides*) and from East Asia (*A. rugosa*). They have been studied for their honey-producing potential in the United States (Ayers and Widrlechner 1994a, 1994b, Pellet 1947) and for production of essential oils (Nykänen et al. 1989, Fuentes--Granados et al. 2000). In Russia, *Agastache* (probably *A. rugosa* called „lofant") is recommended as an extremely valuable honey-producing and medicinal plant. In Poland as a rule, the mentioned hyssop species can only be found in honey plant gardens of some beekeepers.

Records of blooming and foraging by bees were taken using known and currently universally applied methods (Jabłoński and Kołtowski 1996). Nectar production was assessed by the well-known pipette method (Jabłoński and Szklanowska 1979).

RESULTS

Blooming. The screened plants bloomed from mid-May to mid-September (Table 1). The earliest species to bloom was *Salvia pratensis* (on average from 16.05 to 14.06) to be followed in succession by *Archangelica officinalis* (25.05-13.06), *Anchusa azurea* (26.05-29.06), *Agastache anetiodora* (14.06-2.08), *Cichorium intybus* (26.06-22.08), *Malva crispa* (28.06-23.07), *Malva silvestris* (30.06-9.08), *Agastache rugosa* (1.07-14.08), *Agastache nepetoides* (26.07-1.09) and *Polygonum cuspidatum* (27.08-20.09). The length of blooming period was on average ca. 3 weeks for *Archangelica officinalis*, *Polygonum cuspidatum* and *Malva crispa*, 4 weeks for *Anchusa azurea* and *Salvia pratensis*, 5 weeks for *Agastache nepetoides*, 6 weeks for *Agastache rugosa* and *Malva silvestris*, and 7 weeks for *Cichorium intybus* and *Agastache anetiodora*.

The number of flowers produced per unit area of an averagely dense plant stand varied from species to species and from year to year: *Anchusa azurea*, *Malva crispa, M. silvestris* and *Salvia pratensis* produced 10-12 (8-18) thousand flowers on 1 m^2. The flower numbers for the other species were over 30 (20-50) thousand for *Archangelica officinalis*, 50-60 (30-90) thousand for *Cichorium intybus* and *Polygonum cuspidatum*, 90 (50-150) thousand for *Agastache rugosa*, 130-140 (100-180) thousand for *Agastache anetiodora* and *A. nepetoides*.

Foraging. Honeybees and wild bee-like insects (bumblebees and solitary bees) visited the screened plant species. The bees foraged as a rule all day with the peak at mid-day but at different intensities. *Malva crispa* was the least frequently visited species with only few foraging honeybees. Densities of less than a dozen foraging bees per 1 m^2 in peak (morning) flight hours were recorded for *Cichorium intybus* plants. *Anchusa azurea and Salvia pratensis* were visited a little more abundantly. The flowers of the two last species attracted bumblebees and they outnumbered honeybees. On 1 m^2 of blooming *Malva silvestris* there were 6-8(10) foraging bees at a time with an occasional appearance made by a bumblebee. Bees abundantly visited the remaining species (*Archangelica officinalis*, *Agastache sp*. and *Polygonum cuspidatum*) with 20-30(50) foraging insects per 1 m^2 of the blooming plant stand. The flowers of *Agastache sp*. attracted mainly honeybees and bumblebees, and those of *Archangelica officinalis* were mostly visited by honeybees although bumblebees and solitary bees (mostly *Andrena sp*.) were also recorded. The flowers of *Polygonum cuspidatum* were foraged exclusively by honeybees collecting nectar. No insects with pollen loads were recorded on that plant as same as on *Malva sp*. Pollen was collected by bees from the flowers of the remaining 8 species

and the pollen loads were either white or yellowish (*Archangelica officinalis*).

Nectar secretion. The percentage of sugars in the nectar of the investigated plant species varied widely, mostly from 30(40) to 50-60(70)% (Table 1).

The small flowers of *Cichorium intybus* (15-20 flowers in one head) secreted the smallest amounts of nectar, 10 flowers producing on average 0.5 (0.3-0.6) mg sugars. *Malva crispa* was little superior in this respect with an average secretion rate from 10 flowers of 1.1 (0.7-1.6) mg sugars. *Archangelica officinalis, Polygonum cuspidatum* and *Agastache sp*. yielded 3-4 mg sugars in nectar from 10 flowers, *Anchusa azurea*, *Malva silvestris* and *Salvia pratensis* yielded 10-12 mg sugars.

The differences in nectar secretion rate from year to year were generally small. Only for *Malva crispa* and for *Agastache nepetoides* they exceeded 100% whereas for the majority of species they fell within 40-60(70)% and for *Salvia pratensis* they were as little as 15%.

Honey potential. Nectar secreted by all flowers on a unit area (e.g. 1 ha) of bee forage and calculated to honey containing 80% sugars (i.e. equivalent to actual sugar content of honey) is the so-called honey potential of a plant. In order to calculate honey potential, sugar potential must be calculated first. The sugar potential (and honey potential) is not only influenced by the rate of nectar secretion in individual flowers but also by the number of flowers. It can be seen from Table 1 that the sugar potential of an averagely good stand of *Malva crispa* was ca. 10 kg of sugars, that of *Cichorium intybus* - ca. 30 kg, *Anchusa azurea* and *Malva silvestris* - ca. 130 kg, *Archangelica officinalis* and *Salvia pratensis* - ca. 150 kg, *Polygonum cuspidatum* - over 200 kg and *Agastache sp*. - 400-600 kg. If increased by 25% the values become equal to honey potential.

General honey potential divided by the duration of blooming period gives an average daily honey potential. Actual daily amounts of honey are lower than the average in the initial and the final stage of blooming and higher than the average at full blooming. The full blooming is the most important period. To get the best estimate of the actual daily honey potential in that period the general honey potential is divided by the number of days that is equal to 75% of the total length of the blooming period. Thus estimated daily honey potential from 1 ha of forage is 0.7 kg for *Cichorium intybus* and *Malva crispa*, 6(7) kg for *Anchusa azurea* and *Malva silvestris*, 10-15 kg for *Archangelica officinalis*, *Agastache rugosa*, *Polygonum cuspidatum* and *Salvia pratensis*, 20 kg for *Agastache anetiodora* and *A. nepetoides*.

DISCUSSION

The data on honey potential of *Agastache anetiodora* (ca. 600 kg/ha) and *Agastache rugosa* (ca. 400 kg/ha) are in agreement with those obtained earlier (Jabłoński 1986, 1990). Included in the study for the first time *Agastache nepetoides* does not depart in this respect from other representatives of that genus. It was noticed merely that in Poland condition when seeded directly in the field it does not always emerge well and shows more traits of a biennial than of a perennial.

Malva silvestris showed honey potential similar to that of an earlier investigated *Malva mauritiana* - a southern variety (Jabłoński and Kołtowski 1993). *Malva silvestris* and *Malva crispa* furnished further evidence that honeybees do not collect pollen from plants of the *Malvaceae* family.

There are no counterparts in the literature of the remaining species included in the study for the data to be compared. Generally, it can be stated that the honey potential of *Polygonum cuspidatum* may be

Table 1

Time and length of blooming, nectar secretion and sugar potential of 10 species of plants investigated in Puławy in 1991-2000.
Pora i długość kwitnienia oraz nektarowanie i wydajność miodowa 10 gatunków roślin badanych w Puławach w latach 1991-2000.

Plant species Gatunek rośliny	Year of study Rok badań	Blooming period Pora kwitnienia	Number of plants (or sprouts) per 1 m^2 Liczba roślin (lub pędów) na 1 m^2	Number of flowers per 1 m^2 Liczba kwiatów na 1 m^2	Number of days of nectar collection Liczba dni pobierania nektaru	Number of examined flowers Liczba przebadanych kwiatów	Concentration of sugars in nectar Koncentracja cukrów w nektarze	Amount of sugars per 10 flowers in mg Ilość cukrów z 10 kwiatów w mg		Sugar (and honey) potential in kg/ha Wydajność cukrowa (i miodowa) w kg/ha
								min - max min. - maks.	average średnio	
Archangelica officinalis Hoffm. Arcydzięgiel litwor	1996 1998 2000	5.06-20.06 26.05-13.06 17.05-6.06	2 (5) 2 (4) 2 (5)	21 250 32 824 49 564	3 14 3	90 420 90	46 - 54 17 - 55 21 - 70	3.4 - 5.3 1.8 - 5.7 3.6 - 6.1	4.46 3.29 4.92	95 (118) 108 (135) 244 (305)
Cichorium intybus L Cykoria podróżnik	1998 1999 2000	12.07-20.08 24.06-10.08 21.06-5.09	9 (39) 25 (75) 22 (114)	68 037 55 000 34 672	6 6 6	360 540 540	27 - 52 33 - 50 21 - 46	0.3 - 1.0 0.3 - 0.8 0.2 - 0.5	0.54 0.57 0.34	37 (46) 31 (39) 12 (15)
Anchusa azurea L Farbownik lazurowy	1998 1999 2000	22.05-30.06 3.06-3.07 23.05-25.06	4 (14) 4 (16) 4 (18)	10 934 10 576 16 236	6 6 6	180 180 180	33 - 50 31 - 43 31 - 60	6.8 - 16.4 4.9 - 14.9 6.2 - 15.0	10.46 9.47 11.63	114 (143) 100 (125) 189 (236)
Agastache anetiodora (Pursh.) Kuntze Kłosowiec fenkułowy	1997 1998 1999 2000	19.06-8.08 10.06-25.07 18.06-30.07 8.06-5.08	71 (142) 25 (153) 62 (120) 43 (64)	150 125 164 628 138 662 90 368	8 7 6 6	240 210 180 180	33 - 57 45 - 70 67 - 73 42 - 73	1.7 - 5.5 3.6 - 8.1 4.4 - 6.7 2.7 - 5.4	3.49 5.70 5.58 3.58	524 (655) 938 (1173) 774 (967) 324 (405)
Agastache nepetoides (L.) Kuntze Kłosowiec olbrzymi	1994 1997 1998 1999	25.07-1.09 28.07-26.08 23.07-5.09 29.07-30.08	4 (11) 4 (17) 6 (13) 6 (16)	163 448 183 210 108 022 120 348	6 6 7 7	180 180 210 210	28 - 70 45 - 65 31 - 69 42 - 57	2.1 - 5.3 2.7 - 5.3 1.9 - 3.8 3.4 - 6.0	3.07 4.23 2.83 4.44	502 (627) 775 (969) 306 (382) 534 (668)

Agastache rugosa	1997	9.07-27.08	64 (118)	149 575	6	180	28 - 69	1.9 - 5.5	3.43	513 (641)
Kuntze	1998	2.07-5.08	95 (125)	94 858	6	180	44 - 56	1.9 - 5.3	3.29	312 (390)
Kłosowiec	1999	5.07-15.08	80 (80)	85 192	6	180	28 - 70	2.7 - 5.3	4.20	358 (447)
pomarszczony	2000	18.06-10.08	10 (28)	47 852	6	180	54 - 61	4.2 - 8.8	6.71	378 (473)
Polygonum cuspidatum	1998	1.09-24.09	? (4)	46 550	6	360	30 - 56	3.8 - 5.0	4.27	199 (248)
Sieb. et Zucc.	1999	25.08-15.09	? (3)	50 697	9	540	34 - 50	0.9 - 7.4	3.19	162 (202)
Rdest ostrokończysty	2000	23.08-20.09	? (2)	95 200	6	540	33 - 56	2.1 - 3.6	2.98	284 (355)
Malva crispa L.	1991	1.07-21.07	126 (128)	11 172	6	180	21 - 71	0.6 - 1.9	1.55	17 (21)
Ślaz kędzierzawy	1999	20.06-20.07	155 (155)	12 400	8	240	30 - 70	0.6 - 1.0	0.68	8 (10)
	2000	3.07-28.07	144 (146)	9 650	6	180	24 - 69	0.3 - 1.1	0.96	9 (12)
Malva silvestris L.	1989	26.06-5.08	135 (135)	8 100	7	210	45 - 66	6.3 - 26.6	15.44	125 (156)
Ślaz dziki	1992	7.07-10.08	6 (31)	9 200	7	210	24 - 71	9.6 - 19.2	13.93	128 (160)
	1999	28.06-13.08	5 (48)	15 190	6	180	43 - 71	6.8 - 11.7	8.83	134 (168)
Salvia pratensis L.	1998	13.05-3.06	8 (61)	10 908	8	240	35 - 72	6.9 - 20.8	10.68	116 (145)
Szałwia łąkowa	1999	22.05-12.06	6 (63)	12 096	6	180	49 - 58	5.4 - 14.2	10.35	125 (156)
	2000	12.05-28.06	5 (60)	18 285	6	180	45 - 71	4.1 - 20.7	11.87	217 (271)

similar to that of *Polygonum bistorta* or *Fagopyrum esculentum* (Jabłoński 1986 and 1990), that of *Archangelica officinalis* similar to that of *Coriandrum sativum* (Jabłoński and Kołtowski 1992), that of *Anchusa azurea* poorer than that of *Anchusa officinalis*, that of *Salvia pratensis* better than that of *Salvia verticillata* (Demianowicz et al. 1963). The fact, that bees collect only nectar from the flowers of *Polygonum cuspidatum* needs to be explained.

CONCLUSIONS

With respect to the rate of sugar secretion in the nectar of 10 flowers the species can be arranged in the following order (from highest to lowest nectar producers): *Malva silvestris*, *Salvia pratensis* and *Anchusa azurea* 10-12 mg, *Archangelica officinalis*, *Agastache anetiodora* and *Agastache rugosa* 4-5 mg, *Agastache nepetoides* and *Polygonum cuspidatum* ca. 3.5 mg, *Malva crispa* ca. 1 mg, *Cichorium intybus* ca. 0.5 mg.

Arranged in the order of decreasing sugars potential from unit area of a closed stand the line-up will be as follows: *Agastache anetiodora*, *Agastache nepetoides* and *Agastache rugosa* 500-600 kg/ha, *Polygonum cuspidatum* ca. 200 kg/ha, *Archangelica officinalis*, *Anchusa azurea*, *Malva silvestris* and *Salvia pratensis* ca. 140(150) kg/ha, *Cichorium intybus* 30 kg/ha and *Malva crispa* ca. 10 kg/ha.

It seems that the investigated species (with the exception of *Malva crispa* - a poor nectar producer and *Agastache nepetoides* - a poor performer from direct seeding) can be regarded as suitable to be planted on different idle lands in order to improve bee forage and to boost food supply to wild bee-like insects. The density of foraging bees on flowers of those species was from less than a dozen to 50-60 foraging bees per 1 m^2.

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NEKTAROWANIE I WYDAJNOŚĆ MIODOWA ROŚLIN MIODODAJNYCH W WARUNKACH POLSKI

Część XII

B. Jabłoński, Z. Kołtowski

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

W latach 1991-2000 w Puławach badano kwitnienie, nektarowanie i oblot przez pszczoły 10 gatunków roślin zielnych, które rosły w ogródku pszczelarskim na glebie bielicowej lekkiej. Posługiwano się znanymi aktualnie stosowanymi metodami.

Ilość cukrów w nektarze 10 kwiatów ślazu dzikiego, szałwii łąkowej i farbownika lazurowego wynosiła 10-12 mg, arcydzięgla litwora, kłosowca fenkułowego i pomarszczonego 4-5 mg, kłosowca olbrzymiego i rdestu ostrokończystego około 3,5 mg, ślazu kędzierzawego około 1 mg, a cykorii podróżnika 0,5 mg.

Wydajność cukrowa z powierzchni 1 ha dobrze zwartego łanu kłosowców określono na 500-600 kg, rdestu około 200 kg, arcydzięgla, farbownika, ślazu dzikiego i szałwii około 140(150) kg, cykorii około 30 kg, a ślazu kędzierzawego 10 kg.

Najsłabiej oblatywany przez pszczoły był ślaz kędzierzawy (średnie zagęszczenie poniżej 1 zbieraczki pracującej na 1 m^2), a najliczniej kłosowce i rdest (50-60 zbieraczek na 1 m^2). Ze ślazów i rdestu pszczoły nie zbierały pyłku.

Słowa kluczowe: rośliny miododajne, kwitnienie, nektarowanie, oblot przez pszczoły.

AGRONOMIC AND BEEKEEPING VALUE OF SHORT-TUBE POPULATIONS OF RED CLOVER (*Trifolium pratense* L.)

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Summary

Agronomic and beekeeping value of two short-corolla tube populations of red clover (early-maturing – Krw and late-maturing – Krp) was assessed. The two populations were obtained at the Apiculture Division, ISK, Puławy as a result of over thirty years of crossing and selection.

The two short-tube populations were found, when compared with standard cultivars, to bloom equally or more profusely, to have a corolla tube shorter by ca. 20%, a slightly higher nectar bar, 25% shorter nectar depth, slightly higher sugar concentration of nectar, 20% higher nectar secretion rate of individual flowers, 30% higher sugar potential from unit area of crop, foraging rate by honeybees higher by several times and by bumblebees by 50%, similar plant height and equal yield of green weight, slightly higher weight of 1000 seeds and seed yield higher by 20% (assuming that the plots planted to standard cultivars were also fully saturated with pollinator insects).

The short-tube populations seem to have a potential as valuable germplasm to be used in the development of short-tube cultivars that would give sufficiently good yields of herbage and yields of seed superior to those from current cultivars while at the same time providing tolerably good forage to bees.

Keywords: red clover, short-tube clover, blooming, nectar secretion, pollination, seed set, yields.

INTRODUCTION

Red clover is a cross-pollinated (self--sterile) and insect-pollinated species. Wild bees are the major insect pollinators of clover but they have long been too scarce to secure good pollination. Honeybees are reluctant foragers of red clover as they have no free access to the nectar hidden deep at the bottom of the flower tube (Gubin 1947). Even short-tongued bumblebees (*Bombus terrestris* and *B. lucorum*) do not reach the nectar the normal way but drill through the flower tube at the side and the flowers are left unpollinated. For that reason the seed yields are low and unstable varying from several dozen kg per ha (sometimes from nearly zero) to 200 (400) kg (Åkerberg and Stapel 1964, Čumakov and Husarova 1964, Jelinowska 1969, Bawolski et al. 1974, Haragsim 1977). The development of a short-tube cultivar could provide a solution to two problems at once: secure good pollination by honeybees thereby raising seed yields and enrich bee forage and honey yields.

Breeders in many countries (Klingen 1912, Lindhard 1921, Lisycyn 1921 – cited after Smaragdova 1960, Žovka 1963 – cited after Čumakov and Husarova 1964, Kress 1950, Bond and Fyfe 1968) had long been looking for red

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clover types with shortened flower tube. However, it turned out that regular selection for shortened flower tube was associated with a decline in herbage yields. Sokolovska (1958) and Smaragdova (1960) looked for short tube material in nature and found promising types but the ultimate results of that work are not known.

In Poland, Jabłoński (1974) while searching for short-tube red clover found, near Skierniewice at the early 60's, a mutant with split corolla and with substantially reduced stamens. Since then the author has crossed and screened his germplasm, though mainly as his spare time activity, for types with increased availability of nectar to honeybees (Jabłoński 1972, 1975, 1977). The material also caught the interest of the researchers at Institute of Plant Breeding and Acclimatization at Radzików (Mackiewicz and Staszewski 1973) and at the Agricultural University in Kraków (Spiss and Góral 1986, 1989, Góral and Spiss 1992).

The subject of this paper is the evaluation of the agronomic and beekeeping performance of two short-tube red clover populations developed through crossing and selection from the above mentioned split corolla mutant at the Apiculture Division, ISK, Puławy.

METHODS

Two short-tube populations (early maturing - Krw and late maturing - Krp) of two-cut type red clover were compared with two currently best performing standard cultivars i.e. two-cut type cv. Nike and one-cut type cv. Raba. Breeder's seed of the standard varieties was obtained from their respective breeders: cv. Nike from the Plant Breeding Station at Nieznanice and cv. Raba from IHAR Plant Breeding Station at Bartążek.

The nature of the plant (perennial character and the first utilization only in the second year of growth) as well as the terms of financing (three-year research grant awarded late in the season) decided about the scheme of the project: in 1998 one trial was set up on a podsolic soil to be investigated in 1999 and in 1999 two field trials were started to be investigated in 2000: one on a podsolic soil and the other on an alluvial soil. Mineral fertilization was applied pre-plant at a rate of ca. 10 kg N, 30 kg P_2O_5 and 60 kg K_2O per 1 ha.

The trials were laid down as randomized block designs with four replicates for the first trial and five replicates for the second and the third trial. A plot consisted of one row of plants 10 (12) m. long, the rows being spaced 1.5 – 2.0 m. apart depending on local conditions. Pure sowing (without a nurse crop) was done in mid-July. In order to obtain an equal plant number on each plot the plants were thinned down leaving 1 plant at every 5 cm within the row. By the onset of first autumn freezes the plants developed fine rosettes that formed green belts ca. 40 cm in width. Spring and summer cultivation was limited to 2–3 weeding operations using either a hand cultivator or a hoe.

For data collection purposes each plot was divided into three equal parts: $^1/_3$ – seeds from the 1st cut, $^1/_3$ – fresh weight from the 1st cut, seeds from the 2nd cut, $^1/_3$ – fresh weight from 1st and 2nd cut.

The fresh weight was cut at the beginning of blooming, seeds were harvested when the heads turned brown and were sufficiently hard. Freshly harvested herbage was weighed in the field. Harvested seed heads were put in a shed and allowed to dry prior to thrashing. Seed heads were thrashed with flails and the seed pods were ground through sieves to release seeds which were subsequently winnowed. During blooming the plots were passed three times a day and observed for the density of foraging pollinator insects i.e. bumblebees, solitary bees and honeybees.

In order to measure the length of the corolla tube and the height of the nectar (Fig.1) three flower heads on each plot were bagged with tulle covers three times for three days at full blooming. Subsequently, flower heads were brought to the laboratory and three florets from each head were measured using a special home-made slider (Jabłoński 1962). Ten more florets from each head were examined for the amount of nectar and sugar content thereof using a pipette method (Jabłoński and Szklanowska 1979).

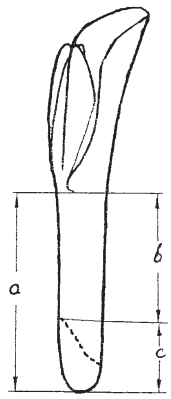

Fig. 1. Schematic diagram of red clover corolla. - Schematyczny rysunek korony kwiatowej koniczyny czerwonej.

Immediately prior to harvest the length of 1 m. of each plot was sampled for plants and flower heads were counted in the samples. Counts were made of seed pods and of florets which failed to set pods in 10 flower heads picked at random from each plot. The weight of 1000 seeds was also measured. In the study 1 m. length of a row was assumed to correspond roughly to 1 m^2 of the clover stand.

The data were subjected to ANOVA. Should the values of zero have occurred for the parameter „density of foraging honeybees per unit area of herbage" a square root conversion was made according to the formula $y \sqrt{x} \; 0.5$. Significance of differences was tested using Duncan's test at 0.05% significance level.

Weather conditions during the growing season of 1999 approximated the nation's average. However, the year 2000 was conspicuous for unusually dry spring. On the alluvial soil the drought set in that caused the plants to wilt at places.

RESULTS

Time and abundance of blooming. Red clover bloomed for ca. 4 wks. or slightly longer. The first cut of the standard cultivar

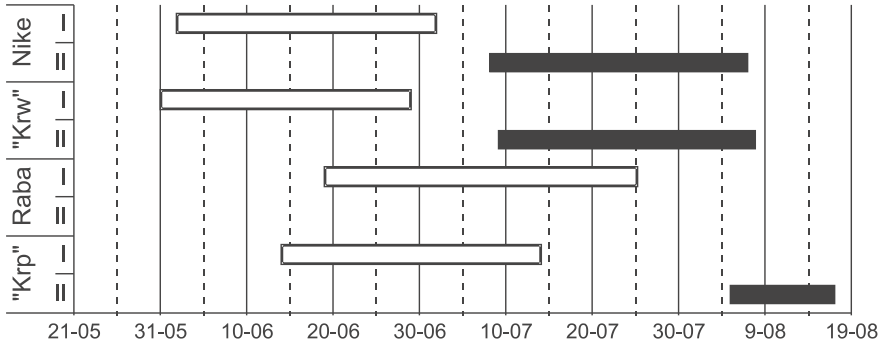

Fig. 2. Blooming time of the red clover populations averaged over the years 1999 - 2000: Krw - early short-tube population, cut I and II; Krp - late short-tube population, cut I and II. - Pora kwitnienia badanych populacji koniczyny czerwonej (na podstawie średnich z lat 1999-2000): "Krw" - populacja krótkorurkowa wczesna, odrost I i II, "Krp" - populacja krótkorurkowa późna, odrost I i II.

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Nike and that of the early short-tube population (Krw) occurred in June, the second cut came about from 10th July to 10th August (Fig.2).

The single-cut cv. Raba bloomed from June 19 to July 25 and the late short tube population Krp bloomed 5 days earlier. The second cut of Krp came to flower only around August 5, which was too late for the seeds to ripen. The poor second cut of cv. Raba produced only leaf rosettes. It must be added that with the dry spring of 2000 the first growth of clover bloomed more than 10 days earlier than it did in 1999 and the second regrowth bloomed only a few days earlier.

The number of heads per 1 m^2 produced by the tested clover populations varied from

Table 1

Blooming abundance of investigated populations of red clover.
Obfitość kwitnienia badanych populacji koniczyny czerwonej.

Cultivar Odmiana	Cut Odrost	Year of study - Lata badań			Average Średnio	Percentage of standard Procent wzorca
		1999 A	2000 A	2000 B		
Number of heads per 1 m^2 - Liczba główek na 1 m^2 łanu						
Nike	I	526 a-c	645 a	639 a	603 a	100
	II	318 a	644 a	721 a	561 a	100
Krw	I	680 c	942 b	735 a	786 b	130
	II	401 ab	677 a	698 a	592 a	106
Raba	I	629 bc	875 b	841 a	781 b	100
Krp	I	499 a-c	898 b	721 a	706 ab	90
Number of flowers per head - Liczba kwiatów w główce						
Nike	I	101 a	110 b	93 a	101 a	100
	II	119 b	113 b	106 bc	113 bc	100
Krw	I	98 a	111 b	100 ab	103 ab	102
	II	119 b	119 b	114 c	117 c	104
Raba	I	99 a	96 a	103 ab	99 a	100
Krp	I	120 b	114 b	106 bc	113 bc	114
Number of flowers per 1 m^2 in 10^3 - Liczba kwiatów na 1 m^2 w tys.						
Nike	I	53.3 ab	71.0 a	59.6 a	61.3 a	100
	II	38.1 a	72.9 a	76.7 ab	62.4 a	100
Krw	I	66.6 b	104.3 b	73.8 ab	81.6 b	133
	II	47.6 ab	80.2 a	79.1 ab	69.0 ab	111
Raba	I	62.1 ab	79.2 a	83.0 b	74.8 ab	100
Krp	I	59.9 ab	99.1 b	76.2 ab	78.4 ab	105

„Krw" – early-maturing short-tube population – populacja krótkorurkowa wczesna
„Krp" – late-maturing short-tube population – populacja krótkorurkowa późna
A – experiment on podsolic soil – doświadczenie na bielicy
B – experiment on alluvial soil – doświadczenie na madzie

300 to ca. 900 (Table 1). The number of heads per unit area produced by Krw was similar to or even higher (cut I) than that of the standard cv. Nike. On the other hand, although blooming as profusely as Krw Krp tended to produce slightly fewer heads per unit area than the single-cut cv. Raba.

The number of florets per head averaged 100 or slightly more. Both short-tube populations showed a weak tendency to produce slightly more florets per head.

The number of florets per 1 m^2 varied from ca. 40 thousand to ca. 100 thousand. In the sunny and warm year 2000 clover flowered a little more profusely than it did in the weather-wise average year 1999. Compared to cv. Nike Krw produced significantly more florets per unit area from both cut I and cut II.

Length of corolla tube. The mean length of the corolla tube of the standard cultivars was ca. 9 mm or slightly more

Table 2

Length of corolla tube and depth to the nectar level for investigated populations of red clover. - Długość rurki kwiatowej i głębokość ukrycia nektaru u badanych populacji koniczyny czerwonej.

Cultivar Odmiana	Cut Odrost	Year of study - Lata badań			Average Średnio	Percentage of standard Procent wzorca
		1999 A	2000 A	2000 B		
Length of corolla tube in mm - Długość rurki korony w mm						
Nike	I	9.17 d	9.88 e	9.37 c	9.47 b	100
	II	8.85 c	9.46 d	9.50 c	9.27 b	100
Krw	I	6.90 a	7.50 b	7.42 a	7.27 a	77
	II	6.81 a	7.26 a	7.40 a	7.16 a	77
Raba	I	8.97 cd	8.97 c	9.03 b	8.99 b	100
Krp	I	7.23 b	7.32 ab	7.31 a	7.29 a	81
Height of nectar column in mm - Wysokość słupka nektaru w mm						
Nike	I	1.37 ab	1.62 a	1.66 ab	1.55 ab	100
	II	1.50 bc	1.75 ab	1.78 bc	1.68 a-c	100
Krw	I	1.35 a	1.98 c	1.92 cd	1.75 bc	113
	II	1.49 bc	1.88 bc	2.03 d	1.80 c	107
Raba	I	1.27 a	1.60 a	1.54 a	1.47 a	100
Krp	I	1.56 c	1.67 a	1.67 ab	1.63 a-c	111
Depth to the nectar level in mm - Głębokość do poziomu nektaru w mm						
Nike	I	7.73 d	8.25 d	7.64 c	7.87 c	100
	II	7.55 c	7.84 c	7.72 c	7.70 bc	100
Krw	I	5.55 b	5.52 a	5.50 ab	5.52 a	70
	II	5.32 a	5.38 a	5.37 a	5.36 a	70
Raba	I	7.70 d	7.37 b	7.49 c	7.52 b	100
Krp	I	5.67 b	5.64 a	5.63 b	5.65 a	75

For explanations see Table 1 - Objaśnienia w tab.1

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Table 3

The abundance of nectar secretion of examined red clover populations.
Obfitość nektarowania badanych populacji koniczyny czerwonej.

Cultivar Odmiana	Cut Odrost	Year of study - Lata badań			Average Średnio	Percentage of standard Procent wzorca
		1999 A	2000 A	2000 B		
Sugar concentration of nectar (%) - Koncentracja cukrów w nektarze w %						
Nike	I	38.7 a	47.4 b	39.3 a	41.8 ab	100
	II	33.3 a	41.6 a	38.3 a	37.7 a	100
Krw	I	41.5 a	47.6 b	44.9 b	44.7 bc	107
	II	37.8 a	41.4 a	39.9 a	39.7 ab	105
Raba	I	42.2 a	49.1 b	53.5 c	48.3 c	100
Krp	I	46.2 a	57.9 c	57.7 c	53.9 d	112
Weight of sugars per 10 flowers (mg) - Ilość cukrów z 10 kwiatów w mg						
Nike	I	1.86 ab	2.37 a	2.74 bc	2.32 ab	100
	II	1.29 a	2.08 a	2.24 ab	1.87 a	100
Krw	I	2.18 ab	2.52 a	3.54 d	2.75 b	119
	II	1.60 ab	2.49 a	3.06 cd	2.38 ab	127
Raba	I	1.57 ab	2.08 a	1.96 a	1.87 a	100
Krp	I	2.39 b	2.55 a	2.27 ab	2.40 ab	128
Yield of sugars per 1 ha (kg) - Wydajność cukrów w kg z 1 ha						
Nike	I	99 ab	169 a	186 a	151 ab	100
	II	49 a	153 a	178 a	127 a	100
Krw	I	144 b	263 b	260 b	222 c	147
	II	76 a	188 a	239 b	168 a-c	132
Raba	I	98 ab	165 a	167 a	143 ab	100
Krp	I	143 b	261 b	171 a	192 bc	134

For explanations see Table 1 – Objaśnienia w tab. 1.

whereas that of short tube populations was slightly over 7 mm (Fig. 3 and Table 2). The differences are ca. 20% and are highly significant.

The height of nectar was ca. 1.5 mm or slightly more. Both short-tube populations tended to have a little bit higher nectar level than the standard cultivars.

The difference between the tube length and the nectar height i.e. the distance from the upper tube rim to nectar level is very important from the standpoint of the accessibility of nectar to the honeybee. The distance or the depth of nectar level averaged from 7.5 to 7.9 mm for the standard cultivars and from 5.4 to 5.6 mm for Kr

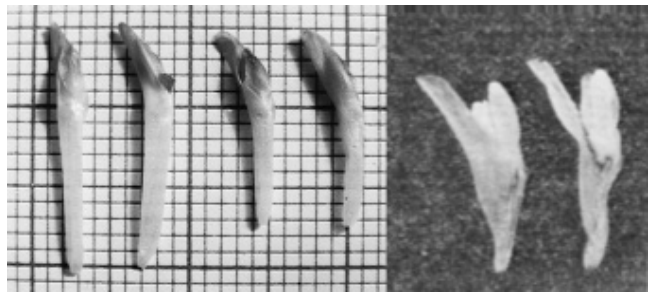

Fig. 3. The flowers of red clover: left – standard cultivar „Nike", center – short corolla tube population, right – mutant with split flower tube found in 1962. - Kwiaty koniczyny czerwonej odmiany standardowej Nike i populacji krótkorurkowej; obok kwiaty znalezionego w roku 1962 mutanta z rozszczepioną rurką kwiatową.

populations. The differences are 25 – 30% and are highly significant. The length of the proboscis of the honeybee is ca. 6 – 7 mm.

Abundance of nectar secretion. Sugar concentration of the nectar samples of the tested populations of red clover ranged from 30% to 60%, and varied most frequently from 40 to 50% (Table 3). Compared with the standard cultivars the short-tube populations tended to have a slightly higher sugar percent content of nectar.

The amount of sugars from 10 flowers varied from 1.3 mg to 3.5 mg and averaged between 1.9–2.8 mg. Compared to the standard cultivars both short-tube populations showed a markedly higher (by ca. 20%) rate of nectar secretion.

Table 4

Composition of insects (%) pollinating red clover in Puławy.
Skład procentowy owadów zapylających koniczynę czerwoną w Puławach.

Pollinating insects Owady zapylające	Year of study - Lata badań			Average Średnio
	1999 A	2000 A	2000 B	
Apis mellifera L. Pszczoła miodna	30.7	8.5	13.9	17.7
Bombus pasquorum Scop. Trzmiel rudy	37.1	42.9	48.2	42.7
Bombus lapidarius (L.) [1)] Trzmiel kamiennik [1)]	22.4	36.8	27.2	28.8
Bombus terrestris (L.) [2)] Trzmiel ziemny [2)]	9.2	8.9	7.7	8.6
Bombus sylvarum (L.) Trzmiel rudo-szary	0.5	2.8	2.9	2.1
Solitary bees [3)] Pszczoły samotnice [3)]	0.1	0.1	0.1	0.1
Total % Razem %	100.0	100.0	100.0	100.0

[1)] - + *Bombus ruderarius* Müller - + trzmiel rudonogi
[2)] - + *B. lucorum* and rarely *B. hortorum* (L.) - + trzmiel gajowy i sporadycznie ogrodowy
[3)] - mostly *Andrenidae* – głównie pszczolinki

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Table 5

Density of pollinating insects on blooming field of investigated red clover populations.
Zagęszczenie (liczba osobników) owadów zapylających na kwiatach badanych populacji koniczyny czerwonej.

Cultivar Odmiana	Cut Odrost	Year of study - Lata badań			Average Średnio	Percentage of standard Procent wzorca
		1999 A	2000 A	2000 B		
Number of honeybees per 100 m^2 of blooming field Liczba pszczół miodnych na 100 m^2 kwitnącego łanu						
Nike	I	2.2 a	0.4 a	0.0 a	0.8 a	100
	II	12.0 a	8.0 b	32.6 b	17.5 b	100
Krw	I	34.2 b	1.8 a	1.2 a	12.4 ab	1550
	II	160.9 c	67.5 c	96.2 c	108.2 c	872
Raba	I	3.8 a	0.0 a	0.6 a	1.4 a	100
Krp	I	33.0 b	0.9 a	0.0 a	11.3 ab	807
Average - Średnio		41.0	13.1	21.8	25.3	-
Number of bumblebees per 100 m^2 of blooming field Liczba trzmieli na 100 m^2 kwitnącego łanu						
Nike	I	53.1 b	174.0 c	123.2 b	116.2 a	100
	II	36.6 ab	76.0 a	67.8 a	60.1 a	100
Krw	I	98.4 c	284.0 d	190.2 c	190.9 b	163
	II	25.0 a	111.8 ab	110.0 b	82.3 a	137
Raba	I	50.9 ab	100.6 a	80.8 a	77.4 a	100
Krp	I	61.1 b	146.6 bc	138.8 b	115.2 a	149
Average - Średnio		53.9	148.8	118.5	106.8	-
Percentage of honeybees on blooming field Udział procentowy pszczół miodnych na kwiatach						
Nike	I	4.1	0.3	0.6	1.7	100
	II	24.7	10.4	32.5	22.5	100
Krw	I	25.8	1.0	0.9	9.2	541
	II	86.6	38.0	46.8	57.1	254
Raba	I	6.9	0.0	1.2	2.7	100
Krp	I	35.1	1.2	0.0	12.1	448
Average - Średnio		30.5	8.5	13.7	-	-

For explanations see Table 1 - Objaśnienia w tab. 1.

Table 6

Plant height and fresh weight yield of investigated red clover populations.
Wysokość roślin i plony zielonej masy badanych populacji koniczyny czerwonej.

Cultivar Odmiana	Cut Odrost	Year of study - Lata badań			Average Średnio	Percentage of standard Procent wzorca
		1999 A	2000 A	2000 B		
Plant height at harvest (cm) - Wysokość roślin w czasie zbioru w cm						
Nike	I	100 c	90 b	90 b	93 c	100
	II	65 b	80 b	75 b	73 bc	100
Krw	I	105 c	95 b	90 b	97 c	104
	II	65 b	85 b	80 b	77 bc	105
Raba	I	150 d	110 c	110 c	123 d	100
	II	30 a	30 a	35 a	32 a	100
Krp	I	150 d	115 c	110 c	125 d	102
	II	60 b	70 b	75 b	68 b	213
Fresh weight yield (kg) from 10 m^2 of clover stand (equivalent to t/ha) Plony zielonej masy w kg z 10 m^2 łanu (odpow. t/ha)						
Nike	I	38.2 c	31.7 c	31.5 e	33.8 cd	100
	II	18.1 b	24.8 b	25.2 cd	22.7 bc	100
Krw	I	39.9 c	32.6 c	28.0 d	33.5 cd	99
	II	18.2 b	27.0 b	24.3 bc	23.2 bc	102
Raba	I	42.1 c	36.0 c	26.3 cd	34.8 d	100
	II	3.8 a	8.2 a	14.4 a	8.8 a	100
Krp	I	38.4 c	33.8 c	27.2 d	33.1 cd	95
	II	7.9 a	12.6 a	21.8 b	14.1 ab	160

For explanations see Table 1 – Objaśnienia w tab.1

The yield of sugars in nectar from 1 ha of the crop stand is related to the abundance of nectar secretion by individual flowers and to the number of flowers per unit area. The sugar yield from 1 ha was 130–150 kg for the standard cultivars and 170 to 220 kg for the Kr populations, the latter outperforming the standard cultivars by a very significant margin of 30%.

Pollination. Among the insects foraging on the red clover populations bumblebees were the dominant group as they accounted for more than 80% of all bee-like insects (Table 4). Honeybees averaged 18% and solitary bees only 0.1% of total visiting insects. In 1999, with weather conditions close to the average, honeybees accounted for ca. 30% and with the warm and dry weather of the year 2000 for ca. 10% of the total number. *Bombus pasquorum* was consistently the most numerous (40% of all foraging insects) to be followed by *B. lapidarius* with a small admixture of *B. ruderarius* (ca. 30%). *Bombus terrestris*, *B. lucorum* and very rarely recorded *B. hortorum* accounted for 9% and

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B. sylvarum for 2% of the total number. Of other species, *B. hypnorum* was spotted once.

The density of honeybee foragers per 100 m^2 of clover stand (measured at peak visitation hours at full blooming) ranged from 0 to 160 and averaged 25 (Table 5). It was consistently several times higher on the second regrowth than on the first one. Likewise, it was several times higher on the Kr populations than on the standard cultivars.

The mean density of bumblebees per 100 m^2 of clover stand was slightly over 50 in 1999 and 2–3 times higher during the dry and sunny weather in 2000. Obviously, the high density of bumblebees on the clover stand in 2000 was the reason for the small density of honeybee foragers since the number of foraging bumblebees reached probably the so-called natural maximum density at which all the nectar and pollen flow is taken by a given group of insects. Of interest is the fact that bumblebees preferred short-tube populations and outnumbered those foraging on standard cultivars by 40–60%.

Honeybees foraging on the stands of the examined clover populations were found to be consistently more numerous in regrowth II compared to regrowth I whereas the reverse was true of bumblebees. Furthermore, honeybees accounted for a higher percentage of pollinating insects on Kr populations compared to standard cultivars. It was particularly conspicuous in the year 1999 in which the prevailing weather was more typical of this country than that of 2000. In 1999, honeybee foragers on regrowth II of Krw accounted for ca. 85% of all pollinating insects.

Yields. In 1999, the first cut of cv. Nike and Krw was made on June 7 and that of cv. Raba and Krp on June 28. In 2000, the cuts were made 1-2 weeks earlier. Likewise, dates of seed harvest were earlier in the warm and dry year 2000 than in the preceding year.

Plant height recorded at the end of full blooming was 90–150 cm for regrowth I and 60–80 cm for regrowth II (Table 6). The second regrowth of the single-cut cv. Raba reached a height of 30 cm. The height of the short-tube populations equaled that of the standard cultivars.

Fresh weight yields of all clover populations averaged 33 tons per 1 ha from cut I and ca. 22 tons per 1 ha from cut II of Nike and Krw. The second cut of Krp yielded ca 14 tons of fresh weight from 1 ha whereas the second cut of the single-cut cv. Raba yielded 9 tons/ha. The short-tube populations nearly equaled the standard cultivars with respect to fresh weight yield. Only with drought setting in on the alluvial soil in 2000 Krw gave a slightly lower yield, especially that from cut I.

The average number of seeds per head varied from 60 to 80 and did not differ much from year to year (Table 7). The Krw population did not differ in this respect from cv. Nike. However, the Krp population produced more seeds per head than did cv. Raba.

The percent seed to floret ratio varied from 60 to 80% and averaged 70%. The Krw population tended to set slightly fewer seeds from the same number of florets as compared to cv. Nike. Conversely, the Krp population showed a slightly better seed set than cv. Raba.

The weight of 1000 seeds varied from 1.5 to 1.8 g. It was slightly higher in the warm and dry year 2000 than in the weather-wise average year 1999. Compared to the standard cultivars both Kr populations showed a slight tendency to produce heavier seeds.

Seed yields varied from 200 to more than 700 kg/ha. In the warm and dry year 2000 they were twice as high as in 1999. Substantially more seeds were taken from cut I than from cut II both in standard cv. Nike and in Krw populations. There was a clear tendency for both Kr populations to

Table 7

Pod setting and seed yield of investigated red clover populations.
Zawiązywanie strąków i plony nasion badanych populacji koniczyny czerwonej.

Cultivar Odmiana	Cut (regrowth) Odrost	Year of study - Lata badań			Average Średnio	Percentage of standard Procent wzorca
		1999 A	2000 A	2000 B		
Number of seeds per head - Liczba nasion w główce						
Nike	I	74.4 bc	85.0 b	70.2 a	76.5 ab	100
	II	82.2 c	75.2 ab	82.8 bc	80.1 ab	100
Krw	I	65.6 ab	82.8 b	78.4 bc	75.6 ab	99
	II	80.0 bc	73.2 ab	85.2 c	79.5 ab	99
Raba	I	56.1 a	66.0 a	76.4 ab	66.2 a	100
Krp	I	88.4 c	77.2 ab	80.4 bc	82.0 b	124
Number of seeds per 100 flowers - Liczba nasion ze 100 kwiatów						
Nike	I	73.4 b	77.4 c	75.2 a	75.3 a	100
	II	68.8 b	66.4 a	78.0 a	71.1 a	100
Krw	I	66.8 b	74.2 bc	78.4 a	73.1 a	97
	II	67.3 b	61.9 a	75.2 a	68.1 a	96
Raba	I	56.9 a	68.4 ab	74.0 a	66.4 a	100
Krp	I	73.7 b	67.2 a	76.1 a	72.3 a	109
Weight of 1000 seeds (g) - Masa 1000 nasion w g						
Nike	I	1.58 a	1.81 c	1.78 b	1.72 cd	100
	II	1.36 a	1.48 a	1.57 a	1.47 a	100
Krw	I	1.77 a	1.89 c	1.79 b	1.82 d	106
	II	1.53 a	1.55 a	1.58 a	1.55 ab	105
Raba	I	1.45 a	1.67 b	1.78 b	1.63 bc	100
Krp	I	1.58 a	1.80 c	1.77 b	1.72 cd	106
Yield of seeds (g) per 10 m^2 (equivalent to kg/ha) Plon nasion z 10 m^2 w g (odpow. kg/ha)						
Nike	I	213 a	563 bc	566 b	447 bc	100
	II	192 a	325 a	379 a	299 a	100
Krw	I	336 b	790 d	597 b	574 c	128
	II	218 a	371 a	396 a	328 ab	110
Raba	I	327 b	532 b	568 b	476 c	100
Krp	I	337 b	690 cd	694 b	574 c	121

For explanations see Table 1- Objaśnienia w tab.1.

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produce seed yields higher than those of the standard cultivars.

DISCUSSION

The data obtained in this study seem to bear out the contention by Sokolovska (1958) and Smaragdova (1960) that there are possibilities to overcome the simple correlation between the length of corolla tube and the yield of fresh weight in red clover. That correlation has so far thwarted all breeding efforts (Kress 1950, Žovka 1963 – cited after Čumakov and Husarova 1964) aimed at the development of valuable cultivars willingly visited by honeybees.

The commonly held views and observations (Bond and Fyfe 1968) have been confirmed that the shortening of the corolla tube in red clover will facilitate access to nectar for bee-like insects and thus foraging by honeybees will be increased and pollination conditions will be improved. Of considerable interest is the fact that a shorter corolla tube makes clover flowers more attractive also to bumblebees, their density having increased by 40 to 60%. It may prove that those insects show some instinct to improve their economic performance.

The increased foraging by pollinator insects on the short-tube populations relative to that on standard cultivars could not have become manifest as an increased seed to floret ratio. It was because the density of pollinators on all plots reached the so-called maximum natural density above which a decline in the rate of seed and fruit set may even occur (Bornus et al. 1977).

The higher seed yields from short-tube populations as compared to those from the standard cultivars were the result of a slightly more abundant blooming and a slightly higher weight of 1000 seeds. On large plantings where normally bumblebees are in short supply and honeybees occur in abundance the differences in yield in favour of short-tube populations can be reasonably expected to be much larger mainly due to inadequate visitations of long-tube populations.

A surprising finding was that the short-tube populations showed sugar yields that were markedly higher than those of their standard counterparts which was attributable to a slightly higher rate of nectar secretion and more profuse flowering. During long years of selection no attention had been paid to nectar secretion rate. Rather, the expectation was that the shortening of the corolla tube would entail smaller amounts of nectar.

The short-tube populations described in this study seem to have a potential as valuable germplasm to breed short-tube cultivars for commercial use - cultivars that will be good yielders of herbage and superior yielders of seeds and at the same time will provide more forage for bees.

SUMMARY AND CONCLUSIONS

The short-tube populations of red clover investigated in this study (early-maturing Krw and late-maturing Krp) when compared with standard cultivars, were found to bloom equally or more profusely, to have a corolla tube shorter by ca. 20%, a slightly higher nectar bar, 25% shorter nectar depth, slightly higher sugar concentration of nectar, 20% higher nectar secretion rate of individual flowers, 30% higher sugar potential from unit area of crop, foraging rate by honeybees higher by several times and by bumblebees by 50%, similar plant height and equal yield of green weight, slightly higher weight of 1000 seeds and seed yield higher by 20% (assuming that the plots planted to standard cultivars were also fully saturated with pollinator insects).

The short-tube populations seem to have a potential as valuable germplasm to be used in the development of short-tube cultivars that would give sufficiently good

yields of herbage and yields of seed superior to those from current cultivars while at the same time providing tolerably good forage to bees.

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ROLNICZA I PSZCZELARSKA WARTOŚĆ KRÓTKORURKOWYCH POPULACJI KONICZYNY CZERWONEJ (*Trifolium pratense* L.)

B. Jabłoński

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

W latach 1998-2000 w Puławach oceniano rolniczą i pszczelarską wartość dwu populacji krótkorurkowych koniczyny czerwonej, które uzyskano w Oddziale Pszczelnictwa ISK w wyniku ponad 30 lat prowadzonej selekcji. Materiałem początkowym był znaleziony w roku 1962 mutant o rozszczepionej koronie kwiatowej oraz krzyżowane z nim wyszukane na polach pojedynki o nieco krótszej rurce korony.

Stwierdzono, że badane populacje krótkorurkowe (wcześniejsza – „Krw" i późniejsza – „Krp"), w porównaniu z aktualnie uprawianymi w Polsce odmianami standardowymi (Nike i Raba), wykazują średnio: podobną lub nieco zwiększoną obfitość kwitnienia, około 20% krótszą rurkę korony, nieco wyższy słupek nektaru, około 25% mniejszą głębokość ukrycia nektaru, nieznacznie wyższą koncentrację cukrów w nektarze, około 20% obfitsze nektarowanie poszczególnych kwiatów, około 30% wyższą wydajność cukrową z jednostki powierzchni uprawy, kilkakrotnie liczniejszy oblot przez pszczoły miodne i około 50% liczniejszy przez trzmiele, podobną wysokość roślin i nie mniejszy plon zielonej masy, podobny stopień zawiązywania nasion, nieznacznie większą masę 1000 nasion i około 20% wyższy plon nasion (przy pełnym nasyceniu owadami zapylającymi także poletek odmian standardowych).

Wydaje się, że badane krótkorurkowe populacje koniczyny czerwonej mogą stanowić cenny materiał wyjściowy do uzyskania przez hodowców odmian o skróconej rurce kwiatowej, dających dobre plony zielonej masy i znacznie wyższe od dotychczasowych plony nasion, a dodatkowo niezły pożytek pszczołom.

Słowa kluczowe: koniczyna czerwona, koniczyna krótkorurkowa, kwitnienie, nektarowanie, zapylanie, zawiązywanie nasion, plonowanie.

GASTRIC TOXICITY TO BEES OF FENROPATRIN – THE ACTIVE INGREDIENT OF DANITOL AND DANIRUN FORMULAS

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S u m m a r y

In the years 1997 – 2000 honeybees were tested for their sensitivity to the toxic gastric action of the formulas Danitol and Danirun. Both formulas contain fenpropathrin – an active ingredient of the pyrethroid group – Danirun also contains another active ingredient, hexythiazox – a heterocyclic compound. When administered to bees in Nissorun hexythiazox did not cause an increase in mortality rate.

Danitol and Danirun were found to be very toxic to honeybees. Average fenpropathrin doses corresponding to three mortality levels were as follows:

mortality	Danitol	Danirun
within error level	0.113 ppm	0.070 µg
up to 50%	0.182 ppm	0.092 µg
above 50%	0.205 ppm	0.161 µg

The mortality rate of bees exposed to the corresponding rates of active ingredient in those formulas varied substantially with the timing of the tests (seasonal variation) and with the provenance of bee colonies.

The toxic effects of fenpropathrin increased with decreasing ambient temperatures which was related to a decrease in phenpropathrin doses tolerated by bees (mortality within error limit).

	Danitol	Danirun
33°C	0.0026 ppm	0.0023 µg
22°C	0.0008 ppm	0.0008 µg

The active ingredient fenpropathrin when ingested in Danirun was more toxic than when ingested in Danitol, possibly because of the interaction with the other active ingredient of Danirun – hexythiazox.

Keywords: honeybee, fenpropathrin, hexythiazox, toxicity, temperature.

INTRODUCT0ION

At the beginning of the 1990's the formula Danitol 10EC was registered for use in Poland to be followed shortly by Danirun 110 EC. Both formulas share the same active ingredient – fenpropatrin – a synthetic pyrethroid. Danirun also contains a biologically active heterocyclic compound named hexythiasox which occurs in its own right in the formula Nissorun (Gromisz 1999).

Danitol and Danirun are formulas to control sucking and biting pests and mites

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in fruit and vegetable crops, herbs and ornamentals and crops grown under cover. They show high toxicity (Tkaczuk, Miętkowski 1995, Maciesiak, Makulski 1996).

The two formulas are highly toxic to honeybees (class I). They have been assigned a 6-hour waiting period which means that they can be applied to blooming insect-pollinated crops in the evening after the cessation of bee flights. Due to their high efficacy they are excellent agents to control pests of ornamental plants scattered across small built-up areas such as rows of trees or hedges. They must be managed properly to be safe for bees. In any case, the formal general guidance is included in label directions (Gromisz 1990). In the case of pyrethroids, such as Danitol and Danirun their management is further complicated by the effect of ambient temperature which may change abruptly during the day. Pyrethroids are characterised by a negative temperature coefficient (Malinowski 1982).

Danitol and Danirun are used at relatively high concentrations for pyrethroids. The effective dosage is most frequently 0.75 l/ha. In fruit crops with the spray mix applied at 750 1000 l/ha the concentration works out at 0.07-0.11%. With more advanced spraying methods which allow the amount of spray mix to be reduced to 300 – 500 l the concentration of the formula is 0.15 to 0.25% (Doruchowski 1996).

This study presents the data from laboratory tests of the sensitivity of honeybees to the gastric action of Danitol and Danirun.

METHODS

In the years 1997 – 2000 the formulas Danitol 10 EC and Danirun 110 EC were tested for their gastric toxicity to honeybees. Danitol contains 10% fenpropathrin as the active ingredient, Danirun contains 80 g fenpropathrin and 30 g hyxythiazox in 1 l. Both formulas are assigned to class I toxicity cathegory to bees and a 6-hour waiting period is imposed.

Test bees of the Caucassian and Carniolan breeds from either natural or artificial queen insemination were sampled from the apiary of the Apiculture Division, ISK. Carbon dioxide was used in portioning the samples. The storage temperature in the laboratory was 22°C, 26°C, 29°C, 33°C.

In each sample there were ca. 25 caged bees. The bees were fed the formula in sugar syrup for 24 hrs. at concentrations ranging from 0.0003 to 0.075%. The amount of dosed active ingredient was calculated by weighing the amount of ingested syrup. Every morning dead bees were counted and removed from cages until their number dropped to 25% of the starting count. The quantitative structure of test bees was made up of 19 series (test dates) and comprised a total of 129 treatments. Each treatment was assigned a defined formula concentration level including zero level for the control groups the number of which corresponded to the number of series. Each treatment was made up of 5 or 6 replications (two breeds x three samples) that corresponded to the number of sampled colonies.

The toxicity indicator was estimated as bee mortality after 72 hrs. If required natural bee loss was compensated for by using Abbot's formula

$$P = 100\ (P_o\text{-}c)\ :\ (100\text{-}c)$$

where P – adjusted mortality, P_o – actual mortality, c – mortality in the control group expressed as percentage

Duncan test at 0.05 confidence level was used to assess the significance of differences. Significantly different mean values were followed by different letters.

RESULTS

The biological action of Danitol relies on the chemical compound named fenpropathrin classified as a synthetic pyrethroid. The same compound is also included in the make up of Danirun where it is aided by the heterocyclic compound hexythiazox, the sole active ingredient of Nissorun. Nissorun was not found to affect the mortality rate of bees (Table 1). The bees ingested similar amounts of syrup with or without the addition of 0.1 – 0.15% of the formula, each bee being exposed to the amount of active ingredient ranging from 2.387 µg to 4.292 µg. Thus it can be reasonably assumed that fenpropathrin is of primary importance in the beekeeping-related assessment of Danirun, the role of hexythiazox being limited to co-action at the most. However, it means that fenpropathrin as the ingredient of Danitol and Danirun may show formulation-dependent effects with respect to doses and bee mortality rate.

In the extensive experiment material the doses of active ingredient were from 0.019 ppm to 0.548 ppm for Danitol and from 0.016 µg to 0.610 µg for Danirun, the mortality rate ranging from 0% to 100% of caged bees and only in very few cases not exceeding significance level.

Generally, both Danitol and Danirun turned out to be highly toxic to bees through stomach poisoning but the amount of ingested active ingredient was not simply related to rise in mortality. There were differences from series to series which were related, among others, to timing of tests and to provenance of bees but first of all to ambient temperature. Figures 1 and 2 show the pattern of the mortality of bees kept at four temperature ranges. A cursory observation shows that there was an increase in mortality rate with increased dose of active ingredient which is obvious. However, when viewed more closely, the pattern of variation curves describing the process shows substantial departures from such a simple scheme. Thus an active ingredient dose that was poisonous to bees under some conditions failed to be significantly hazardous under other conditions. From the beekeper's point of view, it will not be wrong to say that contamination with those formulas may entail different degrees of

Table 1

Sensitivity of bees to gastric action of Nissuron (active ingredient - hexythiazox).
Wrażliwość pszczół na działanie żołądkowe preparatu Nissuron (substancja czynna - heksytiazoks).

Series Seria	No. Of replications Liczba powtórzeń	Concentration Stężenie	Syrup ingestion mg/bee Spożycie syropu mg/pszczołę	Hexythiazox µg/bee Heksytiazoks µg/pszczołę	Bee mortality after 72 hrs. Śmiertelność pszczół po 72 godz. %
60897	6	0.1% 0	64.252 a 55.762 a	2.785	4.35 a 3.61 a
220698	5	0.1% 0	54.426 a 56.198 a	2.387	0.41 a 3.55 a
60798	5	0.15% 0	66.718 a 69.546 a	4.392	27.55 a 27.81 a
200798	5	0.15% 0	59.926 a 72.658 a	3.991	2.00 a 0.29 a

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hazard to individual apiaries and even to individual colonies due to variation in the genetic and non-genetic environment. Thus it is possible that the pesticide-contaminated nectar will be brought to the hive without any poisoning symptoms being developed by bees whereas for some other foragers such a contact will be fatal. Similar situations can be seen in the figures e.g. in Fig. 1 for the range of active ingredient of 0.030 ppm at 22°C. There is very little beekepers can do to assess their own bees in this regard as their general knowledge is not adequate to provide them with a proper key. What they can be certain of is that the ambient temperature effectively modifies the toxicity of Danitol and Danirun. There are marked differences in this respect within the range of 22°C - 33°C which was shown in the graphs by plotting the mortality rates against the amounts of active ingredient. In terms of economics as the temperature rises the costs of killing the bees with those formulas increase. The fundamental role in this reckoning is played by the amount of active ingredient taken up by the bees.

The dosage of active ingredient depends on the pesticide concentration in the syrup and on the amount of syrup ingested by bees. In principle, the concentration can be adjusted at will for experimental purposes but the amount of ingested food always depends on the appetite of bees. Their predispositions in this respect are defined by the amount of sugar syrup without added pesticide that they take up. In 19 experiment series of this study the amounts per one bee were 49.64 to 75.96 mg. Danitol or Danirun-containing syrup was ingested by bees in much smaller amounts, in an extreme case the amount was 7.3% of pesticide-free syrup.

The amount of ingested food was calculated 24 hrs. after the initial bee count in the cages. The aim was to determine how much active ingredient was dosed to the bees. Understandably, the bees that became poisoned already in the first stage had a negative impact on the balance of ingested food regardless of their future appetite should they have survived which was also possible. The relationship can be reduced to a simple scheme: increase in bee mortality suppresses syrup ingestion the more efficiently the higher it is. If this line of reasoning is pushed further on the increase in bee mortality can be attributed to pesticide concentation in the syrup. Those instrumental relationships that ultimately rest on the dosing of active ingredient can be followed in Table 2 for three mortality levels and four temperature levels. Both formulas, Danitol and Danirun, are highly hazardous to bees but the manner in which they are administered modifies their lethal action. The point is that right concentrations should be chosen that would minimize the poisoning hazard to bees. Based on the data listed in Table 2 appropriate calculations can be made. The same amount of pesticide in the syrup that can be ingested by bees *ad libitum* becomes more dangerous as its concentration increases. In conventional label directions the rule finds its practical expression as the warning against increasing pesticide concentration of the spray mix. The matter has become more complicated with new crop protection methods and it is related to the field rate of spraying solution: 700 – 1000 l/ha against 300 – 500 l/ha with new generation machinery. It means that with label rates of application of 0.75 l/ha for Danitol or 0.9 l/ha (0.75-0.9) for Danirun the following pesticide concentrations will be obtained:

	Danitol	Danirun
700-1000 l	0.75-0.11%	0.09-0.13%
300-500 l	0.15-0.25%	0.18-0.3%

Those technological improvements introduce new challenges for bee-safe application of pesticides.

Table 2

Abridged record of testing Danitol i Danirun for gastric toxicity to honey bees (syrup ingestion listed as percentage of ingestion of syrup alone). - Skrócony zapis wyników testowania szkodliwości żołądkowej preparatu Danitol i Danirun dla pszczoły miodnej (spożycie syropu podano w stosunku do spożycia bez domieszki preparatu).

Bee mortality thresholds - Progi śmiertelności pszczół	Temperature Temperatura	Concentration % Stężenie w %	Syrup ingestion % Spożycie syropu w %	fenpropathrin rate ppm Dawka fenpropatryny ppm
Danitol				
within error limits - granice błędu	22°C	0.0003-0.0014	25.4-98.2	0.019-0.030
	26°C	0.0008-0.004	48.8-93.2	0.050-0.180
	29°C	0.00225-0.0058	55.2-80.5	0.084-0.176
	33°C	0.00048-0.0057	62.4-101.0	0.021-0.200
up to 50% do 50%	22°C	0.0012-0.002	32.3-35.1	0.028-0.031
	26°C	0.0024-0.004	56.5-69.9	0.105-0.154
	29°C	0.009-0.0135	46.5-54.8	0.289-0.321
	33°C	0.004-0.011	44.0-75.9	0.185-0.367
above 50% powyżej 50%	22°C	0.0024-0.025	8.4-21.6	0.027-0.102
	26°C	0.004-0.012	24.7-36.6	0.083-0.196
	29°C	0.01-0.018	27.0-46.3	0.222-0.284
	33°C	0.017-0.06	20.8-45.6	0.272-0.548
Danirun				
within error limits - granice błędu	22°C	0.0003-0.0014	44.6-111.4	0.016-0.032
	26°C	0.0006-0.002	57.7-84.4	0.022-0.062
	29°C	0.0027-0.0032	66.8-84.4	0.050-0.100
	33°C	0.0036-0.007	66.7-85.9	0.111-0.170
up to 50% do 50%	22°C	0.002	26.0-44.1	0.020-0.043
	26°C	0.003-0.004	40.4-51.2	0.064-0.097
	29°C	0.004-0.008	41.8-58.7	0.109-0.158
	33°C	0.0053	46.8	0.122
above 50% powyżej 50%	22°C	0.0028-0.025	7.3-26.0	0.024-0.071
	26°C	0.005-0.04	7.9-27.6	0.071-0.164
	29°C	0.01-0.019	21.0-38.5	0.132-0.182
	33°C	0.0108-0.075	13.6-60.5	0.144-0.610

Listed in Table 2 are the average values of pesticide concentration in the syrup at three levels of bee mortality obtained in laboratory tests They are as follows:

mortality	Danitol	Danirun
within error limit	0.026%	0.023%
up to 50%	0.064%	0.043%
above 50%	0.083%	0.082%

In the list the temperature factor was omitted but it is still provides clear reference values to pesticide concentrations in the spraying solution that can be encountered by bees. Under field conditions it can be hoped that the concentrations of pesticides will undergo reduction before, together with nectar, they have made their way to the honey sac of the forager. However, of we abide by this hazard indicator it is a long way to the „mortality within error

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Table 3

Ingestion of pesticide-containing syrups and doses of the active ingredient fenpropathrin (as percentage of ingested no pesticide-containing syrup). - Spożycie syropu z domieszką preparatów i dawki substancji czynnej fenpropatryny (spożycie względne: w stosunku do pobieranego syropu bez domieszki preparatu).

Bee mortality rate - Poziom śmiertelności pszczół	Syrup ingestion - Spożycie syropu		Phenpropathrin ppm/bee - fenpropatryna ppm/pszczołę
	actual mg/bee - rzeczywiste mg/pszczołę	relative - względne %	
Danitol			
Within error limit Granica błędu	45.7	71.2	0.096
up to 50% do 50%	34.0	52.4	0.185
above 50% powyżej 50%	16.3	26.7	0.206
Danirun			
Within error limit Granica błędu	45.2	73.8	0.070
up to 50% do 50%	29.5	43.2	0.092
above 50% powyżej 50%	14.5	24.0	0.161

limits"cathegory when the poisoning hazard becomes minimized. In this respect Danitol seems to be safer to bees (concentration 0.0026%) than Danirun (concentration (0.0023%). That indirect assessment, in spite of active ingredient doses, is well-founded since its practicability can be proved when both formulas are used at the same concentration.

Bees ingested Danitol and Danirun--containing syrup with equal eagerness both with respect to quantity and to limitations resulting from the addition of the pesticide (Tables 2 and 3). Generally, the differences in this respect were small and became negligible at the mortality rate within the error limit (47.7 mg and 45.2 mg). However, the two formulas differed much from each other with the dose of the active ingredient, fenpropathrin, that was ingested by the bees, the ultimate risk being, after all, the same (Table 3). To obtain the kill of 50% (above the error limit) twice as much fenpropathrin was needed in Danitol (0.185 ppm) than that in Danirun (0.092 µg). The analogous ratio for the two remaining mortality levels (above 50% and within error limit) fell short of the two-fold factor but was sufficiently high (1.28 and 1.37). Thus the biological impact of fenpropathrin in Danirun is stronger than that in Danitol. It is to fenpropathrin that the lethal character is attributed regardless of the possible interactions, if any. First of all, hexythiazox, the second biologically active ingredient of Danirun, can be involved here. In this study, the rates of that ingredient were as follows:

mortality:	within error limit	0.026 µg
	up to 50%	0.034 µg
	above 50%	0.060 µg

When fed hexythiazox in Nissorun bees practically did not develop poisoning symp-

toms. In this study we do not attempt to assess its interactive role, it may well be significant or nil. At any rate, it is of little practical importance to the beekeeper. What he needs is the general knowledge about formula's toxicity and toxicity-related hazard to bees. Of practical importance is also the fact that, concentrations being equal, a higher amount of fenpropathrin is introduced to the environment with Danitol (10%) than with Danirun (8%). It may to some extent level off the differences in toxic effects of the two formulas.

The pattern of relationship between the dose of fenpropathrin and bee mortality rate in some cases departed substantially from the average values presented here. The departures can be seen in Fig. 1 and 2. They are related to genetic and non-genetic variation the sources of which have an ultimate impact on the toxicity of the formulas and on the sensitivity of bees. Within a single experiment series, with individual treatments subjected to the same pressure from impact-bearing factors, the graded effects of active ingredient doses on mortality rate are more clearly cut although they do not always follow a straight-line pattern. In order to make this relationship more evident a correlation coefficient is given. However, it relates only to those series in which the formulas were tested alongside each other:

	Danitol	Danirun
22°C	0.493	0.615
26°C	0.705	0.788
29°C	0.596	0.810
33°C	-0.046	0.571

The majority of the coefficients are not high which testifies to a substantial variation in the sensitivity of tested bees to the toxic effects of Danitol and Danirun. Among the factors responsible, the breed of the bees was of secondary importance. With one exception, within individual series there were no statistically valid differences in the number of dead bees following the exposure to the formulas. The analysis of bee longevity yielded similar results.

We endeavoured to differentiate the rates of active ingredients and were successful. However, the corresponding mortality rates were either very high (ca. 90%) or below significance level and few of them fell within the intermediate range (the majority at 33°C. The practical conclusion is that the poisoning symptoms are either very severe or they pass unnoticed. To a large extent the prevailing factor is the current condition of the bee colony and, obviously, it interacts with the amount of ingested active ingredient of the pesticide. As for the rates of active ingredient, ambient temperature is a very important factor. In table 4 are listed average doses of fenpropathrin ingested at temperatures 22°C, 26°C, 29°C i 33°C.

Table 4

Effect of ambient temperature on the relationship between fenpropathrin rates and bee mortality. - Wpływ temperatury otoczenia na związek pomiędzy dawkami fenpropatryny a śmiertelnością pszczół.

Mortality rate	Danitol (phenpropathrin ppm/bee) - (fenpropatryna w ppm/pszczołę)				Danirun (phenpropathrin ppm/bee) - (fenpropatryna w µg/pszczołę)			
	22°C	26°C	29°C	33°C	22°C	26°C	29°C	33°C
Within error limit Granica błędu	0.025	0.110	0.148	0.101	0.025	0.041	0.073	0.140
up to 50% do 50%	0.030	0.124	0.320	0.266	0.031	0.082	0.134	0.122
above 50% powyżej 50%	0.045	0.131	0.246	0.404	0.047	0.104	0.156	0.338

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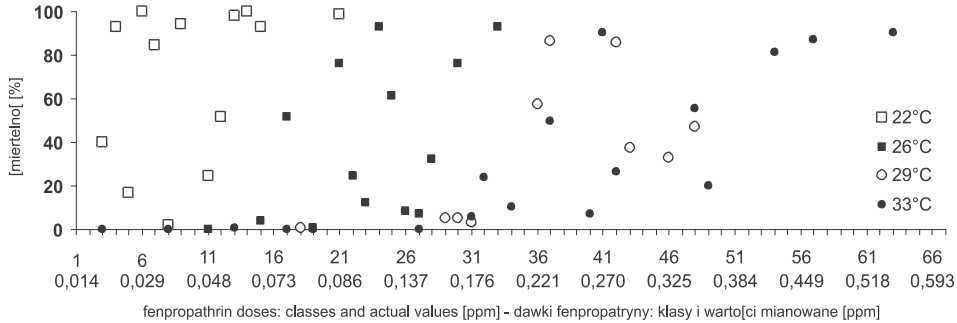
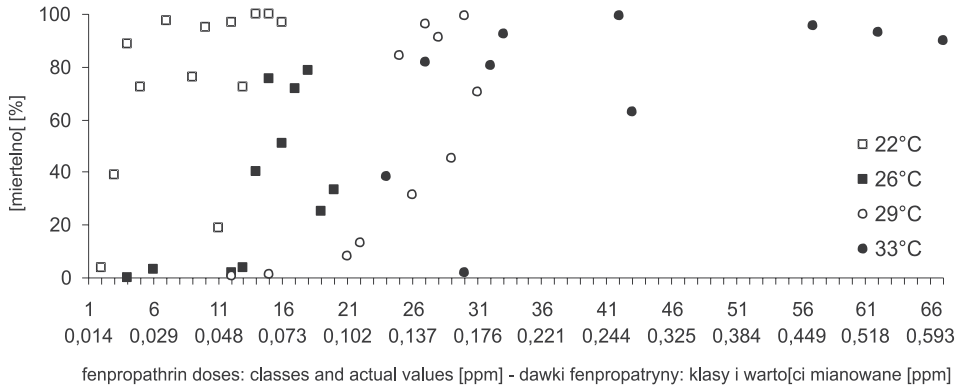


Fig. 1. Danitol: dose of the active ingredient fenpropathrin vs. mortality of bees caged in incubators at 22°C, 26°C, 29°C i 33°C (natural bee losses were eliminated), class intervals correspond to the square root of the dose (actual values in ppm/bee were given under class numbers).
Preparat Danitol: dawka substancji czynnej fenpropatryny (w klasach) i śmiertelność pszczół umieszczonych w cieplarkach utrzymujących temperaturę 22°C, 26°C, 29°C i 33°C (ubytki naturalne pszczół wyeliminowano); przedziały klasowe odpowiadają pierwiastkowi kwadratowemu dawki (wartości mianowane rzeczywiste dawki podano w ppm/pszczołę pod numerami klas).



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CONCLUSIONS

The formulas Danitol 10 EC and Danirun 110 EC are very toxic to honeybees through gastric action.

The mortality rate of bees exposed to the corresponding rates of active ingredient in those formulas varied substantially with the timing of the tests (seasonal variation) and with the provenance of bee colonies.

The toxic effects of fenpropathrin increased with decreasing ambient temperatures. Within the temperature range from 22 to 32°C it was related to a decrease in phenpropathrin doses tolerated by bees (mortality within error limit) from 0.101 ppm to 0.025 ppm for Danitol and from 0.140 μg to 0.025 μg f or Danirun.

The active ingredient fenpropathrin when ingested in Danirun was more toxic than when ingested in Danitol, possibly because of the interaction with the other active ingredient of Danirun – hexythiazox. Hexythiazox did not increase bee mortality when fed in Nissorun.

The concentration of Danitol and Danirun in food ingested by bees may be a useful indicator of poisoning hazard – a positive relationship.

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TOKSYCZNOŚĆ ŻOŁĄDKOWA DLA PSZCZÓŁ FENPROPATRYNY JAKO SKŁADNIKA CZYNNEGO PREPARATÓW DANITOL I DANIRUN

M. Gromisz

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

W latach 1997-2000 przeprowadzono testowanie wrażliwości pszczoły miodnej na toksyczne działanie żołądkowe preparatów Danitol i Danirun. Oba preparaty zawierają w

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swoim składzie fenpropatrynę, związek czynny biologicznie z grupy pyretroidów, poza tym Danirun jest wzbogacony także w drugi składnik czynny heksytiazoks, związek heterocykliczny. Heksytiazoks podany pszczołom w preparacie Nissorun nie wywoływał wzrostu śmiertelności.

Preparaty Danitol i Danirun okazały się wysoce toksyczne dla pszczoły miodnej. Przeciętne

śmiertelność	Danitol	Danirun
w granicach błędu	0,113 ppm	0,070 µg
do 50%	0,182 ppm	0,092 µg
powyżej 50%	0,205 ppm	0,161 µg

dawki fenpropatryny dla trzech poziomów śmiertelności były następujące:

Śmiertelność pszczół odpowiadająca pobieranym dawkom fenpropatryny podlegała jednak dużym wahaniom w związku z terminami testowania (zmienność sezonowa) i pochodzeniu rodzin pszczelich. Działanie toksyczne fenpropatryny zwiększa się z obniżeniem temperatury otoczenia, co się wiąże ze zmniejszeniem jej dawek tolerowanych przez pszczoły (śmiertelność

	Danitol	Danirun
33˚C	0,0026 ppm	0,0023 µg
22°C	0,0008 ppm	0,0008 µg

w granicach błędu):

Fenpropatryna podana w preparacie Danirun działa toksyczniej niż w preparacie Danitol, być może w wyniku współdziałania z drugim składnikiem czynnym, heksytiazoksem.

Słowa kluczowe: pszczoła miodna, fenpropatryna, heksytiazoks, toksyczność, temperatura.

LABORATORY ASSESSMENT OF FASTAC HAZARD TO BEES

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S u m m a r y

Fastac 10 EC is a pyrethroid insecticide containing 10% of alphamethrin as an active ingredient. It is used for pest management in crops at rates from 0.005 to 0.23 l/ha with concentrations of 0.01% to 1.0% (forestry). Tests of 307 bee samples for stomach toxicity of Fastac revealed that a) ingested amounts of alphamethrin in syrup were from 0.055 ppm to 0.287 ppm per bee, b) addition of insecticide to the syrup resulted in the death of 12.2% to 100% of bees (natural deaths were discarded), c) sensitivity of tested bee samples to the toxic effects of Fastac varied over testing dates (seasonal variation) and with different breeds, d) amounts of ingested alphamethrin were essentially restricted by rising mortality rates i.e. by sensitivity to the insecticide. As a stomach poison Fastac retained its toxic properties even one week following its application.

Keywords: honeybee, honeybee breeds, pesticides, Fastac, alphamethrin, toxicity.

INTRODUCTION

Synthetic pyrethroids take an important position among insecticides. They are characterized by a substantial biological activity against insects which allows them to be used in very small doses. Generally, pyrethroids act as strong contact and stomach insecticides. They have little selectivity and thus have a potential of being toxic to beneficial insects including honeybees as has been confirmed by many investigators who reported on the complexity of the process being the result of a specific mode of action of pyrethroid insecticides (Atkins et al. 1978, Gromisz Z., Gromisz M. 1993, Malinowski 1982, Rieth, Levin 1987, Svendsen 1983).

The action of pyrethroid insecticides is based on different active substances. There have been quite a few studies concerning their toxicity to bees. Relatively few of them were concerned with alphamethrin (Wael, Laerc 1987). It is an ingredient of Fastac - a popular plant protection agent.

Fastac is a contact and stomach insecticide. It is used to control biting and sucking pests in agricultural crops, fruit crops, vegetables, herbs, ornamentals and in forestry. In its label description it was classified as practically non-toxic to bees. An additional note said that in recommended dosages there are no restrictions regarding its application to crops in bloom.

The label statements, in themselves rather encouraging to beekepers, were borne out by the results of pilot tests run by the Apiculture Section in 1994 (Gromisz Z., Gromisz M. 1994). However, the tests revealed a marked variability of bee mortality depending on sampling date and bee provenance. The data implied that the toxic effects of Fastac to bees need not be the same in all situations. Obviously the scale of potential hazards could not be determined in that preliminary study.

This paper presents the results from laboratory tests on the contact and stomach toxicity of Fastac to bees.

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METHODS

Bee sensitivity to the toxic action of Fastac 10EC was tested in the years 1994 - 1999. Fastac is a pyrethroid insecticide with stomach and contact activity. It contains alphamethrin as a biologically active ingredient. It is used in plant pest management and is applied at rates of 0.05 to 0.23 l/ha. The concentration of the formula in the tank mix ranges from 0.01 to 1% (forest plantings). It is considered as little toxic to bees, classified as toxicity class III with 1-hour waiting period (Czarnik 1988).

For stomach toxicity tests fastac--cotaining syrup was fed to laboratory-reared bees for 24 hrs. Caged bees were kept in an incubator at a temperature of 24 - 26°C. The tests were run in 17 series (dates) which included treatments that varied for Fastac concentration according to the scheme shown in Table 1. The non-treated group was designated as the zero-group. The number of replications in each treatment was equal to the number of sampled colonies (from 3 to 12). The number of dead bees at the end of a 72-hour observation period was taken as the toxicity indicator. The bees were of the Caucassian, Carniolan and Central European breeds.

Contact toxicity was determined by placing 10 bees on a filter paper disc in a petri dish. The filter paper discs were previously soaked with 1.5 ml aliquots of 0.01%, 0.03% and 0.05% aqueous solutions of the tested formula. Counts of dead bees were made after 24 hrs. The time of filter paper drying, from paper moistening to placement of bees, was differentiated from 0 to 7 days. Eight series were set up in this manner.

The results were subjected to ANOVA and the validity of differences was tested using Duncan's test at 0.05% confidence level. The statistical analysis of the data related to the mortality level (mortality indicator) was performed on conversion values obtained according to Freeman-Tukey formula. The data were adjusted to compensate for the effect of natural bee losses by using Abbott's formula:

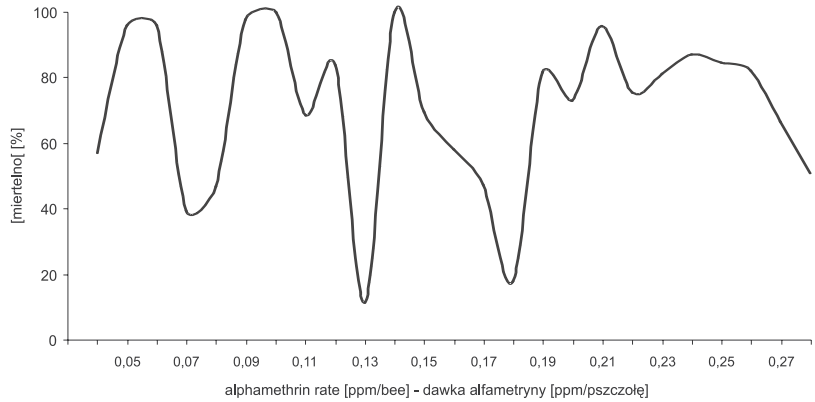
$$P = 100(Po-c):(100-c)$$

Where P - adjusted mortality,
Po - observed mortality, c - mortality in control group expressed as percentages.

RESULTS

Stomach toxicity

At the outset of the study it was assumed that Fastac is little toxic to bees. It soon became apparent, however, that the assumption did not hold good in every instance. Upon exposure to the formula the bee response varied from series to series as did the counts of dead bees. However, the relationship between the dose of the active ingredient and bee mortality was by no means a straightforward one. If viewed from that angle the data listed in Table 1 and in Fig. 1 show an inconsistency that testifies to the effect of some unaccounted for factors on the noxiousness of the formula. E.g. the same number of bees perished when exposed to 0.058 ppm in series 060994 as when exposed to 0.189ppm in series 200694, the mortality rate being as high as over 90% of the cages' initial population. On the other hand, only 18.8% of the bees perished following exposure to 0.171 ppm of alphametrin in series 010894. It must be noted, however, that the bees used in the tests varied for origin, generation and rearing environment. What we dealt with here was an array of different sensitivities to alphamethrin exposure as affected by ambient conditions. Thus, under laboratory conditions, a cross section of possible events was recorded that might accompany the exposure of a bee to the insecticide. The understanding of those events may be useful in practical interpretations so that we



Ryc. 1. Rates of Fastac E 10 active ingredient (ppm/bee) and bee mortality rates (%) Bee deaths due to natural causes have been excluded. - Dawki substancji czynnej (ppm na pszczołę) preparatu Fastac 10 EC i śmiertelność pszczół w procentach (ubytki naturalne pszczół wyeliminowano).

can either better safeguard bee colonies against poisoning or improve the management of poisoning situations once they have occurred.

In the 1994 season Fastac 10EC was tested by sampling Central European bees six times and always from the same 3 colonies. The bees from the first sampling date, 14th June, all died upon ingesting Fastac-contaminated food. The kill was caused by the rate of 0.213 ppm of active ingredient, alphamethrin, per bee, natural losses having been accounted for. When those initial data are compared to the results from the subsequent samplings the following differences in plus or in minus are obtained over a range of active ingredient doses (ppm) and mortality rates (%):

Date	ppm	%
14th June	0.213	100
20th June	+0.071	-2.5
28th June	+0.138	-50.0
6th July	-0.009	-5.5
19th July	+0.101	-12.3
1st August	-0.008	-87.8

The review of those figures provides a sufficient insight into how bee sensitivity to the toxic action of the same formula may vary. What first of all comes into play here is the variability in the physical predisposition of bees to respond to the hazard. We are more interested, though, in the range of that response so that it can be used as a data source to measure and verify formula toxicity. In the case of Fastac any generalizations are not easy to make and may be downright doubtful if the range of possible responses is not stated.

Another source of variation may arise from genotype differences of the bees tested. The bee toxicity results reported here are based on studying responses of three breeds - Central European (*mel*), Caucassian (*cau*) and Carniolan (*car*) - upon exposure to active ingredient (ppm/bee) and the resulting bee deaths (%).

mel	0.134 ppm	94.5%
cau	0.166 ppm	47.9%
car	0.151 ppm	59.4%

Such large breed-to-breed differences do not hold true in every case, at least not when the Carniolan bees are compared with the Caucassian bees. In three experiment series of the years 1997 and 1999 similar numbers of the two races died upon exposure even though the amounts of ingested insecticide were dissimilar. In any case, there is a clear indication that genetic differences should be accounted for in the assessment of pesticide toxicity - in this particular

Table 1

Stomach toxicity of Fastac to bees as overall test results arranged according to increasing bee mortality rates: columns 4 to 8 list extreme treatment values; (a:b) 100 - ratio of ingested insecticide-contaminated (a) to non-contaminated (b) syrup.
Sumaryczny zapis testów szkodliwości żołądkowej preparatu Fastac dla pszczół według gradacji poziomu śmiertelności; w rubrykach od 4 do 8 podano skrajne wartości dla kombinacji; (a:b)100 - relacja spożycia syropu z domieszką (a) i bez domieszki (b) preparatu.

Number of - Liczba			Insecticide concentration Stężenie preparatu %	Syrup ingestion mg/bee - Spożycie syropu mg/pszczołę	(a:b)100 %	Alphametrin rate ppm/bee - Dawka alfametryny w ppm/pszczołę	Mortality rate after 72 hrs % Śmiertelność po 72 godzinach %
Series Serii	Treatment Kombinacji	Replications Powtórzeń					
1	2	3	4	5	6	7	8
16	16	133	0	43.31-84.63	-	0	0-6.01
1	2	15	0.01	21.04-40.20	36.2-40.2	0.134-0.171	12.15-18.82
1	1	6	0	42.84	-	0	28.22
1	1	5	0.03	26.31	33.3	0.069	39.92
2	2	18	0.005-0.01	12.38-19.17	26.4-35.2	0.076-0.158	48.31-49.22
3	3	27	0.004-0.02	16.33-24.09	24.6-48.1	0.055-0.287	52.03-58.89
2	2	14	0.005-0.01	18.24-22.07	27.9-31.7	0.098-0.150	68.78-69.84
2	2	18	0.01-0.015	14.86-25.78	29.7-34.0	0.188-0.212	73.45-75.70
3	4	21	0.01-0.03	7.44-29.62	15.9-35.0	0.132-0.265	82.05-87.74
6	6	50	0.0075-0.02	5.10-12.87	6.7-30.0	0.058-0.189	95.86-100.0

Table 2

Bee performance following exposure to surface contaminated with 0.03 aqueous solution of Fastac. - Skutki kontaktu pszczół z powierzchnią skażoną 0,03% roztworem wodnym preparatu Fastac.

Filter paper drying time Czas wysychania bibuły	Number of replications Liczba powtórzeń	Bee counts after 24 hours Kwalifikacja pszczół po 24 godzinach		Losses of control bees - Ubytki pszczół kontrolnych %
		Dead - Martwe %	Poisoned Z objawami zatrucia %	
1 hour - 1 godzina	12	72.5	14.2	1.7
3 hours - 3 godziny	6	90.0	10.0	0
1 day - 1 dzień	6	90.0	6.7	0
3 days - 3 dni	12	94.1	5.9	0
7 days - 7 dni	6	76.7	23.3	0

case of a pyrethroid insecticide. As far as the demonstration of the phenomenon or, possibly, of its extent is concerned it is immaterial whether some genetic factor acts directly, indirectly or whether some interaction is involved. However, that confusion of effects makes it very difficult to assess the toxicity of the pesticide and to evaluate bee poisoning especially in contentious cases. The range of possibilities is simply too great here.

In our study the addition of Fastac to the syrup led consistently to an increase in bee mortality above the significance level. The statistical verification of differences, the least significant difference of 12.2% of dead bees was in this case a mere formality. The bee mortality was generally so high that it limited syrup ingestion. It is indicated by the following relationships as expressed by correlation coefficients:

mortality rate vs.
alphamethrin dose (ppm) - -0.27
mortality rate vs.
amount of ingested syrup (mg) - -0.490
mortality rate vs.
amount of ingested syrup (%) - -0.678
mortality rate vs.
insecticide concentration of syrup - 0.364

Ingested syrup (%) signifies the amount of syrup ingested as percentage of non-contaminated syrup ingested in the control group, the latter being taken as the measure of bee activity. High by comparison, the correlation coefficient (r = -0.678) demonstrates that it is bee losses that effectively restricted the ingestion of contaminated syrup. We accept the restriction at its face value without, at this stage, seeking the explanation in the repellent properties of the formula cited by the manufacturer. However, no such properties were confirmed at an insecticide concentration of 0.03%. Regardless of what mechanism is involved here the active ingredient of Fastac ingested at a low rate brought about an acute poisoning of bees which is characteristic of synthetic pyrethroids. Because of that in the tabulated list that also comprises variability over experiment series, the increase in alphamethrin dosage and the rise in mortality do not reveal any synchronous pattern as is shown in Fig. 1. However, within individual series such a relationship does show up. E.g. in series no. 100996 the ingestion of alphamethrin in insecticide-contaminated syrup at concentrations of 0.0075%, 0.015% and 0.03% resulted in the following mortality rates:

	mg	ppm	%
0	50.08 c	0	1.0 a
0.0075%	24.09 b	0.153	58.9 bc
0.015%	14.86 a	0.188	73.4 cd
0.03%	10.47 a	0.265	82.4 d

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Contact toxicity

First, the bees were placed on paper filter discs freshly soaked with 0.02% Fastac 10 EC solution. After 24hr 35.9% of the bees were dead and the remaining (64.1%) showed severe poisoning symptoms. In the next experiment (4 replications, 10 bees per dish) the bees were exposed to the insecticide after the discs had been drying for 1 hr which corresponded to the waiting period of the formula. Only 17.9% perished, the remaining bees surviving the test in good health and preserving full mobility.

In the tests run during the next beekeeping season that positive result prompted us to differentiate only formula concentrations while preserving the 1-hour filter paper drying period. However, the observations of test bees were disappointing not because of insecticide concentration (0.01%, 0.03%, 0.05%) but because of the toxicity rates since dead (m.) and poisoning symptoms-bearing bees (z) were prevalent in all contaminated dishes:

	m (%)	z (%)
0.01%	79.4	12.7
0.03%	81.7	16.7
0.05%	93.4	16.4

At the end of July further experiment series were established by differentiating filter drying times within a 7-day interval whilst maintaining pesticide concentration at 0.03%. The results are given in Table 2.

The symptoms of Fastac poisoning usually set in soon after placing bees in petri dishes. In the last experiment series (7-hour drying time) only 11.7% fully motile bees were counted within two hours following the treatment. Another 5% moved languidly in the dish, the remaining ones lay on the paper paralysed, from time to time showing by discordant movements and tremors of their legs and antennae that they were still alive. Some tried to get up but usually to no avail. All bees showing such symptoms would pass away within 48 hrs.

CONCLUSIONS

The sensitivity of bees to stomach poisoning by Fastac is characterized by great variability. That variability confuses the simple relationship between active ingredient dose from 0.055 to 0.287 as applied in this study and the resulting mortality rates from 12.2% to 100%. There is a possibility that the toxicity of the formula might exceed the average level regardless of what the actual underlying causes of such increased toxicity may be related to: the rearing environment, non-heritable factors or inherited traits. The increased risk should therefore be allowed for in devising strategies for protecting the bees from coming into contact with the insecticide on crop fields.

In view of the results from testing the contact toxicity of Fastac it would be advisable to revise its labeled 1-hour waiting period. When exposed to filter paper that had been soaked with the 0.03% aqueous solution of the insecticide and allowed to dry for as many as 7 days the bees still ran a serious risk of being poisoned.

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SZKODLIWOŚĆ DLA PSZCZÓŁ PREPARATU FASTAC W OCENIE LABORATORYJNEJ

M. Gromisz

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

Preparat Fastac 10 EC należy do grupy pyretroidów, zawiera 10% alfametryny, substancji czynnej biologicznie. Stosuje się go w ochronie roślin przed szkodnikami w ilości od 0,005 do 0,23 l/ha, w stężeniu od 0,01% do 1,0% (uprawy leśne). W testowaniu 307 prób pszczół na jego toksyczność żołądkową stwierdzono: a) pobierane dawki alfametryny w syropie wynosiły od 0,055 ppm do 0,287 ppm w przeliczeniu na jedną pszczołę, b) dodatek preparatu do syropu prowadził do śmierci od 12,2% do 100% pszczół (ubytki naturalne wyeliminowano), c) testowane próby pszczół cechowały się dużą zmiennością co do wrażliwości na toksyczne działanie preparatu w zależności od terminu badań (zmienność sezonowa) i pochodzenia (rasy pszczół), d) wielkość pobieranych dawek alfametryny była w zasadzie limitowana wzrostem śmiertelności pszczół, to znaczy stopnia ich wrażliwości. W działaniu kontaktowym preparat Fastac zachowywał znaczące właściwości toksyczne jeszcze w tydzień po zastosowaniu.

Słowa kluczowe: pszczoła miodna; rasy pszczół; pestycydy; Fastac; alfametryna; toksyczność.

BEEKEEPING VALUE AND POLLINATION REQUIREMENTS OF DOUBLE-IMPROVED CULTIVARS OF SPRING RAPESEED (*Brassica napus* L. *var. oleifera* Metzger *f. annua* Thell.)

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S u m m a r y

The beekeeping value and pollination requirements of six cultivars of spring rapeseed (Licosmos, Lisonne, Margo, Sponsor, Star and Unica) were investigated in trials run at the Apiculture Division, ISK, Puławy in the years 1998-2000.

Spring rapeseed was found to have an average blooming period from the second half of June to the beginning of July, producing 10 to 15 thousand flowers per 1 m^2 during the period of 20 days. During their lifetime 10 flowers secreted ca. 4.63 mg of sugars and yielded an average of 8.80 mg of pollen.

Sugar output of the rapeseed cultivars was estimated at 40-80 kg per 1 ha and pollen output was estimated at 85-120 kg. With respect to beekeeping value cv. Licosmos and Unica were the best performers. The composite hybrid cultivar Margo yielded only ca. 30 kg of sugars and as much pollen per 1 ha.

All rapeseed cultivars were abundantly visited by pollinating insects, mainly by the honeybee, the latter accounting for 92% of the total number of foraging insects. At full blooming during the peak foraging hours and with fine weather an average of six foragers were found per 1 m^2 of the blooming rapeseed stand.

The flowers with free access of insects set pods at a rate of 44%. In the absence of insects the pod set was 37% and only 18% for the hybrid cultivar Margo. The pods resulting from free pollination contained an average of 15 seeds whereas those from covered flowers set an average of 10 seeds.

Seed yield from free pollination averaged 25 q/ha. In the absence of pollinating insects they were reduced by 40%. Cv. Margo showed the most substantial yield decrease related to the absence of pollinators (up to 70%) followed by Unica (up to 50%) and by the remaining cultivars (ca. 30%).

Keywords: spring rapeseed, nectar secretion, pollen production, pollination, seed set.

INTRODUCTION

With more and more rapeseed crop being lost to winter freezes spring forms have been attracting more attention over the recent years. Althought they are not so good seed yielders and, consequently, oil producers as the winter forms, they can always be used to re-seed the failed winter crop. As a result, fields in yellow bloom so characteristic of Polish springtime can very often be found only in midsummer, in June. According to statistical data spring rapeseed accounts for ca. 10% of the total rapeseed crop in Poland whereas in such countries as Sweden the relevant figure is ca. 25%. Until now, beekeeping value and pollination requirement studies have been almost exclusively concerned with winter rapeseed. The aim of the present study was to

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find out how much nectar and pollen flow is provided for honeybees by spring forms of rapeseed and to what extent, if any, rapeseed yields are dependent on the presence of pollinator insects.

METHODS

Poland's six most widely grown double-improved spring rapeseed cultivars were singled out for the study. They were mass selection cultivars: German-bred Licosmos and Lisonne, Danish-bred Star and Unica the Swedish-bred Sponsor and the Polish composite hybrid Margo. The trials were run on the ISK experiment field in Puławy in the years 1998-2000. The soil was pseudopodsolic rated as class IV. Conventional agronomic practices as recommended for rapeseed production (Wałkowski et al. 1996) were used. The trials were laid out as completely randomized block designs in four replications. The plot size was 8 m^2. At the first true leaf stage the seedlings were thinned down to obtain roughly the same number of plants per plot in a given year.

Nectar secretion rate by the flowers of individual cultivars was assessed using the micropipetting method (Jabłoński and Szklanowska 1979). Ten flowers from each plot were sampled for nectar six times at full blooming. At this stage 30 flower buds were also collected from each plot to measure pollen production according to the Warakomska method modified by Szklanowska (Warakomska 1972, Szklanowska and Pluta 1984). The density of pollinating insects on rapeseed flowers was measured for 8-10 days during fine weather. Each day in the noontime on each plot three counts were made of foraging honeybees, bumblebees, solitary bees and flies.

In order to keep out pollinating insects and to check for pod and seed set rate under self-pollination a portion of each plot was covered with air-permeable plastic gauze. At technical maturity 15 plants were sampled in both portions of each plot (insect-isolated and insect non-isolated) for biometrical analyses. Each plant sample was analysed for plant height, branching, flower number, pod number and seed number. Seeds from each sample were weighed and 1000 seed weight was calculated.

The results were subjected to ANOVA and the significance of differences was tested using Duncan's test at confidence level of $\alpha = 0,05$.

WEATHER DURING THE TRIALS

The weather conditions in the growing seasons of 1998-2000 varied and only in the first year were conducive to normal seed yields. In that year the blooming of spring rapeseed coincided with a cool spell but with little rainfall which allowed the plants to set seeds well. In the next year (1999) it was very warm, minimum temperatures at the blooming period reaching 16°C. However, although heavily overcast skies and abundant rainfall prevented the plants from drying, they did not favour good nectar secretion and pollination. In the year 2000 it was moderately warm and humid at the time of blooming.

At the seed developing stage in 1998 the weather was normal - similar to the long-term average. In 1999 it was very hot with mean maximum temperatures above 28°C over all decades of July and in the first decade of August. The result was poor development and withering of seeds. In the year 2000 the temperatures recorded for that period came within the long-term average. However, the extremely rainy July brought on a high infestation of rapeseed fields by mildew resulting in depressed seed yields.

RESULTS

BLOOMING OF THE SPRING RAPESEED CULTIVARS

Blooming date. Spring rapeseed blooms in the second half of June. Cvs. Sponsor and Licosmos were the first to bloom on the 10th June on average (Fig.1) to be followed in short intervals by cvs. Star, Unica, Lisonne and Margo. Averaged across cultivars, rapeseed bloomed on the earliest date in 1999 - 10th July. In the remaining years (1998 and 2000) it came to flower slightly later. Apart from the weather the much different seeding dates - 27th April, 2nd April and 19th April influenced blooming date substantially. The data show that cv. Licosmos showed the least and the cvs. Lisonne and Margo the greatest variation of blooming date over the years.

The duration of blooming of the spring rapeseed cultivars averaged 20 days. Below that average was the blooming duration of cvs. Margo, Star and Lisonne. In the warm season of 1999 spring rapeseed was characterized by the shortest duration of blooming.

Abundance of blooming.

Number of plants per 1 m^2. Since the seedlings were thinned down there were no major differences from cultivar to cultivar in the number of plants per unit area in a given year or in the ratio of male sterile to male fertile plants (7 to 3) in the composite hybrid cultivar Margo. However, there were substantial differences from year to year. In the first year the plant density per 1 m^2 was the highest averaging 78 plants (Table 1). In the course of the study it transpired that the density was too high as the whole stand lodged. In the subsequent years the rapeseed stands were thinned down to lower densities of 56 and 35 plants in the years 1999 and 2000, respectively. The plots were very uniform and no differences in plant number per unit area were found between the portion freely accessed by insects and that covered by plastic gauze.

Plant height in cm. Plant height is a cultivar-dependent trait. The plants of cv. Sponsor were the shortest over all study years and those of cv. Margo were the tallest though sometimes equalled by those of Unica (Table 2).

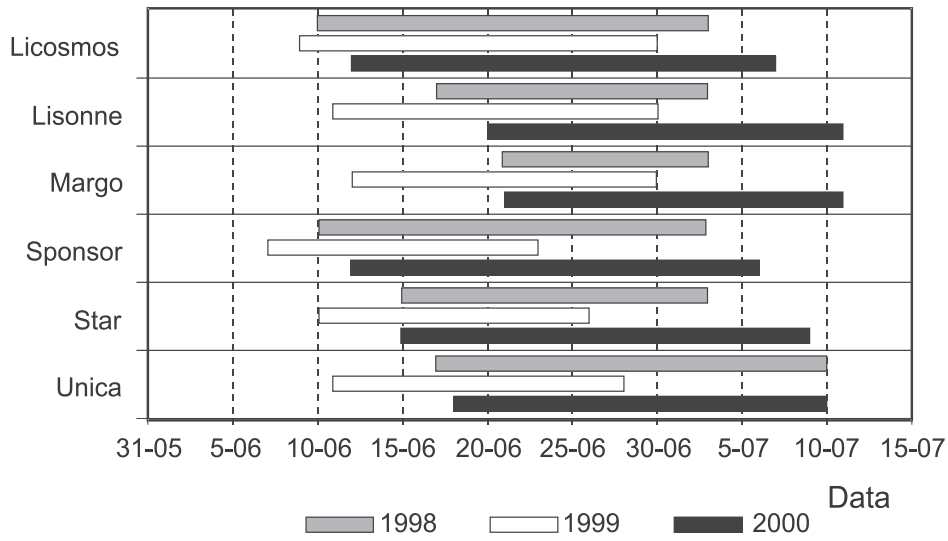

Fig. 1. Date and duration of blooming of the spring rapeseed cultivars.
Pora i długość kwitnienia badanych odmian rzepaku jarego

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Table 1

Number of plants per 1 m^2 of rapeseed stand. - Liczba roślin na 1 m^2 łanu rzepaku.

Cultivar Odmiana	Open pollination - Swoboda				Gauze cover - Izolator			
	1998	1999	2000	Average Średnia	1998	1999	2000	Average Średnia
Licosmos	88	60	38	62.0 cd	88	60	39	62.3 d
Lisonne	68	60	29	52.3 ab	68	60	25	51.0 ab
Margo	69	50	37	52.0 ab	69	50	32	50.3 a
Sponsor	82	60	38	60.0 cd	82	60	30	57.3 b-d
Star	80	52	37	56.3 a-d	80	52	32	54.7 a-c
Unica	80	54	39	57.7 b-d	80	54	32	55.3 a-c
Average Średnia	77.8 d	56.0 c	36.3 b	56.7 a	77.8 d	56.0 c	31.7 a	55.2 a

Table 2

Height of plants in cm. - Wysokość roślin w cm.

Cultivar Odmiana	Open pollination - Swoboda				Gauze cover - Izolator			
	1998	1999	2000	Average Średnia	1998	1999	2000	Average Średnia
Licosmos	150	110	146	135.3 b-d	143	108	151	134.0 b-d
Lisonne	149	107	140	132.0 bc	147	107	146	133.3 b-d
Margo	155	120	152	142.3 ef	163	122	160	148.3 f
Sponsor	138	88	131	119.0 a	135	92	137	121.3 a
Star	144	99	144	129.0 b	141	100	152	131.0 b
Unica	156	109	155	140.0 de	151	105	160	138.7 c-e
Average Średnia	148.7 bc	105.5 a	144.7 b	132.9 a	146.7 bc	105.7 a	151.0 c	134.4 a

Likewise, the two experiment variants (open pollination vs. gauze cover) did not vary for that trait with the exception of the year 2000. However, different weather conditions in individual study years significantly affected plant height. The conditions were unequivocally adverse in 1999 resulting in plants only slightly in excess of 1 m in height. In the remaining years the plants grew to the height of nearly 150 cm.

Number of lateral branches per plant. There were hardly any differences among the cultivars for that trait (Table 3). Likewise, no differences were recorded between the experiment variants each plant averaging 8-9 branches. It is only in the year 2000 that the plant habit departed from normal. The plants branched off more profusely especially in the lower part of the stalk. In that year the number of lateral branches reached 12 - 13 whereas in the remaining years it was 6 to 7. It could also be due to a low plant number per 1 m^2.

Table 3

Number of branches per plant. - Liczba rozgałęzień na roślinie.

Cultivar Odmiana	Open pollination - Swoboda				Gauze cover - Izolator			
	1998	1999	2000	Average Średnia	1998	1999	2000	Average Średnia
Licosmos	7.7	6.3	14.1	9.4 bc	7.7	5.9	13.6	9.1 bc
Lisonne	7.5	6.1	13.4	9.0 bc	7.7	6.9	14.2	9.6 c
Margo	7.8	6.4	10.7	8.3 a-c	8.3	7.0	12.8	9.4 c
Sponsor	6.4	5.6	11.6	7.9 ab	6.4	5.9	12.6	8.3 a-c
Star	6.4	5.6	10.0	7.3 a	6.4	5.6	12.5	8.2 a-c
Unica	7.6	6.4	11.3	8.4 a-c	7.3	5.9	12.2	8.5 a-c
Average Średnia	7.2 b	6.1 a	11.9 c	8.4 a	7.3 b	6.2 a	13.0 d	8.8 a

Table 4

Number of flowers per plant. - Liczba kwiatów na roślinie.

Cultivar Odmiana	Open pollination - Swoboda				Gauze cover - Izolator			
	1998	1999	2000	Average Średnia	1998	1999	2000	Average Średnia
Licosmos	194	207	394	265 cd	186	175	372	245 b-d
Lisonne	136	175	349	220 a-c	148	179	393	240 a-d
Margo	219	271	321	270 d	363	268	362	331 e
Sponsor	160	159	266	195 a	163	154	303	207 ab
Star	171	169	255	198 ab	147	163	338	216 ab
Unica	158	199	332	230 a-d	158	157	346	220 a-c
Average Średnia	173 a	196 a	319 b	230 a	194 a	183 a	352 c	243 a

Number of flowers per plant. A single spring rapeseed plant produced an average of 240 flowers in our experiments (Table 4). However, the trait varied both among cultivars and from year to year. The plants of cv. Margo produced the highest number of flowers - 300 on average. It is only in the first two years that the values substantially departed from those recorded for the remaining cultivars (from 140 to 200). In the year 2000, due to a high degree of lateral branching, all cultivars produced nearly twice the number of flowers per plant as compared to the previous years. Cv. Margo measured half-way down the scale in this respect.

Cvs. Sponsor and Star produced by far the smallest number of flowers per plant - 200 on average. In this respect those cultivars performed below the total average.

Number of flowers per 1 m^2 of crop stand. This trait gives the most accurate

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Table 5

Number of flowers per 1 m^2 in 10^3. - Liczba kwiatów na 1 m^2 łanu w tysiącach.

Cultivar Odmiana	Open pollination - Swoboda				Gauze cover - Izolator			
	1998	1999	2000	Average Średnia	1998	1999	2000	Average Średnia
Licosmos	17.1	12.4	14.2	14.6 d	16.4	10.5	14.1	13.7 cd
Lisonne	9.3	10.5	9.8	9.9 a	10.1	10.7	9.8	10.2 ab
Margo	15.1	13.5	11.8	13.5 cd	25.1	13.4	11.1	16.5 e
Sponsor	13.1	9.5	10.0	10.9 ab	13.4	9.3	8.8	10.5 ab
Star	13.7	8.8	9.3	10.6 ab	11.8	8.4	10.8	10.3 ab
Unica	12.6	10.8	12.5	12.0 bc	12.6	8.5	10.7	10.6 ab
Average Średnia	13.5 b	10.9 a	11.3 a	11.9 a	14.9 c	10.1 a	10.9 a	12.0 a

assessment of the flower abundance of a taxon since it is the joint outcome of the number of plants per unit area and flower abundance of individual plants.

The data listed in Table 5 indicate that both under open pollination and under cover the numbers of produced flowers were very similar. However, there were significant differences among the cultivars. Cvs. Margo and Licosmos produced the highest number of flowers per unit area: 14,000-15,000 per 1 m^2 whereas the remaining cultivars produced 10,000--11,000. In 1998, probably due to favourable weather conditions, the number of flowers was the highest and averaged over 14,000 per 1 m^2. In the remaining years it was 10,000-11,000 per 1 m^2.

NECTAR SECRETION AND POLLEN PRODUCTION OF SPRING RAPESEED CVS.

Nectar secretion and pollen production rate. The weight of sugars secreted in the nectar from 10 flowers averaged 4.63 mg (Table 6). There were small though significant differences among cultivars. Cvs. Lisonne, Licosmos and Unica were better nectar producers than the remaining ones averaging 5.2 mg of sugars in nectar from 10 flowers, cvs. Margo and Star producing 4.3 mg and cv. Sponsor only 3.7 mg. Those differences were consistent throughout the study.

There were also significant year-to-year differences. The performance was particularly low in 1999 with 10 flowers secreting only 1.36 mg of nectar-borne sugars. It was the reflection of the cloudy and rainy weather prevailing during rapeseed blooming. In 1998 and 2000 the values were much closer averaging 6.75 and 5.77 mg. However, also in that case the difference was statistically proved.

Weights of pollen from 10 flowers were not found to vary substantially both when averaged across cultivars and across years of study (Table 6). Cvs. Unica and Star were the best pollen producers averaging 9.58 and 9.35 mg, respectively, and cvs. Sponsor and Licosmos the poorest (8.20 and 8.32 mg) and only those cultivars differed significantly for that trait. Intermediate values were recorded for the remaining cultivars. The year 1999 turned out to be the least beneficial also for that trait with average pollen yields from 10 rapeseed flowers of 7.32 mg as contrasted with 8.56 mg for 1998 and 10.54 for 2000. The differences were statistically valid.

Table 6

Weight of sugars and pollen per 10 flowers in mg.
Masa cukrów i pyłku z 10 kwiatów w mg.

Cultivar Odmiana	Sugars - Cukry				Pollen - Pyłek			
	1998	1999	2000	Average Średnia	1998	1999	2000	Average Średnia
Licosmos	7.37	1.57	6.62	5.19 b	8.25	7.08	9.63	8.32 a
Lisonne	7.67	1.52	6.46	5.22 b	8.78	7.08	10.10	8.65 a-c
Margo *	5.87	1.14	5.88	4.30 a	8.19	6.60	11.38	8.72 a-c
Sponsor	5.52	1.17	4.28	3.66 a	8.45	6.30	9.85	8.20 a
Star	6.48	1.13	5.17	4.26 a	8.06	8.65	11.33	9.35 bc
Unica	7.61	1.65	6.18	5.15 b	9.62	8.20	10.93	9.58 c
Average Średnia	6.75 c	1.36 a	5.77 b	4.63	8.56 b	7.32 a	10.54 c	8.80

*- Male fertile plants - Rośliny komponenta męskopłodnego

Table 7

Sugars and pollen yield of spring rapeseed cultivars in kg/ha.
Wydajność cukrowa i pyłkowa badanych odmian rzepaku jarego w kg/ha.

Cultivar Odmiana	Sugars - Cukry				Pollen - Pyłek			
	1998	1999	2000	Average Średnia	1998	1999	2000	Average Średnia
Licosmos	126.0	19.5	94.0	79.8 d	141.1	87.8	136.7	121.9 c
Lisonne	71.3	16.0	63.3	50.2 b	81.7	74.3	99.0	85.0 b
Margo	45.6	7.9	35.7	29.7 a	37.5	27.0	40.7	35.1 a
Sponsor	72.3	11.1	42.8	42.1 b	110.7	59.9	98.5	89.7 b
Star	88.8	9.9	48.1	48.9 b	110.4	76.1	105.4	97.3 b
Unica	95.9	17.8	77.3	63.7 c	121.2	88.6	136.6	115.5 c
Average Średnia	83.3 c	13.7 a	60.2 b	52.4	100.4 b	68.9 a	102.8 b	90.7

Sugar and pollen output from unit crop area. The number of flowers per unit area and nectar secretion rate have the decisive impact on the amount of nectar supply available to insects. Once the calculations were made spring rapeseed cultivars were found to supply an average of 50 kg of sugars per 1 ha (Table 7).

Licosmos was the highest sugar yielder averaging 80 kg followed by Unica with a sugar yield of 64 kg. The poorest sugar yields were recorded for the hybrid cultivar Margo. Even though its male fertile flowers secreted nectar fairly well there were only 30% of them and male sterile flowers were much poorer nectar secretors. The remaining three cultivars were intermediate in this

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Table 8

Composition of particular groups of pollinating insects visiting spring rapeseed flowers.
Udział procentowy poszczególnych grup owadów zapylających na kwiatach rzepaku jarego.

Pollinating insects Owady zapylające	Years of study - Lata badań			Average Średnia
	1998	1999	2000	
Honeybees - Pszczoła miodna	97.40	91.61	86.86	91.96
Solitary bees - Pszczoły samotnice	1.20	3.07	7.89	4.05
Bumblebees - Trzmiele	0.50	2.34	4.28	2.37
Flies - Muchówki	0.90	2.98	0.97	1.62
Total % - Razem %	100.00	100.00	100.00	100.00

respect. Averaged across cultivars, the sugar yields for 1999, the least favourable year, were the lowest falling short of 14 kg/ha. The remaining years 1998 and 2000 can be considered as more typical and the sugar yields were 83 and 60 kg, respectively.

With respect to pollen yields cvs. Licosmos and Unica were again the best performers (122 and 116 kg) and cv. Margo was the poorest (35 kg). The remaining cultivars yielded on average ca. 90 kg of pollen per 1 ha and the total average reached a similar value. Over the consecutive years of the study pollen yields followed a pattern similar to that of sugar yields. In 1999 the average pollen output was the poorest - 69 kg whereas in the remaining years it fluctuated around 100 kg. It must be noted however that the low values for cv. Margo resulted in an underrated total average and that the average values for the remaining cultivars and especially for the two best-performing ones were substantially higher.

POLLINATION OF SPRING RAPESEED CULTIVARS

Insect pollinators of rapeseed. The amounts of nectar and pollen forage supplied by the spring rapeseed cultivars turned out to be very attractive to insect pollinators. Honeybees accounted for the majority of them - nearly 92% followed by solitary bees - 4%, bumblebees - 2.4% and flies - 1.6% (Table 8). In 1998 honeybees effectively displaced the remaining insect groups accounting for 97% of all insects foraging on rapeseed flowers. The values for the year 1999 approximated the average figures whereas in 2000 more solitary bees and bumblebees were counted.

Density of honeybees on the blooming rapeseed stand. Since there was an overwhelming majority of honeybees among the insects occurring on rapeseed flowers their number per unit area served as the estimate of the visitation intensity of individual cultivars.

Rapeseed flowers were visited most frequently between 9 hrs and 16 hrs. Given the weather that favoured insect flights more than 6 insects per 1 m^2 of rapeseed stand were found at that time of day (Table 9). The data show that the flowers of cvs. Licosmos, Star and Unica were among those most keenly visited to be followed by the flowers of Lisonne and Sponsor. Due to scant pollen and nectar the hybrid cultivar Margo was the least frequently visited. Interestingly, in 1998 the foraging of rapeseed flowers was relatively poor with fewer than 5 bees per 1 m^2 even though the yields of sugar and pollen were fairly high. In

Table 9

Density of honeybees per 1 m^2 of the blooming plot of spring rapeseed cultivars.
Zagęszczenie pszczół miodnych na 1 m^2 kwitnącego łanu badanych odmian rzepaku jarego.

Cultivar Odmiana	Years of study - Lata badań			Average Średnia
	1998	1999	2000	
Licosmos	6.5	6.9	9.4	7.60
Lisonne	4.1	5.8	9.4	6.43
Margo	1.5	2.2	4.0	2.57
Sponsor	5.2	5.4	8.2	6.27
Star	6.2	6.8	8.6	7.20
Unica	4.7	7.2	9.3	7.07
Average - Średnia	4.70	5.72	8.15	6.19

Table 10

Number of fruit per plant for investigated spring rapeseed cultivars.
Liczba łuszczyn na roślinie badanych odmian rzepaku jarego.

Cultivar Odmiana	Open pollination - Swoboda				Gauze cover - Izolator			
	1998	1999	2000	Average Średnia	1998	1999	2000	Average Średnia
Licosmos	77.4	82.0	207.0	122.1 f	70.5	66.9	186.7	108.0 d-f
Lisonne	65.4	85.0	197.4	115.9 f	68.1	76.6	170.4	105.0 d-f
Margo	76.0	91.2	159.2	108.8 ef	57.6	73.9	45.9	59.1 a
Sponsor	62.8	55.4	133.1	83.8 bc	53.0	49.8	142.6	81.8 b
Star	68.0	67.2	135.8	90.3 b-e	55.8	59.2	164.5	93.2 b-e
Unica	60.7	78.4	168.6	102.6 c-f	55.2	56.6	152.2	88.0 bd
Average Średnia	68.4 ab	76.5 b	166.9 d	103.9 b	60.0 a	63.8 ab	143.7 c	89.2 a

1999 during fine weather the number of foraging bees was nearly 6 per 1 m^2 and more than 8 in the 2000 season. Those differences could be due to the condition of bees in nearby apiaries, a factor not dealt with in this study.

POD SET AND YIELDS OF SPRING RAPESEED CULTIVARS

Number of pods per plant. Under free access of pollinating insects the plants produced an average of 104 pods whereas when isolated produced 89 pods (Table 10). The differences were statistically significant. Within individual cultivars only for cv. Margo the difference was significant: 109 pods under open pollination vs. 59 pods under cover. In each study year the plants produced more pods when grown under open pollination although the difference was proven only for 2000. It is in that year that the highest number of pods per plant was recorded - an average of ca. 155 which was significantly different from the 1998

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Table 11

Number of pods per 100 flowers for spring rapeseed cultivars.
Liczba łuszczyn ze 100 kwiatów badanych odmian rzepaku jarego.

Cultivar Odmiana	Open pollination - Swoboda				Gauze cover - Izolator			
	1998	1999	2000	Average Średnia	1998	1999	2000	Average Średnia
Licosmos	40.4	39.9	52.8	44.4 e	38.2	37.8	50.3	42.1 c-e
Lisonne	48.0	47.9	57.2	51.0 f	46.1	42.6	44.2	44.3 e
Margo	35.0	35.0	50.0	40.0 b-d	16.1	27.5	11.4	18.3 a
Sponsor	39.6	34.9	50.0	41.5 c-e	32.4	32.1	46.6	37.0 b
Star	39.9	39.8	53.8	44.5 e	38.2	36.4	49.1	41.2 c-e
Unica	38.4	39.2	50.8	42.8 de	35.0	36.2	44.4	38.5 bc
Average Średnia	40.2 b	39.5 b	52.4 c	44.0 b	34.3 a	35.4 a	41.0 b	36.9 a

Table 12

Number of seeds per pod. - Liczba nasion w łuszczynie.

Cultivar Odmiana	Open pollination - Swoboda				Gauze cover - Izolator			
	1998	1999	2000	Average Średnia	1998	1999	2000	Average Średnia
Licosmos	17.0	15.2	15.7	16.0 c	13.6	12.1	7.4	11.0 b
Lisonne	16.3	16.4	15.4	16.0 c	12.6	13.0	5.0	10.2 b
Margo	16.4	15.1	13.2	14.9 c	6.6	10.2	1.4	6.1 a
Sponsor	16.0	15.4	15.2	15.5 c	13.3	14.1	5.6	11.0 b
Star	18.0	15.8	15.9	16.6 c	14.3	14.2	6.2	11.6 b
Unica	16.8	15.6	15.0	15.8 c	12.9	12.1	4.2	9.7 b
Average Średnia	16.8 d	15.6 cd	15.1 c	15.8 b	12.2 b	12.6 b	5.0 a	9.9 a

(64) and 1999 (70) figures. There were significant differences among the cultivars for that trait. The plants of cvs. Licosmos and Lisonne produced the most pods - 115 and 110 on average whereas those of the remaining cultivars produced from 84 to 95 pods.

Pod to flower ratio. Based on the ratio of set pods to the number of flowers one can analyse the effect of pollinator insects on yields. The data shown in Table 11 indicate that the number of pods developed from 100 flowers was consistenly higher under open pollination than under cover, the difference being significant for years and for most cultivars. In the year 2000 the degree of pod set was significantly higher than that in the previous years (by as much as 26%).

Number of seeds per pod. The number of seeds in the pods of plants freely accessed by insect pollinators averaged 15.8 and did not vary substantially from cultivar to cultivar or from year to year (Table 12). However, in plants that bloomed under gauze cover as few as 10 seeds per pod were recorded, the cultivar-to-cultivar and year-to-year differences being significant.

Table 13

Weight of 1000 seeds (g) of spring rapeseed cultivars.
Masa 1000 nasion badanych odmian rzepaku jarego w g.

Cultivar Odmiana	Open pollination - Swoboda				Gauze cover - Izolator			
	1998	1999	2000	Average Średnia	1998	1999	2000	Average Średnia
Licosmos	3.586	2.665	2.454	2.902 a-c	4.281	3.290	1.672	3.081 a-d
Lisonne	3.675	2.798	2.022	2.832 ab	4.735	3.158	1.834	3.242 d-f
Margo	3.802	3.290	2.305	3.132 b-d	3.456	3.772	3.205	3.478 ef
Sponsor	3.820	2.976	2.402	3.066 a-d	4.681	3.198	1.745	3.208 c-e
Star	4.278	3.278	2.228	3.261 d-f	5.205	3.724	1.699	3.543 f
Unica	3.948	2.582	1.835	2.788 a	4.696	3.444	1.200	3.113 b-d
Average Średnia	3.852 e	2.932 c	2.208 b	2.997 a	4.509 f	3.431 d	1.893 a	3.278 b

Table 14

Seed yields of spring rapeseed cultivars in q/ha.
Plony nasion badanych odmian rzepaku jarego w q/ha.

Cultivar Odmiana	Open pollination - Swoboda				Gauze cover - Izolator			
	1998	1999	2000	Average Średnia	1998	1999	2000	Average Średnia
Licosmos	37.8	20.2	29.2	29.1 f	34.2	16.0	8.9	19.7 cd
Lisonne	35.8	23.7	17.7	25.7 ef	26.7	19.0	3.8	16.5 bc
Margo	31.7	22.8	18.0	24.2 e	7.5	14.2	0.7	7.5 a
Sponsor	33.3	15.1	18.3	22.2 de	25.2	13.2	4.3	14.2 b
Star	38.4	18.2	18.7	25.1 ef	31.9	15.9	5.6	17.8 bc
Unica	38.9	17.2	18.5	24.9 ef	25.1	12.6	2.5	13.4 b
Average Średnia	36.0 e	19.5 c	20.1 c	25.2 b	25.1 d	15.2 b	4.3 a	14.9 a

The hybrid cultivar Margo showed the strongest response to the absence of pollinating insects by producing only 6 seeds per pod. The remaining cultivars averaged 10-11 seeds per pod. In the year 2000 plants blooming under cover produced 5 seeds per pod whereas in the previous years the figure was above 12.

Weight of 1000 seeds. The weight of 1000 seeds obtained under open pollination averaged 3.0 g whereas that obtained under cover was nearly 3.3 g (Table 13). The differences among cultivars were small but significant - cvs. Star and Margo produced the largest seeds. However, the differences from year to year were very large. Actually, it is only in 1998 that the seeds fully ripened and were adequately developed (1000 seed weight was 4.2 g). In 1999, very adverse weather conditions (little sunshine and a lot of rain) prevailed at the ripening stage. It resulted in the 1000 seed weight of only 3.2 g. In the year 2000, due to the same reasons and, additionally, because of mildew

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infestation the average 1000 seed weight was little more than 2 g.

Seed yield. The prevailing weather conditions had a significant effect on seed yields. Under open pollination they averaged 25 q/ha ranging from 22 to 29 q/ha for individual cultivars (Table 14). In 1998 the yields were the highest (36 q on average) whereas in the successive years they averaged ca. 20 q. Under the plastic gauze that kept out insects the seed yields per 1 ha were significantly lower and fell short of 15 q/ha or 60% of what they were under open pollination. The highest insect pollinator--dependent yield reduction was shown by cv. Margo - 69% to be followed by cv. Unica - 46% and by the remaining cultivars with yield reductions of 29 - 36%. It is noteworthy that seed yields obtained under cover in 2000 were very low and averaged only 21% of the yields obtained under open pollination.

DISCUSSION

The results on the beekeeping value and pollination requirements of six spring rapeseed cultivars presented in this study can be confronted only with literature data concerning mainly winter rapeseed since, save for the report by Bobrzecka and Bobrzecki (1973), no information relative to spring forms was found.

In Puławy, the blooming of spring rapeseed occurred in the second half of June and in the beginning of July very much like in England (Williams 1985) but its duration was shorter (15-25 days) than in the north of this country (30-40 days) (Bobrzecka and Bobrzecki 1973).

The branching of rapeseed plants was much better in the third study year than in the remaining years. The fact should be explained by the well-known tendency for plants to grow sturdier and more profusely branched with lower densities.

The blooming abundance of ivestigated in Puławy spring rapeseed cultivars fluctuated between 10,000 and 15,000 flowers per 1 m^2. It was higher than reported by Maksymiuk (1958) and Demianowicz (1968) for winter rapeseed and also for spring rapeseed in north Poland conditions (Bobrzecka and Bobrzecki 1973), but similar to noted by Jabłoński and associates (1985).

The average amount of sugars secreted in the nectar from 10 flowers was 4.6 g for the examined cultivars varying from 1.36 to 6.75 mg over the years of the study. Those quantities are smaller than the majority of those reported for winter rapeseed e.g. by Maksymiuk (1958), Demianowicz (1968), Bobrzecka and Bobrzecki (1973), Kubišova et al. (1980), Jabłoński et al. (1985), Williams (1985). They are also inferior to those obtained by Bobrzecka and Bobrzecki (1973) in the north of this country and to those reported by Davis et al. (1994) for allotetraploid rapeseed (4n = 38). The data from this study cannot be compared with some literature data since our data refer to total sugar quantities secreted in nectar by flowers whereas the latter refer to the so-called diurnal quantities (Kamler 1980, Mesquida et al. 1988a, Mohr and Jay 1990) or even to nectar volume expressed as ml (Pierre et al. 1999).

Poorer nectar secretion by flowers of individual spring rapeseed cultivars may be attributable not so much to inherited traits as to the weather conditions under which they come to bloom, and specifically to elevated temperatures prevailing in summer. This suggestion is supported by similar nectar secretion rates recorded for winter and spring cultivars in the north of Poland (Bobrzecka and Bobrzecki 1973) where the summer is cooler. Furthermore, the nectar secretion of all six cultivars examined in this study was three times more abundant in the cool and moderately humid

seasons of 1998 and 2000 than in the very warm and cloudy season of 1999.

Pollen production rate estimates of nearly 9 mg per 10 flowers for the cultivars examined in this study did not show as much dependence on weather as did nectar secretion rates and ranged over the years from 7.3 mg to 10.5 mg. The quantities are similar to those reported by Jabłoński and associates (1985) for winter rapeseed cultivars.

The sugar output or the quantity of sugars yielded by the unit area (1 ha) of the crop during the blooming season depends on the number of flowers and nectar secretion thereby. The spring rapeseed cultivars yielded on average ca. 60 (40-80) kg of sugars per 1 ha which is less than the amounts reported for winter rapeseed, the figures coming within the range of 60-120 kg per 1 ha and sometimes more (Maksymiuk 1958, Demianowicz 1968, Bobrzecka and Bobrzecki 1973, Kubišova et al. 1980, Williams 1980, Jabłoński et al. 1985).

With an average pollen output of 90 (80-120) kg per 1 ha the spring rapeseed cultivars were not inferior to winter rapeseed cultivars investigated by Jabłoński and associates (1985). It is only the hybrid cultivar Margo that was a poor pollen yielder (30-40 kg of pollen per 1 ha). It should be remembered, however, that the cultivar is made up of ca. 70% male sterile plants, which do not produce pollen. Apart from that, male sterile plants secrete less nectar, hence the lowest sugar output of that cultivar.

Available literature consistenly reports that all winter rapeseed cultivars but also spring rapeseed (Bobrzecka and Bobrzecki 1973) are dominated by honeybees. The present study with spring rapeseed yielded similar data with honeybees accounting for more than 90% of all pollinating insects. At full blooming during the peak foraging hours 5-6 foragers were working the flowers within an area of 1 m^2 at the same time, a density similar to that observed by Bobrzecki and Bobrzecka (1973). The relatively low density on cv. Margo was due to the presence of male sterile plants, the latter being not only devoid of pollen but, on top of that, poor nectar secretors.

Many investigators demonstrated a positive impact of pollinating insects on rapeseed yields (Mesquida and Renard 1981, Woźnica 1982, Fries and Stark 1983, Jabłoński et al. 1985, Mesquida et al. 1988b). This study was in full agreement with those findings. A higher rate of open pollination pod setting in the year 2000 compared to that in previous years could be explained by higher insect densities in that year. However, neither the number of flowers per 1 m^2 nor nectar secretion rate were above the average in that year. Even more pronounced was the positive effect of pollinator insects on the number of seeds per pod. Whereas under open pollination the pods contained the same number of seeds in 2000 as in the preceding years the pods developed under cover contained $^2/_3$ fewer seeds than those developed under open pollination.

CONCLUSIONS

Spring rapeseed is a valuable honey crop for central Poland enriching the main honey flows as its blooming period extends over the second half of June and the beginning of July and lasts for ca. 20 days. During that time spring rapeseed yields ca. 30-80 kg of sugars and 35-120 kg of pollen depending on cultivar.

Spring rapeseed stands from low seeding densities were found to branch profusely. Owing to that the flower density of ca. 11,000 flowers per 1 m^2 was maintained at both 56 and 32 plants per 1 m^2.

Cvs. Licosmos and Unica were found to be the best performers as honey and pollen

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plants, the hybrid cultivar Margo was the poorest.

Each flower of the spring rapeseed cultivars can produce ca. 0.5 mg of sugars in nectar and yield an average of 0.9 mg of pollen during their lifetime. The flowers are eagerly visited by pollinating insects of which 90% are honeybees. At full blooming during the peak foraging hours there was an average of 6 foragers per 1 m^2 of blooming rapeseed stand.

A beneficial effect of pollinating insects on seed setting was proven for all spring rapeseed cultivars but the highest it was for the hybrid cultivar, which relied most heavily on pollen transfer for seed production. The rate of pod setting was 44% for free insect access and only 37% for restricted access situation. For the hybrid cv. Margo the respective figures were 40% and as little as 18%.

Not only did open pollination result in 10% more pods but the pods contained 50% more seeds as compared to pods developed under exclusion of insects.

The absence of pollinating insects resulted in seed yield reduction that was highest for cv. Margo - 69%, 46% for cv. Unica and within 29-36% for the remaining cultivars.

Compared to winter forms spring rapeseed cultivars had similar pollination requirements, yielded similar amounts of pollen but their nectar output was lower by $^1/_3$.

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WARTOŚĆ PSZCZELARSKA I POTRZEBY ZAPYLANIA PODWÓJNIE ULEPSZONYCH ODMIAN RZEPAKU JAREGO (*Brassica napus* L. *var. oleifera* Metzger *f. annua* Thell.)

Zbigniew Kołtowski

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

W doświadczeniach przeprowadzonych w Oddziale Pszczelnictwa ISK w Puławach w latach 1998-2000 badano wartość pszczelarską i wymogi zapylania 6 odmian rzepaku jarego (Licosmos, Lisonne, Margo, Sponsor, Star i Unica).

Stwierdzono, że średnio rzepak jary kwitnie w drugiej połowie czerwca i początkach lipca, przez okres około 20 dni i wytwarza na 1 m^2 od 10 do 15 tysięcy kwiatów. Dziesięć kwiatów wydzielało w nektarze w ciągu swojego życia średnio 4,63 mg cukrów i dostarczało średnio 8,80 mg pyłku.

Wydajność cukrową populacyjnych odmian rzepaku jarego określono na 40-80 kg z 1 ha, a wydajność pyłkową na 85-120 kg. Najlepsze pod względem wartości pszczelarskiej okazały się odmiany Licosmos oraz Unica. Odmiana mieszańcowa złożona Margo dostarczała tylko około 30 kg cukrów i tyleż pyłku z 1 ha.

Wszystkie odmiany rzepaku jarego były chętnie odwiedzane przez owady zapylające, a głównie przez pszczołę miodną stanowiącą 92% wszystkich owadów spotykanych na kwiatach. W okresie pełni kwitnienia, podczas szczytowych godzin oblotu, przy ładnej pogodzie spotykano średnio 6 zbieraczek na 1 m^2 kwitnącego łanu.

Kwiaty swobodnie oblatywane przez owady zawiązywały łuszczyny średnio w 44%, a w warunkach braku obecności owadów średnio w 37%, natomiast mieszańcowa odmiana Margo tylko w 18%. Łuszczyny po swobodnym zapyleniu kwiatów zawierały średnio po 15 nasion, a powstałe z kwiatów izolowanych średnio po 10 nasion.

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Plony nasion na swobodzie wynosiły średnio 25q/ha, a w warunkach izolacji o około 40% mniej. Największy spadek plonu wywołany brakiem owadów zapylających (do 70%) wykazała odmiana Margo, następnie Unica (do 50%), a pozostałe odmiany w granicach około 30%.

Słowa kluczowe: rzepak jary, nektarowanie, pylenie, zapylanie, zawiązywanie nasion.

TOXICITY OF Agrosol Z WITH ADDITION OF THE MIXTURE OF THE FERTILIZERS AND PESTICIDES TO THE HONEY BEES IN LABORATORY CONDITIONS

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Summary

The aim of the study was to determined the effect of 0.33% and 1% Agrosol Z in the mixtures with urea, fungicide, and insecticide. The research was conducted in two variants:

1. Agrosol Z 0.33% and 1% with: 10% urea solution, Aminopielik, Dithane, Fastac;
2. Agrosol Z 0.33% and 1% + 10% urea solution with: Aminopielik, Dithane, Fastac.

Aminopielik, Dithane, and Fastac were administered in doses than recommended by the manufacturer. The mortality of bees and intake of food and water were determined during 10 days of experiment. The histological examination was performed, and a paraffin method and Novum azane staining were applied. The presence of basic and acidic phosphatase and nonspecific esterase in epithelial cells of the midgut was checked histochemically.

The intake the sugar syrup with Fastac caused the highest increas in bee mortality. The histological and histochemical examination showed some disturbances in functioning of the midgut. These changes, however, were not permament, and ceased after 48 hours.

Keywords: honey bee, Agrosol Z, Aminopielik, Dithane, Fastac.

INTRODUCTION

Mixed fertilizers are to be the substance not dangerous for bees. In papers there is only few information about its destructiveness however the problem of bees intoxication by fertilizers was the subject of trials (Gromisz Z. 1980). Some danger for bees is top-dressing of forests, meadows and fields when fertilizers stay on the plants and with water can be taken and carried by bees to the hive. Mineral fertilizers are on the leaves and on the soil. According to economically purpose fertilization on the leaves is recommended. Danger of intoxication of bees is pointed out by Kostecki and Lipa (1969).

That fertilizers are special compounded concentrates for proper growth and need of plants in appropriate agriculture. Fertilizers let the plant take the nutritious components in the periods of intensive vegetation. Advantage of these fertilizers is easy to dose and usage in agriculture. It is said that they are safer for environment. In order to lower the costs of plant agriculture the tendency of combined usage of various mixtures of these fertilizers with pesticides (herbicides, fungicides, insecticides) is observed.

Most of on the leaves fertilizers as a pure substance and in fertilizer-pesticide mixture are used in a splay way on plants in the period of time from the end of tillering to the beginning of heading. Splayed agriculture with the appearing dew-drops can be potential source of water for bees in the period of its lack around apiary.

The aim of the study was to estimate the potential mesenteric toxicity of Agrosol Z with additions of urea, fungicide, herbicide

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and insecticide for bees and observation of the influence of these substance on the intestinal tract, specially digestive intestine in experimental bees.

MATERIALS AND METHODS

Agrosol Z in a concentrate of fertilizer for on the leaves administration of corn grain and seed grasses. It contains 12% (gravimetric) of nitrogen, 1.8% of potassium and 5% of magnesium. According to producer's advice it can be mixed with urea and pesticides necessary in the situation.

Study was carried out in the year 2000 and 0.33% (minimal) and 1% (maximal) solution of Agrosol Z was used as a working concentration. As an additives an urea (10% solution), fungicide (Dithane M-45), herbicide (Aminopielik) and insecticide (Fastac 10 EC) were used. Selection and concentration of used products were according to recommended in agriculture (Mrówczyński et al. 1991).

there were two levels of experiment. First concerned using Agrosol Z in both concentrations with additives: urea, Dithane, Aminopielik, Fastac 10 EC. During the second level mentioned pesticides were added to the mixture of Agrosol Z and urea. 17 groups of experimental and control bees were prepared according to the following outline:

Group	Notes
I- [Ag 0.33]	0.33% Agrosol Z
II- [Ag 1]	1% solution Agrosol Z
III- [Ag 0.33+H]	0.33% solution Agrosol Z with Aminopielik
IV- [Ag 1+H]	1% solution Agrosol Z with Aminopielik
V- [Ag 0.33+F]	0.33% solution Agrosol Z with Dithane
VI- [Ag 1+F]	1% solution Agrosol Z with Dithane
VII- [Ag 0.33+I]	0.33% solution Agrosol Z with Fastac
VIII- [Ag 1+I]	1% solution Agrosol Z with Fastac
IX- [Ag 0.33+U]	0.33% solution Agrosol Z with urea
X- [Ag 1+U]	1% solution Agrosol Z with urea
XI- [Ag 0.33 +U+H]	0.33% solution Agrosol Z with urea and Aminopielik
XII- [Ag 1+U+H]	1% solution Agrosol Z with urea and Aminopielik
XIII-[Ag 0.33+U+F]	0.33% solution Agrosol Z with urea and Dithane
XIV-[Ag 1+U+F]	1% solution Agrosol Z with urea and Dithane
XV- [Ag 0.33+U+I]	0.33% solution Agrosol Z with urea and Fastac
XVI- [Ag 1+U+I]	0.33% solution Agrosol Z with urea and Fastac
XVII- Control	Sugar syrup

Notes: Ag - Agrosol, H - Aminopielik (Herbicide), F - Dithane (Fungicide), I - Fastac (Insecticide), U - urea

Sugar syrup with adequate contents of Agrosol Z and additives was given only once and was taken away after 24 hours. After that experimental groups were given 50% syrup and water to the end of experiment. Control groups were supplied with pure syrup and water during all the days of experiment.

Observations last for 10 days. Feed and water consumption per one bee and mortality in experimental and control groups were observed. To the research 51 group of 50 bees each were used and all series were repeated three times.

To histological and histoenzymatic tests new 24 experimental and 3 control groups were formed. All these groups were given feed only with higher concentration of Agrosol Z (1%) and the combination of additives was the same like in first two levels of experiment. In these groups after 24 hours feed was taken away and the bees were given pure syrup and water. After 24, 48 and 96 hours after eating the feed with additives bees from each group were taken to examination. 15 to take midgut to

List 1

Consumption of sugar syrup with admixtures in nitrogen (N), magnesium (Mg) and potassium (K) (µg/bee). - Spożycie pokarmu z dodatkami w przeliczeniu na azot (N), magnez (Mg) i potas (K) (µg/pszczołę).

Group/component Grupa/składnik		N	+/-	Mg	+/-	K	+/-
I	Ag 0.33	16.40	0.00	3.93	0.00	2.30	0.00
II	Ag 1	38.20	1.85	9.12	0.46	5.23	0.25
III	Ag 0.33+H	25.90	16.25	6.20	3.88	3.60	2.24
IV	Ag 1+H	65.29	33.26	15.61	8.93	9.12	4.65
V	Ag 0.33+F	13.84	2.29	3.32	0.54	1.93	0.31
VI	Ag 1+F	59.76	34.66	14.29	8.29	8.35	4.84
VII	Ag 0.33 +I	3.64	0.63	0.86	0.15	0.50	0.09
VIII	Ag 1 + I	5.53	1.91	1.31	0.46	0.76	0.26
IX	Ag 0.33+U	942.94	0.00	2.62	0.00	1.52	0.00
X	Ag 1+U	772.16	96.52	6.35	0.80	3.71	0.47
XI	Ag 0.33+U+H	502.90	54.44	1.39	0.15	0.81	0.09
XII	Ag 1+U+H	289.56	48.35	2.38	0.80	1.09	0.52
XIII	Ag 0.33+U+F	687.52	193.91	2.82	1.89	1.64	1.10
XIV	Ag 1+U+F	241.30	68.24	1.98	0.56	1.15	0.33
XV	Ag 0.33+U+I	188.59	0.00	0.52	0.00	0.30	0.00
XVI	Ag 1+U+I	96.52	0.00	0.79	0.00	0.46	0.00

Notes: Ag - Agrosol, H - Aminopielik (Herbicide), F - Dithane (Fungicide), I - Fastac (Insecticide), U - urea

histological tests and 15 to histoenzymatic tests. In histological tests the paraffin method was used with Novum azane staining and frozen technique to histoenzymatic tests. Histoenzymatic tests of epithelial midgut cells estimated the activity of three enzymes: basic and acidic phosphatases and non-specific esterase alpha using the method described in previous own research (Tomaszewska, Chorbiński 1999).

Consumption of feed with Agrosol Z with additives was calculated according to feed of nitrogen, magnesium and potassium in mg per one bee in order to standardize the results and for interpretation (Tomaszewska, Chorbiński 1997, 1999) (list 1). Mean percentage of bees mortality for listed groups was calculated for counting according to Abbot formula (Lipa, Śliżyński 1973). Variance analysis (α=0.05) with regards to corrected values according to Abbot formula and real values were made for all groups for the of 10 days period of experiment. Results were statistically calculated and showed in lists.

RESULTS AND DISCUSSION

Once given feed with addition of pure Agrosol Z (groups I and II) did not cause the increase of mortality in experimental bees. Equally the addition of pesticide

List 2

Total percent of bees mortality after single application of Agrosol Z in the fertilizer-pesticide mixtures. - Ogólny procent śmiertelności pszczół po jednorazowym podaniu Agrosolu Z w mieszankach nawozowo-pestycydowych.

Group/days Grupa/dni		1	2	3	4	5	6	7	8	9	10
I	Ag 0.33	1.2	1.2	1.2	1.8	2.4	3	5	7.6	8.8	10.8
II	Ag 1	0	0.6	0.6	1.8	1.8	1.8	2.4	2.4	3	3.6
III	Ag 0.33+H	0	0.6	0.6	0.6	0.6	0.6	0.6	1.8	1.8	3
IV	Ag 1+H	2	3.2	3.2	3.8	3.8	4.4	4.4	4.4	5	7
V	Ag 0.33+F	0.6	1.2	3.2	4.4	4.4	5	7	7	11	13.4
VI	Ag 1+F	4	5.2	7.6	10	12	16.6	26	29.4	32.8	38.8
VII	Ag 0.33 +I	94.6	94.6	94.6	94.6	94.6	94.6	94.6	94.6	94.6	94.6
VIII	Ag 1 + I	89.3	89.3	89.3	89.9	91.1	91.7	91.7	93.7	93.7	93.7
IX	Ag 0.33+U	0	0	0.6	4	4.6	4.6	4.6	4.6	7	10.4
X	Ag 1+U	0	1.2	1.8	2.4	2.4	2.4	2.4	2.4	3	3
XI	Ag 0.33+U+H	2.6	5.2	5.2	5.2	6.4	7.6	8.8	10	10.6	11.2
XII	Ag 1+U+H	3.2	3.2	3.8	5.8	5.8	7.8	8.4	9.6	10	12
XIII	Ag 0.33+U+F	1.2	1.8	4.4	6.4	7.6	10.8	14	14.6	15.8	18.4
XIV	Ag 1+U+F	0	0.6	1.8	1.8	1.8	5	5.6	5.6	6.2	6.8
XV	Ag 0.33+U+I	70.6	70.6	70.6	70.6	70.6	72.2	72.8	74	76	79.2
XVI	Ag 1+U+I	58.6	58.6	58.6	58.6	59.2	59.2	60.4	61.6	64	64.6
XVII	Control Kontrola	1.5	1.5	1.5	3.5	4.5	7	8	10.5	11.5	13

Notes: Ag - Agrosol, H - Aminopielik (Herbicide), F - Dithane (Fungicide), I - Fastac (Insecticide), U - urea

(groups III and IV) did not increase the mortality either. But Agrosol Z in mixture with fungicide and insecticide (Fastac) caused significant increase of mortality in experimental bees. In group VII and VIII (with Fastac) the death ratio was more than 90% (list 2). Fastac chemically based on synthetic pyretroid is known as non toxic midgut activity substance for bees. Gromisz Z. and Gromisz M. (1994) in their research pointed out that during testing of Fastac the high variability in behaviour and mortality were observed. Perhaps in our experiment there was the addition effect of two active preparation that influenced on bees. Wallner (1999) points out about intoxication of bees in the fields during simultaneously used Fastac with fungicides (Folicur and Caramba).

In the groups being given 10% additives of urea in first 5 days of experiment significant decrease of bees mobility assembling in the bottom of the cage and diarrhoea were noticed. Feed with urea addition was taken in lower number which might be the reason of lower mortality of bees in group XV and XVI even after Fastac addition (list 2 and 3). Aversion of bees to take feed with higher concentration of urea and presence of diarrhoea were observed in previous own

List 3

Consuption of sugar syrup after single application of Agrosol Z in the fertilizer-pesticide mixtures (µl/bee). - Spożycie pokarmu cukrowego po jednorazowym podaniu Agrosolu Z w mieszankach nawozowo-pestycydowych w (µl/pszczołę.

Group/days grupy/dni		1	2	3	4	5	6	7	8	9	10
I	Ag 0.33	46	21	30	30	32	29	30	36	37	38^{e}
II	Ag 1	50	18	29	35	23	34	30	40	37	40^{e}
III	Ag 0.33+H	37	21	23	28	20	28	29	33	28	33^{cde}
IV	Ag 1+H	39	21	24	24	20	27	25	35	29	35^{cd}
V	Ag 0.33+F	47	26	26	36	28	31	35	46	35	33^{e}
VI	Ag 1+F	51	24	31	37	26	33	31	41	27	34^{e}
VII	Ag 0.33 +I	20	20	20	20	20	20	20	20	20	20^{ab}
VIII	Ag 1 + I	30	30	30	30	30	30	30	30	30	30de
IX	Ag 0.33+U	37	17	15	17	11	15	17	19	17	23^{a}
X	Ag 1+U	32	19	19	18	13	14	16	21	17	25^{ab}
XI	Ag 0.33+U+H	26	18	16	16	7	12	10	20	18	26^{a}
XII	Ag 1+U+H	33	26	22	24	17	18	22	32	28	30^{bc}
XIII	Ag 0.33+U+F	36	15	13	14	9	12	14	23	19	23^{a}
XIV	Ag 1+U+F	46	16	14	17	17	13	22	22	25	26^{ab}
XV	Ag 0.33+U+I	45	63	19	19	38	15	30	30	34	33^{e}
XVI	Ag 1+U+I	48	18	12	15	22	16	28	24	22	30^{ab}
XVII	Control Kontrola	47	21	34	37	23	30	27	35	32	39^{e}

Notes: Ag - Agrosol, H - Aminopielik (Herbicide), F - Dithane (Fungicide), I - Fastac (Insecticide), U - urea

research. The lesions and effects of syrup with urea consumption are highly correlated with the volume of feed taken (Chorbiński, Tomaszewska 1995).

During 10 days of experiment the significant variability in syrup consumption volume which was the effect of calculation of microliters per one experimental bee. Statistically significant lower consumption in comparison to the control group was noticed in the group number VII - IX - XIV and XVI, mainly in the groups fed with urea addition. Water consumption was various and statistically significant in groups number VII, VIII, X, XV. Bees from these groups took much more water than other experimental and control groups (list 4).

In histological examination it was noticed that pure Agrosol Z with additions of Aminopielik did not cause visible changes in histological view of midgut. In most of cases an intensive shelling of apical parts of epithelial cells and increase of peritrophic membranes production were observed. These lesions were specially visible in all groups which took feed with addition of urea and in groups being given Agrosol Z with fungicide addition (Dithane).

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List 4

Consuption of water after single application of Agrosol Z in the fertilizer-pesticide mixtures (µl/bee). - Spożycie wody po jednorazowym podaniu Agrosolu Z w mieszankach nawozowo-pestycydowych w (µl/pszczołę).

Group/days Grupy/dni		1	2	3	4	5	6	7	8	9	10
I	Ag 0.33	4	3	2	3	2	3	1	3	3	3[a]
II	Ag 1	3	3	1	4	2	2	1	3	2	2[a]
III	Ag 0.33+H	5	2	3	3	3	3	2	2	2	2[a]
IV	Ag 1+H	2	2	2	2	2	3	1	1	1	1[a]
V	Ag 0.33+F	2	2	1	2	3	2	2	2	1	2[a]
VI	Ag 1+F	2	2	1	2	3	2	2	2	1	2[a]
VII	Ag 0.33 +I	5	5	5	5	5	5	5	5	5	5[d]
VIII	Ag 1 + I	5	5	5	5	5	5	5	5	5	5[d]
IX	Ag 0.33+U	3	4	2	3	3	4	2	3	3	3[a]
X	Ag 1+U	4	3	2	2	3	6	3	3	3	3[b]
XI	Ag 0.33+U+H	3	2	2	3	3	2	2	2	2	2[a]
XII	Ag 1+U+H	2	2	2	3	3	3	2	4	2	3[a]
XIII	Ag 0.33+U+F	2	3	2	2	3	4	1	3	2	3[a]
XIV	Ag 1+U+F	3	4	2	2	4	2	4	4	2	3[ab]
XV	Ag 0.33+U+I	12	14	6	4	4	2	1	1	2	3[c]
XVI	Ag 1+U+I	2	4	3	3	4	2	2	1	2	2[a]
XVII	Control Kontrola	3	3	1	2	2	3	2	2	1	2[a]

Notes: Ag - Agrosol, H - Aminopielik (Herbicide), F - Dithane (Fungicide), I - Fastac (Insecticide), U - urea.

Described lesions were temporary and regenerative processes like highly active reparation centres were seen in the 96th hour of experiment.

Fluctuating activity of chosen enzymes in midgut epithelial cells of experimental bees (evaluation: +, ++, +++) was highest marked in the 24th and 28th hour of experiment and were temporary similarly to histological lesions (list 5).

Only in group VIII [Ag 1+U+I] (experimental bees to histological and histoenzymatic tests fed with 1% Agrosol Z and 10% addition of urea and Fastac) that was impossible to do such a tests because of very high mortality level.

Results from laboratory conditions tests indicates for danger of bees intoxication correlated with usage Fastac and Dithane in ferrilizer-pesticedes mixtures on the leaves of plants.

CONCLUSIONS

1. Usage of on the leaves fertilizers in agriculture in mixtures with Fastac 10 EC and Dithane can be a reason of bees intoxication.

List 5

Activity of nonspecific esterase alpha (EN), acidic phosphatase (FK) and basic phosphatase (FZ) in epithelial cells of the midgut of honeybee after single application of Agrosol Z in the fertilizer-pesticide mixtures. - Aktywność esterazy niespecyficznej alfa (EN), fosfatazy kwaśnej (FK) i zasadowej (FZ) w jelicie środkowym pszczoły miodnej po jednorazowym podaniu 1% roztworu Agrosolu Z w mieszankach nawozowo-pestycydowych.

Enzyme Enzym		EN - EC			FK - EC			FZ - EC		
Group/hour Grupa/godz.		24	48	96	24	48	96	24	48	96
I	Ag 1	++	+	++	++	+	++	+++	++	++
II	Ag 1 + H	++	++	++	++	++	++	+++	++	++
III	Ag 1 + F	++	+++	++	++	++	++	++	++	+
IV	Ag 1 + I	+++	+++	++	++	+	++	+++	++	++
V	Ag 1 + U	+++	+++	++	+++	+	++	++	++	+
VI	Ag 1 + U + H	+++	+++	+	+	++	++	++	+++	++
VII	Ag 1 + U + F	++	+++	++	+	++	+	++	+++	++
VIII	Ag 1 + U + I	+	0	0	+	0	0	++	0	0
IX	Control Kontrola	++	++	++	++	++	++	+++	++	++

Notes: Ag - Agrosol, H - Aminopielik (Herbicide), F - Dithane (Fungicide), I - Fastac (Insecticide), U - urea, 0 - not observed (very high mortality level)

2. Using fertilizers specially with addition of insecticides the prevention procedures must be strongly obeyed and also phenomenon of synergism of mixture compounds must be taken under condition.

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TOKSYCZNOŚĆ DLA PSZCZÓŁ Agrosolu Z Z DODATKIEM MIESZANEK NAWOZOWO-PESTYCYDOWYCH W WARUNKACH LABORATORYJNYCH

B. Tomaszewska, P. Chorbiński

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

Celem badań było określenie w warunkach laboratoryjnych wpływu na pszczoły 0.33% i 1% Agrosolu Z w mieszankach z mocznikiem, fungicydem i insektycydem. Badania prowadzono w dwóch wariantach:

1/ Agrosol Z 0.33% i 1% z dodatkami: 10% roztwór mocznika, Aminopielik, Dithane, Fastac

2/ Agrosol Z 0.33% i 1% + 10% roztwór mocznika z dodatkami: Aminopielik, Dithane, Fastac.

Aminopielik, Dithane i Fastac podawano w syropie cukrowym, w ilościach odpowiadających stężeniom zalecanym w rolnictwie. W ciągu 10 dni obserwacji notowano liczbę martwych pszczół oraz spożycie pokarmu i wody. Wyniki badań poddano obliczeniom statystycznym z zastosowaniem analizy wariacji (α=0.05). Najwyższy procent śmiertelności zanotowano w pierwszej dobie doświadczenia u pszczół otrzymujących w pokarmie dodatek preparatu Fastac. Wykonano również badania histologiczne i histoenzymatyczne jelita środkowego pszczół. W badaniach histoenzymatycznych uwzględniono aktywność esterazy niespecyficznej, fosfatazy kwaśnej i zasadowej. Stwierdzono, że obserwowane zmiany histologiczne w obrębie nabłonka jelita środkowego jak i wahania aktywności enzymatycznej miały charakter przejściowy.

Słowa kluczowe: pszczoła miodna, Agrosol Z, mocznik, Aminopielik, Dithane, Fastac.

HARMFULNESS OF THE SELECTED FOLIAR FERTILIZERS TO THE HONEY BEES IN LABORATORY CONDITIONS

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S u m m a r y

The stomachic toxicity of three foliar fertilizers - Insol TP, Agrosol Z and RSM has been established in laboratory conditions. The experimental and control groups were composed of 50 bees. The 0.5% and 1.5% solution of the Insol TP, 0.33% and 1% of the Agrosol Z, 2% and 4% of the RSM was applied in 50% sugar syrup to experimental insects once. In the 10-day experiment the bees mortality and food and water consumption was monitored. The real and corrected data were statistically analyzed according to the Abbot formula. The physiological condition of the midgut of the experimental bees was examined histologically and histochemically. No sudden increase in mortality of honey bees after consuming any of the fertilizers was observed. The intake in the sugar syrup with RSM (0.147 mgN/bee) caused the highest increas in bee mortality (significant difference, the real data). The histological and histochemical examination showed some disturbances in functioning of the midgut. These changes, however, were not permament, and ceased after 48 hours.

Keywords: honey bee, toxicity, Insol TP, Agrosol Z, RSM.

INTRODUCTION

Foliar fertilizers are the concentrates speciallyproduced to provide the needs of growth of appropriate agriculture. They let the plants be supplied with necessary ingredients during intensive vegetation periods. The advantage of these preparations is easy way of dosage and usage in the agriculture. Also they are considered to be safe for environment.

Most of foliar fertilizers are used in a splay way in the period of time from the end of tillering to the beginning of heading. Splayed agriculture can be potential source of water for bees in the time of its lack around apiary. taking the water from the plants bees also carry fertilizers diluted in the liquid.

Most dangerous for insects can be foliar fertilizers being used on grounds, corns and weed areas.

The aim of the study was to estimate stomachic toxicity of three concentrated foliar fertilizers - Insol TP, Agrosol Z and RSM for bees in laboratory conditions.

MATERIAL AND METHODS

Insol TP is foliar fertilizer used for fertilization of meadows and grass-lands. Agrosol Z is a concentrate devoted to foliar supply of corm and seed grasses and RSM is a saltpetre-urea fertilizer used for top-dressing during vegetation period of plants.

Experimental groups consisted of 50 bees and came from one hive. Solutions of fertilizers as additions were given once with 50% sugar syrup. Following concentrations of fertilizers were used: Insol TP -0.5% and 1.5%, Agrosol Z -0.33% and 1%, RSM -

List 1

Groups Grupy	Fertilizer Nawóz	Consumption of fertilizers in mgN/bee and mgMg/bee Spożycie nawozu w mgN/pszczołę i mgMg/pszczołę)
I	Insol TP	0.0035 mgN (+/- 0.0005); 0.009 mgMg (+/- 0.0001)
II	Insol TP	0.0069 mgN (+/- 0.0006); 0.0025 mgMg (+/- 0.0002)
III	Agrosol Z	0.0076 mgN (+/- 0.002); 0.0031 mgMg (+/- 0.0008)
IV	Agrosol Z	0.0155 mgN (+/- 0.0031); 0.007 mgMg (+/- 0.0062)
V	RSM	0.0775 mgN (+/- 0.0219)
VI	RSM	0.1745 mgN (+/- 0.0012)
VII	Control Kontrola	-

2% and 4%. Concentration are adequate to the values used in agriculture.

Feed with fertilizers was taken away after 24 hours and for following 10 days of experiment bees were being given pure sugar syrup and water ad libitum, as well as bees from control groups. Every day the number of dead bees and water and syrup consumption were noticed. The study was carried out in the year 1999 and experiment was repeated three times. During the study 48 groups of control and experimental bees were used.

Because of huge differences in consumption of feed with addition of fertilizers in appropriate experimental groups the intake of feed was calculated to elements consumption (Chorbiński, Tomaszewska 1995, Tomaszewska, Chorbiński 1997, 1999). In the case of Insol TP and Agrosol Z the consumption of nitrogen and magnesium was calculated per one bee, and in the case of RSM it was calculated in the mg of nitrogen per one bee. Independently from fertilizers concentrations given in sugar syrup but according to intake of nitrogen and magnesium per experimental bees, 6 experimental groups were created (list 1).

The average percentage of bee mortality adequate to fertilizers intake in experimental groups was corrected according to Abbot formula (Lipa, Śliżyński 1973). Variance analysis ($\alpha = 0.05$) with regards to corrected values to Abbot formula and real values was made for all groups for 10 days period of experiment. results were statistically calculated and shown in lists.

Also histological and histoenzymatic examinations were made on the midgut of experimental bees. To these examinations 18 experimental and 3 control groups of bees were created additionally. After 24, 48 and 96 hours after taking the feed with addition of fertilizers (concentrations given above) 15 bees from each group were taken and midgut were tested. In order to estimate the status of midgut in histological examination the paraffin method was used. Histoenzymatic tests of midgut epithelial cells were provided with the use of frozen technique described in former papers (Tomaszewska, Chorbiński 1997, 1999). Histoenzymatic tests of midgut epithelial cells estimated the activity of three enzymes: non-specific esterase alpha, basic and acidic phosphatase. The activity of enzymes was described as +, ++, +++.

RESULTS AND METHODS

Addition of Insol TP in feed did not cause significant increase of bee mortality in groups I and II but statistically significant decrease of mortality was noticed (according to values corrected with Abbot formula) in group II in comparison to the control group. Also addition of Agrosol Z did not cause significant increase of mortality level in experimental groups II and IV either.

Statistically significant increase of mortality level (according to real values) was noticed after giving the feed with RSM addition for the bees in group VI (0.174 mgN) in comparison to the group V (0.077 mgN) and to control group (list 2).

Water and sugar syrup consumption in the groups given Insol TP and Agrosol Z (groups I-IV) did not vary in comparison to the control group. Inthe case of RSM addition group VI (0.174 mgN) showed statistically lower sugar syrup consumption to group V (given RSM in lower concentration) and to the control group too. feed intake results are shown in list 3.

Water consumption in all experimental groups (I-VI) did not vary from consumption in control group (list 4).

List 2

Percent mortality of bees after single application of the Insol T, Agrosol Z and RSM.
Śmiertelność pszczół po jednorazowym podaniu Insolu TP, Agrosolu Z i RSM w syropie cukrowym (w %).

Groups Grupy	Number of examined bees (groups) Liczba testowanych owadów (grup)	Days of experiment Dni doświadczenia									
		1	2	3	4	5	6	7	8	9	10
Control Kontrola	600 (12)	1	1	2	4	4	4	6	6	6	8
I 0.0035 mgN 0.009 mgMg	300 (6)	1.5	2	2.5	2.5	3.5	4	5	5	5	5.5
II 0.0069 mgN 0.0025 mgMg	300 (6)	1	1	1	1	1	1	1	1	1	2*
III 0.0076 mgN 0.0031 mgMg	300 (6)	1	1.5	1.5	2	3	3.5	3.5	4.5	4.5	4.5
IV 0.0155 mgN 0.007 mgMg	300 (6)	1	1	2	3	3	4	5	7	9	9.5
V 0.0775 mgN	300 (6)	0	2.5	5	6	6	6.5	7	7	7	7
VI 0.1745 mgN	300 (6)	3	3	9	13	14	15	16	16	18	20#

Notes: Objaśnienia:
Groups according to consumption of fertilizers in mgN/bee and mgMg/bee
Grupy zestawione wg spożycia nawozów w przeliczeniu na mgN/pszczołę i mgMg/pszczołę.
* - significant difference, the data corrected to the Abbot formula (alfa = 0.05)
* - różnica statystycznie istotna dla wartości skorygowanych wg wzoru Abbota (alfa = 0.05)
# - significant difference, the real data (alfa = 0.05).
# - różnica statystycznie istotna dla wartości rzeczywistych (alfa = 0.05)

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List 3

Consumption of sugar syrup by bees after single application of the Insol TP, Agrosol Z and RSM (in µl/bee). - Spożycie pokarmu przez pszczoły po jednorazowym podaniu Insolu TP, Agrosolu Z i RSM (w µl/pszczołę)

Groups Grupy	Number of examined bees (groups) Liczba testowanych owadów (grup)	Days of experiment Dni doświadczenia									
		1	2	3	4	5	6	7	8	9	10
Control Kontrola	600 (12)	30	30	26	25	26	26	23	40	26	33
I 0.0035 mgN 0.009 mgMg	300 (6)	28.5	22.5	25	29	24.5	24	28	31.5	26.5	32.5
II 0.0069 mgN 0.0025 mgMg	300 (6)	28	26	23	32	34	30	29	37	30	37
III 0.0076 mgN 0.0031 mgMg	300 (6)	37	25	26	21	27	28	28	29	23	29
IV 0.0155 mgN 0.007 mgMg	300 (6)	28	22	24	26	22	27	26	26	26	30
V 0.0775 mgN	300 (6)	40	22	28	25	30	24.5	23	30	23	32
VI 0.1745 mgN	300 (6)	37	12	18	20	20	23	16	23	15	28*

Notes: Objaśnienia:
Groups according to consumption of fertilizers in mgN/bee and mgMg/bee
Grupy zestawione wg spożycia nawozów w przeliczeniu na mgN/pszczołę i mgMg/pszczołę.
*- significant difference (alfa = 0.05).
* - różnica statystycznie istotna (alfa = 0.05)

During experiment there was no sign of aversion of bees to take the feed with addition of fertilizers. Gromisz M. (1999) testing the influence of consumption of feed with addition of Insol 5 and Insol 6 on bees observed that bees can limit the intake of feed with microelements by themselves showing it as a variability in feed consumption. In our experiment with the use of Insol TP there was no such a limitation.

Histological tests of midgut of experimental bees done in 24th, 48th, and 96th hour after taking away the feed with fertilizers did not show significant impact of tested preparation on the enterocytes.

Only small dysfunction in peritrophic membrane production in midgut in the 24th hour of experiment in bees given RSM in both concentration (2% and 4%). Also the residues of high shelling activity of midgut epithelium after taking the feed with addition of Agrosol Z in both concentration was observed. there was no differences in histological view of midgut in experimental and control groups of bees in 48th and 96th hour.

List 4

Consumption of water by bees after single application of the Insol TP, Agrosol Z and RSM (in µl/bee). - Spożycie wody przez pszczoły po jednorazowym podaniu Insolu TP, Agrosolu Z i RSM (w µl/pszczołę).

Groups Grupy	Number of examined bees (groups) Liczba testowanych owadów (grup)	Days of experiment Dni doświadczenia									
		1	2	3	4	5	6	7	8	9	10
Control Kontrola	600 (12)	1	2	3	4	3	2.5	2	2	1	3.5
I 0.0035 mgN 0.009 mgMg	300 (6)	2.5	1.5	3.5	1.5	2.5	2	2.5	0.5	2.5	3.5
II 0.0069 mgN 0.0025 mgMg	300 (6)	3	2	2	1	2	2.5	2	1	4	4
III 0.0076 mgN 0.0031 mgMg	300 (6)	3	2.5	3	3	3	3	2.5	2	2.5	2.5
IV 0.0155 mgN 0.007 mgMg	300 (6)	3	2.5	3	4	3	4.5	2.5	2	2.5	3
V 0.0775 mgN	300 (6)	3	3	3	2	2.5	3	3	1.5	3	3
VI 0.1745 mgN	300 (6)	1	1.5	3	2	3	3.5	3	3	3	4

Notes see list 3. Objaśnienia patrz tab. 3

List 5

Activity of the nonspecific eterase (EN), and the acidic (FK) and basis (FZ) phosphatase after single application Insol TP, Agrosol Z and RSM in the midgut epithelial cells of the experimental bees.
Aktywność esterazy niespecyficznej (EN), fosfatazy kwaśnej (FK) i zasadowej (FZ) po jednorazowym podaniu Insolu TP, Agrosolu Z i RSM w komórkach nabłonkowych jelita środkowego doświadczalnych pszczół.

Enzyme - enzym	EN - EC			FK - EC			FZ - EC		
Time (h) Czas (h) Groups Grupy	24	48	96	24	48	96	24	48	96
Control Kontrola	++	+++	+++	++	+++	++	+++	+++	+++
Insol TP - 1.5%	++	+++	++	+++	+++	+++	+++	+++	++
Insol TP - 0.5%	+++	+++	+++	+++	++	+++	+++	++	+++
Agrosol Z - 1%	+++	+++	++	+++	+++	++	++	++	++
Agrosol Z - 0.33%	++	+++	+++	+++	+++	+++	+++	+++	++
RSM - 4%	+++	++	++	+++	++	++	++	+	++
RSM - 2%	++	+++	+++	+++	+++	++	++	++	+++

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Histoenzymatic tests estimating the activity of three enzymes (non-specific esterase alpha, basic and acidic phosphatase) in midgut epithelial cells showed variability in activity. But most important is to be the decrease of basic phoshpatase activity after the intake of feed with 1% Agrosol Z and feed with 2% and 4% addition of RSM. It shows that the temporary dysfunction of metabolic reactions in midgut of experimental bees can occur (list 5)

Histological lesions in midgut epithelial cells and variability in enzymatic activity in experimental bees show on some dysfunction in metabolism. But these dysfunction do not have a significant influence on outliving of bees and disappear after 48 hours after taking away the feed with addition of fertilizers.

CONCLUSIONS

1. Single consumption the feed with addition of Insol TP, Agrosol Z and RSM fertilizers by bees in concentration used in agriculture is safe for the bees.
2. Histological changes in bee's midgut being given feed with mentioned fertilizers addition s are temporary and disappear after 48 hours.
3. Variability of enzymatic activity in midgut epithelial cells of experimental bees show on temporary dysfunction of digestion process.

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OCENA SZKODLIWOŚCI WYBRANYCH NAWOZÓW DOLISTNYCH DLA PSZCZÓŁ W WARUNKACH LABORATORYJNYCH

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

W warunkach laboratoryjnych prowadzono badania nad toksycznością żołądkową dla pszczół trzech nawozów dolistnych: Insol TP, Agrosol Z i RSM. Doświadczalnym owadom podawano jednorazowo nawozy w następujących stężeniach: Insol TP - 0,5% i 1,5%, Agrosol Z - 0,33% i 1%, RSM - 2% i 4%. Obserwacje prowadzono przez 10 dni notując procent śmiertelności oraz spożycie pokarmu cukrowego i wody. W badaniach histologicznych posłużono się metodą parafinową przy zastosowaniu barwienia azanem Novum. Wykonano

również badania histoenzymatyczne na obecność esterazy niespecyficznej alfa oraz fosfatazy kwaśnej i zasadowej w komórkach nabłonkowych jelita środkowego pszczół.

Na podstawie przeprowadzonych badań nie stwierdzono gwałtownego wzrostu śmiertelności pszczół po spożyciu wszystkich trzech nawozów. Statystycznie istotny wzrost śmiertelności pszczół (wg wartości rzeczywistych) zanotowano jedynie po podaniu pokarmu z dodatkiem RSM w dawce 0,174 mgN na pszczołę. Nie stwierdzono różnic w intensywności pobierania wody przez doświadczalne owady w porównaniu z pszczołami grup kontrolnych. Statystycznie istotne niższe spożycie pokarmu cukrowego obserwowano w grupie pszczół otrzymującej RSM w podanej wyżej dawce. Badania histologiczne i histoenzymatyczne wykazały pewne upośledzenie funkcjonowania jelita środkowego u doświadczalnych pszczół, ale zjawisko to miało charakter przejściowy i ustępowało po 48 godzinach.

Słowa kluczowe: pszczoła miodna, toksyczność, Insol TP, Agrosol Z, RSM.

PREVENTION OF NATURAL MATING OF INSTRUMENTALLY INSEMINATED QUEEN HONEYBEES BY PROPER METHOD OF INSTRUMENTAL INSEMINATION

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S u m m a r y

Black coloured queens were instrumentally inseminated with semen from yellow coloured drones, and were allowed to mate naturally in an area containing black drones. Since yellow body colour is dominant over black, any black coloured worker progeny would indicate additional natural mating of the mother-queen. Together 308 queens were investigated, and body colour of 188 300 workers was determined.

Nine groups of queens were investigated. Queens of the control group mated solely naturally. The others were instrumentally inseminated either with 8 µl semen and treated with CO_2 or not, or were inseminated twice, with 4 µl of semen each time.

The results show, that among queens inseminated once with 8 µl of semen at the age of 6, 8 and 14 days, 69, 53, 29% respectively mated also naturally, and among those treated additionally with CO_2 two days later, only 14%. Queens treated with CO_2 two days before insemination with 8 µl of semen, as well as those inseminated twice with 4 µl of semen did not mate naturally in addition.

The highest losses occurred within the three groups of queens inseminated exclusively with 8 µl of semen, - 14.0, 13.6 and 10.0% respectively. Within all groups, as more queens flew out for natural mating, more of them were lost ($r = 0.96$). Three times more queens were lost within those which mated solely naturally, or were inseminated exclusively with 8 µl of semen, than within queens inseminated twice with 4 µl of semen.

Queens of the control group, which mated solely naturally, started to lay eggs 3 days after the queen excluders were removed from the entrances. Queens inseminated with 8 µl of semen and treated with CO_2, or inseminated twice with 4 µl of semen started oviposition significantly later (6 - 8 days), and those inseminated exclusively with 8 µl of semen started to lay eggs latest of all (10 - 11 days).

Queens of the control group produced exclusively black worker progeny. Queens inseminated instrumentally exclusively with 8 µl of semen at the age of 6, 8, and 14 days produced 20, 18, and 19% of black coloured worker bees respectively. Queens treated with CO_2 two days after insemination produced 11% of black progeny. Thus queens of the three first groups mated on average also naturally probably with 2 drones, and of the last group with 1 drone.

Due to low losses and absence of additional natural mating within high number of queens inseminated instrumentally twice, the method of double instrumental insemination of queens with 4 µl of semen with subsequent open entrances can be recommend for practical beekeepers.

Keywords: queen honey bees, natural mating, instrumental insemination, CO_2 treatment.

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INTRODUCTION

Instrumental insemination of queen bees is widely applied by beekeepers in Poland. About 30,000 queens are inseminated instrumentally per year. Honey production of colonies headed by queens of particular crosses of breeding lines is much higher than that of colonies headed by sister queens mated naturally. The wide use of instrumentally inseminated queens by beekeepers requires simplifications of dealing with instrumentally inseminated queens. The practice of fixing queen excluders before the entrances of nuclei, in order to prevent natural mating of instrumentally inseminated queens is very inconvenient. Worker bees have difficulties going through the entrances, they loose pollen loads, ventilation is reduced and so on.

Roberts (1944) showed, that queens which mated naturally in one mating flight do not stop to fly out of the hive. Out of 110 observed queens 55 flew for their next mating flights and mated again. Similarly, S. and F Ruttner (1953-54) and Alber et al (1955) proved that queens which mated in one flight mated again in the following flights. Those authors thought that the queens mate with only one drone in one flight. However, Triasko (1951), Taber (1954) and Woyke (1955) showed that queens mate with several drones during a single mating flight. According to Woyke (1956), queens returned from mating flights with 1.13 to 22.39 µl of semen in their oviducts. They had to mate with from 1 to at least 13 drones. Woyke (1958, 1964) showed that queens which stored 5.6 mil spermatozoa in their spermatheca after the first mating flight did not fly out of the hives. Those which stored 4.2 mil flew out but did not mate, and those which stored 3.9 mil spermatozoa mated again in a second mating flight. Thus, the next flights depended upon the number of spermatozoa stored in the spermatheca after the first mating flight. According to Woyke (1960) the queens mate with up to 17 drones, and on average with 8 to 9 drones during one mating flight.

Mackensen (1947) showed that CO_2 treatment of both, virgin and instrumentally inseminated queens accelerated beginning of egg laying. Woyke (1963, 1966) showed that additional CO_2 treatment of queens inseminated once with 4 µl of semen accelerated onset of oviposition, however, did not accelerate when queens were inseminated with 8 µl of semen. But Ebadi and Garry (1980) and Konopacka (1991) reported that queens inseminated once with 8 µl and treated additionally with CO_2 also began oviposition earlier then those untreated additionally. The first authors concluded that 75% CO_2 would be better than 100% for narcotising queen honeybees.

According to Woyke (1963, 1966) queens inseminated twice began oviposition earlier than inseminated once with the same total amount of semen. However, Prabucki et al (1987) and Woyke and Jasiński (1990) reported that queens inseminated twice did not begin oviposition earlier than inseminated once. Jasiński (1993) showed, that second insemination of queens which did not lay eggs during 3 weeks resulted in onset of oviposition.

Skowronek (1976) and Kaftanoglu and Peng (1982) reported that virgin queens treated with CO_2 flew out of the hives less frequently than the untreated ones.

The first investigations on natural mating of instrumentally inseminated queens were started by Woyke (1963, 1966). He showed that queens inseminated instrumentally also fly out of the hives and mate naturally. He inseminated queens with 1, 2, 4, 8, 12, and 16 µl of semen. As the dose increased, the percentage of queens flying out and mating naturally, decreased. Further investigations concerned queens insemi-

nated instrumentally with 8 µl of semen. Woyke and Jasiński (1992) and Woyke et al (1995) found that 50 to 60% of queens inseminated instrumentally with 8 µl of semen fly out of hives and mate naturally. However, queens inseminated twice with 4 µl of semen, and those treated additionally twice with CO_2, before and after insemination with 8 µl did not mate naturally.

Therefore we now investigated more differentiated and more numerous groups in order to find the influence of different factors on the behaviour of instrumentally inseminated queens. We hoped to find combinations of procedures which would eliminate natural mating of instrumentally inseminated queens.

MATERIAL AND METHODS

The investigations were conducted in the Apiculture Division of the Agricultural University in Warsaw in the years 1992 - 1995. In order to find out which instrumentally inseminated queens mate afterwards naturally, black coloured queens were reared. They were inseminated instrumentally with semen from yellow coloured drones, and were allowed to mate naturally in an area containing black drones. Since yellow body colour is dominant over black, any black coloured worker progeny would indicate additional natural mating of the mother-queen.

Together 308 queens were investigated of which 267 started to lay eggs. Body colour was determined in 188 300 workers originating from 202 queens which had the possibility to fly. On average, 932 workers per queen were examined.

Hybrid black *Apis mellifera mellifera* and *Apis mellifera caucasica* queens were reared. On 11^{th} day after grafting, queen cells were introduced into trapezoid Kirchhein mating nuclei, already filled with worker bees. Next, the nuclei were kept for 48 hours in a cool room. Afterwards they were placed in an apiary with black drones. Queen excluders have been fixed to entrances of all nuclei.

A control group of 23 queens was created. Those queens were not instrumentally inseminated. They were allowed to mate solely naturally. They served to test the absence of yellow coloured drones in the mating area, and to compare some other characters.

Yellow coloured Italian drones *A. m. ligustica* were used for instrumental insemination of the rest of the queens. The queens were treated as is presented in Table 1. With the exception of queens from two groups (No 3 and 4), all other were instrumentally inseminated or treated with CO_2 at the age of 6 days. The queens were inseminated with 8 µl of semen either once or twice with 4 µl of semen. The CO_2 treatment was applied either before or after instrumental insemination (tab. 1).

At the queen age of 6 days, queen excluders were removed from entrances of the control nuclei. Excluders from entrances of other nuclei were removed after the last queen treatment. However, the excluders were not removed from nuclei with instrumentally inseminated queens of the last group (No 9). Queens of that group, as well as those with cut wings (of group No 8, tab. 1), could not fly for mating flights, and could not mate naturally. They served to test mortality of instrumentally inseminated queens, without interference of losses during flights.

The beginning of oviposition was checked by examination of mating nuclei every 3 days. The exact day start of egg laying was calculated from the age of larvae.

A goodness-of-fit test was applied to compare the frequency distribution of the number of instrumentally inseminated queens with the number of queens mated also naturally, and the number of lost queens. Test-t was used to determine signif-

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Table 1

Way of insemination and number of queens investigated in particular years.
Sposób unasieniania i liczba matek w poszczególnych latach badań.

Group No Nr grupy	Way of insemination Sposób unasienienia	Year of investigation - Rok badań				Total Razem
		1992	1993	1994	1995	
1	Naturally mated 6-D* Unasienione naturalnie			23		23
2	8µl** 6-D	27	23			50
3	8µl 8-D	22				22
4	8µl 14-D		20			20
5	8µl + CO_2 6-D				24	24
6	CO_2 + 8µl 6-D				24	24
7	4µl + 4µl 6-D	24	31	19	23	97
8	4µl + 4µl 6-D Cut wing Obcięte skrzydło			24		24
9	4µl + 4µl 6-D Excuder on entrance Okratowany wylot			24		24
Total Razem		73	74	90	71	308

* D Age of queens (in days) at first treatment; in group No 1, queen age at removal of queen excluder from entrances.
* D Wiek matek (w dniach) w chwili pierwszego zabiegu; w grupie nr 1, wiek matek w dniu usunięcia kraty z wylotków.
** Amount of semen used for instrumental insemination of queens. - Ilość nasienia użyta do sztucznego unasienienia.

icant differences between two means. Correlation coefficient between percentages of queens which mated also naturally and the lost ones was calculated. Binomial confidence intervals (α one sided = 0.05) were applied to show results which could happen if large number of similar experiments were performed. ANOVA was applied to results of several groups concerning beginning of oviposition and the percentage of worker offspring originating from additional natural mating. The percentages for statistical calculations were transformed according to the Bliss function. Newman-Keuls test was used to determine statistically significant differences between particular means.

RESULTS

Natural mating of queens

Out of 23 queens of the control group of queens mated solely naturally, all produced exclusively black worker offspring. This indicates that yellow drones were absent in the mating area of investigated queens.

Table 2 shows, that among queens inseminated with 8 µl of semen at the ages of 6, 8, and 14 days, 69.2, 52.6 and 29.4% produced black worker offspring, originating from natural mating. As the age of instrumentally inseminated queens increased, the percentages of natural matings decreased. The goodness-of-fit test χ^2 showed that the frequency distribution of the number of laying queens (39 : 19 : 17) and the number of mated in addition natu-

Table 2

Natural mating of instrumentally inseminated queens.
Naturalne dounasienianie sztucznie unasienionych matek.

Group No Nr grupy	Way of insemination Sposób unasienienia	Number of queens Liczba matek			Mated naturally Dounasienionych	
		Instrument. Insemin. Sztucznie unasien.	Laying eggs Czerwiących	Mated natural. Dounas natural.	Percent Procent	#Bin. conf. Interval Dwumian. prz. ufności
1	Natur. Mated 6D* Natur. unasien.	(23)**	18	18**	100**	
2	8µl 6D	50	39	27	69.2	55 - 79
3	8µl 8D	22	19	10	52.6	30 - 67
4	8µl 14D	20	17	5	29.4	23 - 53
5	8µl + CO_2 6D	24	22	3	13.6	4 - 28
6	CO_2 + 8µl 6D	24	22	0	0	0 - 13
7	2 × 4µ 6D	97	87	0	0	0 - 4
8	2 × 4µl 6D cut wing obcięte skrzydło	24	22	0	0	
9	2 × 4µl 6D excluder on entr. krata na wylotku	24	21	0	0	
Total - Razem		308	267			

* D Age of queens (in days) at first treatment; in group No 1, queen age at removal of queen excluder from entrances
* D Wiek matek (w dniach) w chwili pierwszego zabiegu; w grupie nr 1, wiek matek w dniu usunięcia kraty z wylotków
** Queens mated solely naturally. Matki unasienione tylko naturalnie
# Binomial confidence interval. Dwumianowy przedział ufności

rally (27 : 10 : 5) differed significantly (χ^2 = 9.13, df. = 2, p = 0.011). This indicates that the ageing of queens significantly decreased the proportion of queens which mated naturally after being inseminated instrumentally.

Comparison of two groups of queens inseminated with 8 µl of semen at the age of 6 days (groups 2 and 5) revealed that additional CO_2 treatment decreased the percentage of naturally inseminated queens from 69.2 to 13.6%. The χ^2 test showed that the distribution of the number of laying queens in both groups (39 : 22) and the number of mated also naturally (27 : 3) differed significantly (χ^2 = 46.05, df. = 1, p = 0.000). Thus, additional CO_2 treatment after instrumental insemination decreased significantly the proportion of naturally mated queens.

Queens treated with CO_2 before insemination did not mate naturally, while 13.6% of those treated after insemination mated naturally. Thus, CO_2 treatment before insemination was more effective then after in decreasing the percentage of naturally mated queens. No one queen inseminated twice with 4 µl of semen, each time, mated naturally.

If a large number of similar experiments were performed, the percentages of natu-

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Table 3

Natural mating of queens inseminated with 8 µl of semen at the age of 6 days during 2 seasons. - Naturalne dounasienianie się matek unasienionych 8 µl nasienia w wieku 6 dni, w dwóch sezonach.

Season Sezon	No of queens Licz. matek		% mated naturally % dounasienionych
	Laying eggs Czerwiących	Mated naturally Dounasienionych	
1992	21	15	71.4
1993	18	12	66.7
Total - Razem	39	27	69.2

Table 4

Losses of insminatad queens. - Straty matek po unasienianiu.

Group No Nr grupy	Way of insemination Sposób unasienienia	No queens Liczba matek	Lost queens Zginęło matek		Absconded nuclei Uciekło roików		Total losses Razem straty	
			N**	%	N	%	N	%
1	Natur. Mated 6D* Natur. unasien.	23	3	13.0	2	8.7	5	21.7
2	8µl 6D	50	7	14.0	4	8.0	11	22.0
3	8µl 8D	22	3	13.6	0	0	3	13.6
4	8µl 14D	20	2	10.0	1	5.0	3	15.0
5	8µl + CO_2 6D	24	1	4.2	1	4.2	2	8.3
6	CO_2 + 8µl 6D	24	1	4.2	1	4.2	2	8.3
7	2 × 4µ 6D	97	4	4.1	6	6.2	10	10.3
8	2 × 4µl 6D cut wing obcięte skrzydło	24	1	4.2	1	4.2	2	8.3
9	2 × 4µl 6D excluder on entr. krata na wylotku	24	3	12.5	0	0	3	12.5
Total - Razem		308						

* D Age of queens (in days) at first treatment; in group No 1, queen age at removal of queen excluder from entrances

* D Wiek matek (w dniach) w chwili pierwszego zabiegu; w grupie nr 1, wiek matek w dniu usunięcia kraty z wylotków

** Number, Liczba

rally mated queens may fall within the binomial confidence intervals presented in table 2 ($\alpha = 0.05$, one-sided). Thus, (upon the number of investigated queens) it is not excluded that within the CO_2 + 8 µl group, 0 - 13% queens may mate naturally and within the 2 x 4 µl group, 0 - 4 % may mate naturally.

Queens 6 days old were inseminated with 8 µl of semen during two seasons. Table 3 shows that percentages of queens mated also naturally were similar (71.4 and 66.7%) in both years. The frequency distri-

butions of the number of queens laying eggs in both seasons (21 : 18) and the number of mated also naturally (15 : 12) did not differ significantly ($\chi^2 = 0.05$, df = 1, p = 0.830). Thus, the seasons did not interfere significantly with the results.

Losses of queens

Two types of losses occurred. A loss of queens was considered when a dead queen was found in the nuclei, or when a queenless colony was found. The second type concerned the absconding of all bees from the nuclei. In the last case, we were not sure whether the queens died, or absconded together with the worker bees.

Table 4. shows, that among the control group of naturally mated queens, 13.0% were lost. Among 6 groups of instrumentally inseminated queens, which had the possibility to fly out (groups No 2 - 7), 14.0 to 4.1% were lost. The frequency distribution of the number of instrumentally inseminated queens (50 : 22 : 20 : 24 : 24 : 97) and the number of lost ones (7 : 3 : 2 : 1 : 1 : 4) differed significantly ($\chi^2 = 83.71$, df = 5, p = 0.000). Thus, the manner of instrumental insemination affected significantly the proportion of lost queens.

On average 12.5% of queens were lost out of those inseminated instrumentally, at different ages, solely with 8 µl of semen (groups 2 - 4), and only 4.2% out of those treated additionally with CO_2, or inseminated twice (groups 5 - 7). The losses within queens inseminated exclusively once were three times as high as within queens treated twice. The t-test showed highly significantly lower losses within queens of the last groups (t = 6.6, p = 0.003, after Bliss transformation).

The highest loss of 14.0% was found in group of queens inseminated with 8 µl of semen at the age of 6 days (tab. 4) In the same group, the highest proportion of 69.2% of naturally mated queens was noticed (tab. 2). The comparison of results presented in tables 2 and 4 revealed that in all groups, the decrease of the percentage of

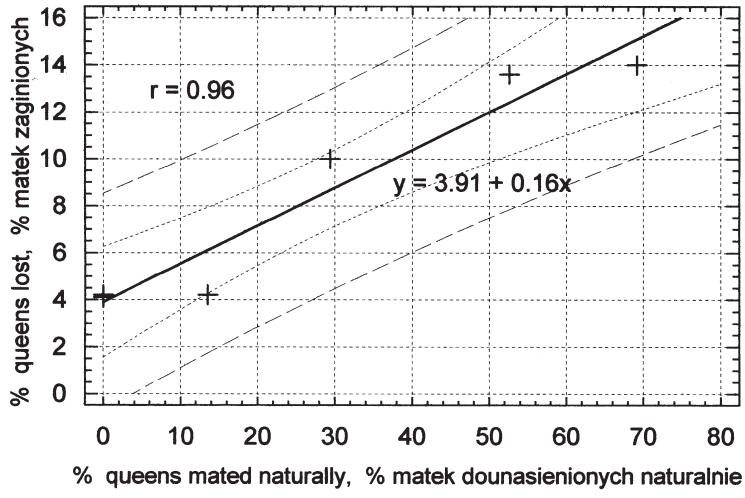

Fig. 1. Regression between percentage of instrumentally inseminated queens, mated in addition naturally, and percentage of queen losses in six groups. Dotted curves indicate 95.0% confidence intervals for the mean of many observations. Dashed curves indicate 95.0% prediction intervals for new observations.
Regresja między procentem sztucznie unasienionych matek dounasienionych naturalnie, a procentem ich strat w sześciu grupach. Krzywe kropkowane oznaczają 95.0% przedziały ufności dla średniej z wielu powtórzeń. Krzywe przerywane oznaczają 95.0% przedziały dla przewidywanych nowych obserwacji.

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Table 5

Beginning of egg laying by queens, number of days from first treatment.
Początek czerwienia, liczba dni od pierwszego zabiegu.

Group No Nr grupy	Way of insemination Sposób unasienienia	No queens Liczba matek	No of days - Liczba dni		
			Range Od-do	Mean Średnia	Stand. Deviation Odchylenie standardowe
1	Natural. Mated 6D* Natural. unasien.	18	1 ÷ 10*	3C	2.49
2	8µl 6D	39	5 ÷ 29	11A**	4.89
3	8µl 8D	19	3 ÷ 16	10A	4.48
4	8µl 14D	17	6 ÷ 19	11A	3.56
5	8µl + CO_2 6D	22	6 ÷ 10	8B	1.22
6	CO_2 + 8µl 6D	22	5 ÷ 9	7B	1.17
7	2 × 4µ 6D	87	3 ÷ 16	7B	1.89
8	2 × 4µl 6D cut wing obcięte skrzydło	22	3 ÷ 11	6B	1.86
9	2 × 4µl 6D excluder on entr. krata na wylotku	21	5 ÷ 14	6B	2.27

* D Age of queens (in days) at first treatment; in group No 1, queen age at removal of queen excluder from entrances

* D Wiek matek (w dniach) w chwili pierwszego zabiegu; w grupie nr 1, wiek matek w dniu usunięcia kraty z wylotków

** Different letters after means indicate highly significant differences p = 0.01 Różne litery za średnimi oznaczają wysoko istotne różnice p = 0.01

queens which also mated naturally 69.2, 52.6, 29.4, 13.6, 0.0, 0.0 was related to the decrease of the percentage of the lost ones 14.0, 13.6, 10.0, 4.2, 4.2, 4.1. The correlation coefficient between the percentages of naturally mated and lost queens was, r = 0.964, (r = 0.934, after Bliss transformation). The regression equation was y = 3.91 + 0.16 x % mated queens (fig. 1). When no additional natural mating occurred about 4% of queens were lost. The increase of 10% of additional natural mating was related to the loss of 1.6% of queens. Thus, the higher percentage of naturally mated queens was correlated with the higher percentage of lost queens. This indicates that the queens were lost during their flights. It is interesting to note, that 3 times more queens were lost within those mating solely naturally (13.0%) than within those inseminated instrumentally twice (4.1%).

Some queens were lost due to the absconding of whole colonies. Thus the numbers of total losses were higher. However, table 4 shows, that the pattern of total losses in particular groups was similar to the pattern of queen losses. Total losses within group of queens inseminated twice were lower than within queens inseminated only once or mated solely naturally.

Beginning of oviposition

Table 5 shows that queens from the control group of naturally mated started to lay eggs 3 days after being released by entrance opening. Those queens started to lay eggs highly significantly earlier, than any one of instrumentally inseminated.

Table 6

Beginning of oviposition in different seasons, number of days from first insemination.
Początek czerwienia w różnych sezonach, liczba dni od pierwszego unasieniania.

Group No Nr grupy	Season Sezon	No queens Licz. matek	Range Od - do	Mean Średnia	Standard dev. Odchylenie std.
Queens instrumentally inseminated with 8 mm^3 of semen at age of 6 days Matki unasieniane 8 mm^3 nasienia w wieku 6 dni					
2	1992	21	6 ÷ 20	9.9A*	3.58
2	1993	18	5 ÷ 29	11.2A	6.11
Queens instrumentally inseminated 2 x 4 mm^3 of semen Matki sztucznie unasieniane 2 × 4 mm^3 nasienia					
7	1992	21	3 ÷ 9	6.1B	1.42
7	1993	27	5 ÷ 14	7.1B	1.8
7	1994	17	5 ÷ 16	6.7B	2.62
7	1995	22	6 ÷ 11	7.8B	1.34

** different letters after means indicate highly significant differences p = 0.01
Różne litery za średnimi oznaczają wysoko istotne różnice p = 0.01

Table 7

Worker offspring of instrumentally inseminated queens which also mated naturally.
Potomstwa sztucznie unasienionych matek które dounasieniły się naturalnie.

Group No Nr grupy	Way of instrumental insemination Sposób sztucznego unasienienia		No. Queens Liczba matek	% offspring from natural mating % potomstwa z dounasienienia		
				Range Od - do	Mean Średnio	Standard dev. Odchyl.std.
2	8µl	6D*	27	2.05 ÷ 90.43	20.33	20.55
3	8µl	8D	10	1.63 ÷ 59.59	17.85	16.74
4	8µl	14D	5	2.41 ÷ 38.00	18.87	14.87
5	8µl + CO_2	6D	3	9.40 ÷ 12.90	10.70	1.89

* D age of queens (in days) at first insemination
* D wiek matek (w dniach) w chwili pierwszego unasienienia

Queens inseminated with 8 µl of semen at the age of 6 to 14 days, without additional CO_2 treatment, started to lay eggs 10 to 11 days after the first treatment (groups 2 - 4). This is the latest start, highly significantly later than in queens of any other groups. The highest range in the period of the beginning of oviposition was noticed within queens inseminated at the youngest age of 6 days. Queens treated additionally with CO_2, or inseminated twice with 4 µl of semen, started oviposition 6 to 8 days after the first treatment (groups 5 - 9). No significant difference was found between those 5 groups. However, those queens started to lay eggs highly significantly later than those mated only naturally, but highly significantly earlier than those from the 3 groups inseminated exclusively with 8 µl of semen. Thus, three super groups occurred concerning the period of the beginning of oviposition. Queens mated naturally started to lay eggs earlier than commenced queens inseminated once and treated additionally

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Table 8

Effect of season on the ratio of worker progeny from queens instrumentally inseminated with 8 mm^3 of semen at the age of 6 days, which also mated naturally.
Wpływ sezonu na udział potomstwa pochodzącego z naturalnego dounasieniania się matek sztucznie unasienionych 8µl nasienia w wieku 6 dni.

Season Sezon	Number queens Liczba matek	% progeny from natural mating % potomstwa z dounasienienia		
		Range Od - do	Mean Średnio	Standrard dev. Odchylenie std.
1992	15	6.09 ÷ 82.90	20.88	18.80
1993	12	2.05 ÷ 90.43	19.62	23.39
Total - Razem	27		20.33	20.55

with CO_2, or inseminated twice. Queens inseminated exclusively once initiated egg laying the latest. The results of the second super group suggest that additional CO_2 treatment is as valid as second insemination in accelerating the start of oviposition.

Queens inseminated ones with 8 µl of semen at the age of 6 days were investigated during two seasons and those inseminated twice with 4 µl of semen during four seasons (tables 1 and 6). ANOVA showed highly significant variations between those two groups. However, the Newman-Keuls test did not showed statistically significant differences within both groups. Thus, queens inseminated twice with 4 µl of semen started to lay eggs significantly earlier than those inseminated once with 8 µl of semen. However, the effect of season on the beginning of oviposition was not found, even when investigations were repeated during four years.

Worker offspring from naturally mated queens

Table 7 shows that, out of queens inseminated exclusively with 8 mm of semen 5 - 27 mated also naturally and out of those treated afterwards with CO_2 only 3 mated naturally. Those queens produced on average 10.70 to 20.33% of black worker offspring originating from drones which mated the queens naturally. Queens inseminated exclusively with 8 µl of semen produced, on average, twice as many offspring from natural mating as those treated additionally with CO_2. However, ANOVA did not show significant variances between those groups. This was probably due to the very high variation in the percentage of workers originating from natural mating. Table 7 shows that particular queens inseminated with 8 µl of semen at the age of 6 days produced from 2 - 90% of offspring originating from natural mating (group 2). A tendency is visible to decrease the variation in the percentage of offspring from natural mating in queens inseminated at an older age.

Queens inseminated with 8 µl of semen at the age of 6 days mated in addition naturally during two seasons (tab. 8). ANOVA did not show a significant variation between the percentages of queens which mated naturally during both seasons Thus the seasonal conditions did not interfere with the percentages of queens which mated also naturally.

Assuming that a queen mates with 10 drones, the queens inseminated exclusively with 8 µl would mate naturally, on average, with 2 drones, and queens treated additionally with CO_2 would mate additionally with 1 drone. However, taking into account the variation in the percentage of offspring production, it must be accepted that some queens inseminated instrumentally with 8 mm^3 of semen at the age of 6 days, mated in addition naturally with 1 up to 9 drones.

DISCUSSION

Present investigation showed, that none of 22 instrumentally inseminated queens of the CO_2 + 8 mm^3 group mated in addition also naturally. However, the binomial confidence interval indicated that it is not excluded that 0 - 13% queens may mate naturally, if a large number of similar experiments were performed. According to Woyke and Jasiński (1992) 3 (25%) out of 12 queens inseminated this way mated also naturally and Woyke et al (1995) reported that 2 (9.1%) out of 22 mated naturally. Thus this method of instrumental insemination did not exclude additional natural mating of queens.

None of the queens instrumentally inseminated twice with 4 µl of semen mated in addition also naturally during the whole four years of presnted investigations. Also, none of double inseminated queens mated naturally in investigations conducted by Woyke and Jasiński (1992) and Woyke et al (1995). If all those queens, of the six year observations, are aggregated, 12 (1992) + 24 (1995) + 87 (2001) = 123, then the binomial confidence interval is 0 - 2.5%. Thus, if a large number of similar experiments were performed it could not be excluded that 2.5% of double instrumentally inseminated queens would mate also naturally. This does not prove that so many instrumentally inseminated queens will mate naturally, but this is only a very low possibility. Thus it can be assumed that, in practice, queens inseminated instrumentally twice with 4 µl of semen, each time, would not mate in addition also naturally.

Total losses of queens double instrumentally inseminated with 4 µl of semen were similar in groups which had the possibility to fly out of the hives (10.3% - tab. 4, group 7) and those which could not fly (8.3 and 12.5% - groups 8 and 9) due to cut wing or queen excluder on the entrances. Losses in those three groups of queens were half as high as within queens which mated solely naturally (21.7% - group 1).

Thus, due to low losses and absence of additional natural mating within high number of queens inseminated instrumentally twice, during six years, the method of double instrumental insemination of queens with 4 µl of semen with subsequent open entrances can be recommend for practical beekeepers.

CONCLUSION

Queens inseminated instrumentally with 8 µl of semen at age of 6 days mate afterwards also naturally in 70% of cases.

Instrumental insemination of older queens at age of 8 or 14 days decreases the percentage of queens mating also naturally to 53 or 39% respectively.

CO_2 treatment two days after insemination decreases even further the percentage of queens mating also naturally to 14%

CO_2 treatment two days before instrumental insemination as well as double insemination with 4 µl of semen eliminates natural mating of queens.

Binomial confidence intervals show, that if a large number of similar experiments is performed, it cannot be excluded that 0 - 13% queens of the CO_2 + 8 mm^3 group and 0 - 2% of double instrumentally inseminated queens would mate also naturally.

Queens which could fly out of the hives are lost 3 times more often after being inseminated once with 8 µl of semen (12.5%) than after two inseminations with 4 µl of semen each (4.1%).

A high correlation ($r = 0.96$) exists between the percentage of instrumentally inseminated queens which mated also naturally and the percentages of lost queens. This indicates that the losses occur during the flights.

Losses within queens mating solely naturally (13.0%) are 3 times higher than

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within queens inseminated instrumentally twice with 4 µl of semen (4.1%).

Queens mated solely naturally start to lay eggs 3 days after removal of queen excluders from hive entrances. Queens inseminated instrumentally twice, or inseminated once and treated additionally with CO_2, start oviposition significantly later, 7 - 8 days after the first treatment. Queens inseminated solely once with 8 µl of semen start to lay eggs still significantly later, 10 - 11 days after the first treatment.

Queens inseminated once with 8 µl of semen which mated also naturally produce, on average, 20% of worker progeny originating from drones which mated the queens. They probably mated with 2 drones. Queens which after one instrumental insemination were treated with CO_2 and mated also naturally, produce on average 11% of progeny from drones which mated the queens. They probably mate naturally with 1 drone.

Due to low losses and absence of additional natural mating within high number of queens inseminated instrumentally twice, the method of double instrumental insemination of queens with 4 µl of semen with subsequent open entrances can be recommend for practical beekeepers.

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ZAPOBIEGANIE NATURALNEMU DOUNASIENIANIU SIĘ SZTUCZNIE UNASIENIONYCH MATEK PSZCZELICH PRZEZ ZASTOSOWANIE ODPOWIEDNIEJ METODY SZTUCZNEGO UNASIENIANIA

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

Badania prowadzono w Zakładzie Hodowli Owadów Użytkowych, Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie w latach 1992 - 1995. Celem pracy było zbadanie naturalnego dounasieniania się sztucznie unasienionych matek, oraz znalezienie metody, która zapobiegałaby dounasienianiu matek mających swobodę wylatywania z ula.

Do badań użyto ciemno ubarwione matki pszczele, które unasieniano sztucznie nasieniem żółto ubarwionych trutni i pozwalano im na naturalne dounasienianie się w okolicy, gdzie znajdowały się ciemne trutnie. Ponieważ żółta barwa ciała pszczół jest dominującą nad ciemną, potomstwo w postaci ciemno ubarwionych robotnic wskazywałoby na naturalne dounasienienie się matek.

W sumie zbadano 308 matek oraz określono ubarwienie 188 300 robotnic. Mateczniki w wieku 11 dni poddawano do nasiedlonych trapezowych ulików weselnych. Wylotki były zabezpieczone kratą odgrodową. Utworzono dziewięć grup matek: 1./ kontrolna - matki wyłącznie naturalnie unasienione, 2./ unasienione sztucznie 8 µl nasienia w wieku 6 dni, 3./ - w wieku 8 dni, 4./ i - w wieku 14 dni, 5./ unasienione sztucznie 8 µl nasienia i dwa dni później uśpione CO_2, 6./ najpierw uśpione CO_2, a po dwu dniach unasienione sztucznie 8 µl nasienia,

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7./ unasienione dwukrotnie po 4 µl nasienia, 8./ tak samo jak 7, jednak jedno skrzydło przycięte, 9./ tak samo jak 7, jednak krata odgrodowa pozostawała przez cały czas na wylotku. Kraty z ulików weselnych grupy kontrolnej odejmowano gdy matki osiągnęły wiek 6 dni, a w pozostałych siedmiu grupach (nr 2 - 8) po ostatnim zabiegu unasieniania lub traktowania CO_2. Co 3 dni sprawdzano rozpoczęcie składania jaj przez matki. Gdy kryty czerw był bliski wygryzienia, plastry wkładano do izolatora i umieszczano w cieplarce. Po wygryzieniu robotnic, liczono ile było ubarwionych ciemno, a ile żółto.

Uzyskane wyniki wykazały, że wszystkie robotnice od matek z grupy kontrolnej były ubarwione ciemno. Dowodzi to, że w okolicy unasieniania nie było żółto ubarwionych trutni. Spośród matek unasienionych sztucznie wyłącznie 8 µl nasienia w różnym wieku oraz tych potraktowanych CO_2 w dwa dni po unasienieniu, 69, 53, 29, i 14 % produkowało ciemne potomstwo. Oznacza to, że dounasieniły się one naturalnie. Matki traktowane CO_2 w dwa dni przed sztucznym unasienieniem, oraz matki unasienione dwukrotnie po 4 µl nasienia produkowały wyłącznie żółto ubarwione robotnice. Oznacza to, że nie dounasieniły się one naturalnie

Największe straty stwierdzono w trzech grupach matek unasienionych wyłącznie 8 µl nasienia (grupy 2, 3, 4,straty - 14,0 13,6 i 10,0%). Zauważono, iż we wszystkich grupach matek, które miały możliwość wylatywania, procent strat wzrastał w miarę wzrostu procentu naturalnie dounasienionych matek. Stwierdzono wysoką korelację między procentami dounasienionych i zaginionych matek r = 0.96. Wynika z tego, że matki ginęły głównie w czasie wylotów z ulików. Interesujący jest wynik, zgodnie z którym matki unasieniane wyłącznie naturalnie, oraz te które unasieniano tylko 8 µl nasienia ginęły trzykrotnie częściej niż matki unasieniane dwukrotnie po 4 µl nasienia.

Matki grupy kontrolnej, które unasieniały się wyłącznie naturalnie, rozpoczęły czerwić 3 dni po otwarciu wylotka. Później (6 - 8 dni) zaczęły czerwić matki unasienione 8 µl nasienia i potraktowane CO_2, oraz matki unasienione dwukrotnie po 4 µl nasienia. Najpóźniej (10 - 11 dni) rozpoczęły czerwienie matki unasienione wyłącznie 8 µl nasienia.

Matki unasienione 8 µl nasienia w wieku 6, 8 i 14 dni, które dounasieniły się naturalnie produkowały średnio 20, 18, i 19% ciemno ubarwionych robotnic. Oznacza to, że kopulowały one średnio prawdopodobnie z 2 trutniami. Jednak zakres liczby kopulacji wahał się od 1 do 9. Matki potraktowane CO_2 w dwa dni po unasienieniu produkowały 11% ciemnych robotnic, co oznacza, że kopulowały one średnio prawdopodobnie z 1 trutniem. Matki traktowane CO_2 przed sztucznym unasienianiem 8 µl nasienia oraz unasienione dwukrotnie po 4 µl nasienia produkowały wyłącznie żółto ubarwione robotnice. Oznacza to że nie dounasieniały się one naturalnie pomimo otwartych wylotków.

Ponieważ straty wśród matek unasienianych dwukrotnie są najmniejsze, a żadna spośród dużej liczby zbadanych matek nie dounasieniła się naturalnie, można polecać dla praktyki dwukrotne unasienianie matek po 4 µl nasienia, bez kratowania wylotka po ostatnim zabiegu.

Słowa kluczowe: matki pszczele, naturalne unasienianie, sztuczne unasienianie, traktowanie CO_2.

YOUTH PREFERENCES IN HONEY CONSUMPTION

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Summary

A survey on honey consumption preferences was conducted among 235 college students. The average per capita consumption was found to approximate the nation's average and was 305 g. Female students ate honey at larger quantities and more frequently, 325 g, than did male students, 284 g. Female students who consumed honey on a weekly basis accounted for 61%. Liquid honey is preferred (87.6%) to crystallized honey (12.4%) and light honey is preferred (98.1%) to dark honey (9.9%). The top ranking kinds of honey were lime honey (34.5%) and multiflower honey (34.2%). Honeydew honey was not popular. Clear glassware containers are preferred types of packaging.

Keywords: honey preferences, honey consumption, youth.

INTRODUCTION

There has been an increasing variety of honey types offered on the Polish marketplace. The consumers shop for honey according to their own preferences of which the producers are often ignorant (Zmarlicki 1996). The preferences are dependent on a variety of factors including consumer age. Consumer preferences are manageable. In Israel many beekeepers supply honey to schools and to kindergartens free of charge in order to get the children used to honey consumption from an early age. Children are consumers of the future. In some countries systematic surveys are conducted in that field. In Germany, such a survey was concerned with honey consumption preferences among children 9 to 14 years of age (Meinhardt, Keller, Bienenfeld, 1997). The survey revealed that rape honey was the preferred kind among youths. Another German survey conducted among apiary-touring youths showed that summer harvested honey was most appreciated by youngsters of the same age group.

Consumer preferences change over time. Several years ago a prevalent assumption in Poland was that dark honey was the preferred kind. Results from a recent study carried out in central Poland suggest that it is light honey that is more attractive to consumers (Pidek 1998).

The objective of this study was to assess honey consumption preferences among college students. It is all the more important since they may affect demand for a long time.

METHODS

The survey was carried out using the questionnaire method and a panel study. It involved a group of 235 students of the Horticulture Department, School of Economics and Humanities in Skierniewice. By responding to the questionnaires the students defined their honey-buying preferences with regard to colour, place of purchase, consistency, packaging and type.

The survey was conducted at the beginning of the academic year among the 2-nd and 3-rd year internal and extramural stu-

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dents before taking a course in apiculture. The responses were anonymous and entirely voluntary. The questionnaire data were tabulated and compared for years, student age and gender. Only most characteristic items of the questionnaire were chosen for publication.

RESULTS

Preferences of 235 persons were analyzed. It was a largely homogeneous group with regard to provenance but it varied for age. The youngest person to be enquired was 20 years old the oldest 40 years old. All respondents lived in the countryside or in towns of no more than 50,000 residents. They were internal or extramural students, the latter being employed in different trades, mostly agriculture-related.

The average consumption of honey by the students was 305 g being not very different from Poland's average. Over the years 1998 - 2000 the average was 337 g, 273 g, and 305 g, respectively. Women consumed more honey (325 g) then men did (284 g). The majority of surveyed students (72.4% consumed from 100g to 500 g of honey per year (Table 1).

Liquid honey was preferred by the majority of students (87.6%). There was little variability in this respect over the years, the percentage ranging from 80 to 92. Only one out of seven persons preferred crystallized honey.

Table 1

Annual consumption of honey (%). - Roczne spożycie miodu (%).

g for/1 person g/1 osobę 1	Woman Kobiety	Man Mężczyźni
0-100	15.5	12.1
101-300	24.6	35.5
301-500	31.5	29.8
>500	28.4	22.6
Together Razem	100.0	100.0

Table 2

Preferred colour of honey (%). - Struktura preferencji barwy miodu (%).

Colour of honey Kolor miodu	Woman Kobiety	Man Mężczyźni
Light jasny	4.6	15.2
light amber jasno-złocisty	35.9	31.7
Amber złocisty	46.9	46.0
Dark ciemny	12.6	7.1
Together Razem	100.0	100.0

Table 3

Frequency of honey consumption (%). Struktura częstotliwości spożywania miodu (%).

Frequency Częstotliwość	Woman Kobiety	Man Mężczyźni
Every day Codziennie	15.7	12.1
Every week Tygodniowo	45.2	38.6
Every month Miesięcznie	13.7	32.6
Occasionally Okazyjnie	25.4	15.7
Together Razem	100.0	100.0

Table 4

Preferred type of honey (%). - Struktura preferowanych odmian miodu (%).

Frequency Częstotliwość	Woman Kobiety	Man Mężczyźni
Multiflower Wielokwiatowy	42,0	26,3
Lime Lipowy	35,8	33,3
Buckwheat Gryczany	5,2	12,4
Honeydew Spadziowy	1,5	7,1
Robinia Akacjowy	15,5	20,4
Heather Wrzosowy		2,7
Rape Rzepakowy		8,8
Together Razem	100.0	100.0

The surveyed persons preferred light-coloured honey to dark honey (Table 2). Light, golden light and golden honey were the kinds of choice for 83.8% of the respondents whereas 16.2% would opt for dark honey. The degree of preference for dark honey varied substantially over the years and ranged from 5.6% to 21.4%. When asked to choose from the presented offer the students opted for light amber and amber honey.

The surveyed persons ate honey with different frequency (Table 3). Women were more frequent eaters of honey than men. 61.1% of women consumed honey more than once a week. The majority of men consumed honey less than once a week.

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Men's choice of honey was more varied than women's. Women preferred multiflower honey whereas men gave more preference to lime honey (Table 4). Those two kinds of honey jointly were given preference by 68.3% of men and 67.8% of women. The least popular kind of honey was buckwheat honey with men and honeydew honey with women. The low degree of approval for honeydew honey is puzzling since it is of particular value. The unwillingness to buy it can be due to some extent to its colour and also to the absence of proper consumer habits.

If given a choice all surveyed persons opted for honey in glass packaging as opposed to plastic packaging. Over the years of the study glass packaging was chosen by 89.1% to 98.1% of the respondents. According to 89.3% of the respondents the glass used in packaging should be light-coloured.

DISCUSSION

The average amount of honey consumed by students during the year was 305 g per person and did not differ much from the nation's average. In the European Union nations the statistical inhabitant consumes 0.7 kg of honey. In order to meet consumer preferences the honey should be light--coloured, liquid preferably multiflower or lime and in a clear transparent glassware container. Previous surveys showed that persons more than 40 years of age preferred dark and crystallized honey (Pidek 1998, Zmarlicki 1996). A similar pattern occurs In Germany. Very light honey, particularly rape honey is preferred by German youngsters of up to 15 years of age (Meinhardt, Keller, Bienenfeld 1997, Meinhardt, Keller 1997).

Compared to the results from the previous study (Pidek 1998, Zmarlicki 1996) the percentage of persons who prefer dark honey has been reduced by half (9.8%). The opinion that women prefer light-coloured honey has not been confirmed. Students (average age of 23.5) are more used to systematic eating of honey than elderly persons (Pidek 1998, Zmarlicki 1996). This is more the case with women than with men. The reason for that may be that women are more prone to pay attention to healthy eating habits. Because of the same considerations consumers are inclined to choose glass packaging as optimal for preserving honey's properties. It is not reasonable to use transparent and clear containers for honey as it loses its properties when exposed to sunlight. The use of such containers stems from the need of visual assessment by the consumer. From the standpoint of honey quality it would be more advisable to use dark containers or put clear glass containers in extra cardboard packaging. Cardboard would protect the honey against sunlight.

CONCLUSIONS

There is a need to advertise honey among young people because of low honey consumption by this social group.

Light honey is purchased more readily than dark honey.

Women consume more honey and are more frequent honey consumers than men.

Lime and multiflower honey are most attractive to consumers

Preferred honey packaging is made of glass, light and transparent

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PREFERENCJE KONSUMPCJI MIODU PRZEZ MŁODZIEŻ

A. Pidek

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

Przeprowadzono badania ankietowe wśród 235 studentów odnośnie preferencji miodu. Stwierdzono, że roczne spożycie w przeliczeniu na 1 osobę było zbliżone do średniego poziomu w Polsce i wynosiło 305 g. Więcej i częściej miód spożywały studentki, rocznie 325 g, mniej studenci 284 g. Co tydzień miód spożywało 61% studentek. Miód płynny jest bardziej preferowany (87,6%) niż skrystalizowany (12,4 %), a miód jasny (89,1 %) bardziej od ciemnego (9,9 %). Najbardziej preferowanymi odmianami miodu były: miód lipowy (34,5%) i wielokwiatowy (34,2%). Mało popularny był miód spadziowy. Miód powinien być pakowany w opakowania szklane i jasne.

Słowa kluczowe: preferencje miodu, konsumpcja miodu, młodzież.

EFFECT OF SOME FACTORS ON BROOD SURVIVAL IN A BEE COLONY

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S u m m a r y

The effect of different genetic and environmental factors on the survival rate of brood in honeybee colonies was assessed. The survival rate assessment was made in 2 - 3 day-old and in 7-8 day-old larvae and in pupae the day before the bees emerged from the comb.

The survival rate of brood was not found to be significantly affected by season of brood rearing, breed or queen age even though a slightly higher survival rate was recorded for the brood reared from the eggs laid by queens of younger age and of the Caucasian breed. There was a significant beneficial effect of increased brood compactness and of increased number of eggs laid in the comb. The queens laid eggs in the comb cells with the foundation-supporting wire threaded underneath but more than half of the young brood was removed from the cells by worker bees.

Kaywords: brood rearing, survival, environmental factors.

INTRODUCTION

The number of reared bees in the colony is the outcome of colony strength, queen fertility, and brood survival rate, the latter being affected by different genetic and environmental conditions. Well known is the reduction in brood survival rate brought about by the homozygosity of sex alleles (Woyke 1963, 1984, 1996, Page, Laidlaw, Erickson 1981). Among the environmental factors the shortage or poor quality of food, (Taber 1977, Skowronek 1979, Woyke 1979, Soszka 1996), the failure to maintain the right nest temperature (Stoner et al. 1979), and a contamination of the environment (Gromisz M. 1999, Gromisz Z., Gromisz M. 1996) are the most frequent causes of reduced brood survival.

The aim of the study was to examine the factors that may influence the survival of honeybee brood. Attention was also paid to other less known or unknown factors that might affect brood survival.

MATERIAL AND METHODS

The study was conducted in the apiaries of the Apiculture Division, Research Institute of Pomology and Floriculture, in the years 1998 - 2000. Two honeybee breeds were included in the study: Caucasian (*A. m. caucasica*) and Carniolan (*A. m. carnica*). Alongside with that a comparison was made of the survival of brood hatched from the eggs laid by one-year old vs. two-year old queens. The brood was scored for survival rate in three different periods of the season: intensive colony development (eggs laid in the second half of May), intensive nectar flow (second half of June) and after the nectar flow ends (end of July and August). A total of 92 honeybee colonies were assessed for brood survival.

The experimental brood was obtained by isolating the queen with an excluder

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screen for 24 hrs on an empty comb placed in the brood nest center. Upon the queen's release the eggs and after five days the 2 -3-day old larvae hatched therefrom were counted. The number of eggs laid by the isolated queen varied over the replications from 225 to 1156. Once the assessment was made the experiment comb was taken out of the excluder and left in the nest among other brood-containing combs. If left in the excluder the brood would not have had proper conditions for its development. In order to prevent the queen from laying additional eggs on the assessment comb she was separated on other combs. The subsequent assessment of brood survival was made after the next 5 days when the larvae reached the age of 7 - 8 days (sealed brood). The final records of brood survival were taken a day before the expected date of emergence from the cells.

RESULTS AND DISCUSSION

The brood survival rate assessed for the whole period of development and based upon all observations was 75.1% (Fig. 1). The greatest brood losses occurred from egg laying to the stage of 2-3 day old larva (17.4%) and they included non-hatched eggs and young larvae removed from the cells. Among those losses are also young larvae eaten by the bees as the result of the homozygosity for sex alleles. Another 6.9% of the larvae perished in the stage before brood sealing. During the sealed brood stage the losses were minimal (0.6%). It may be expected that some losses will occur also at the stage of bee emergence. However, that could not be demonstrated because of technical difficulties. Attempts to estimate the numbers of emerging bees by placing the comb in an incubator shortly before the expected date of completed development failed to yield positive results. Under laboratory conditions a certain number of well-developed individuals failed to cut their way out of the cells. However, in a beehive situation, assisted by their fellow bees present on the comb, those individuals would have emerged as fully capable adults. On the other hand there is also a certain error involved when combs remaining in the beehive are assessed for bee emergence rate - counts of emerged individuals might include those that died in the cells and were subsequently removed. Because of those considerations the assessment of brood survival rate was ended with a count of pupae on the last day before the expected date of adult bee emergence.

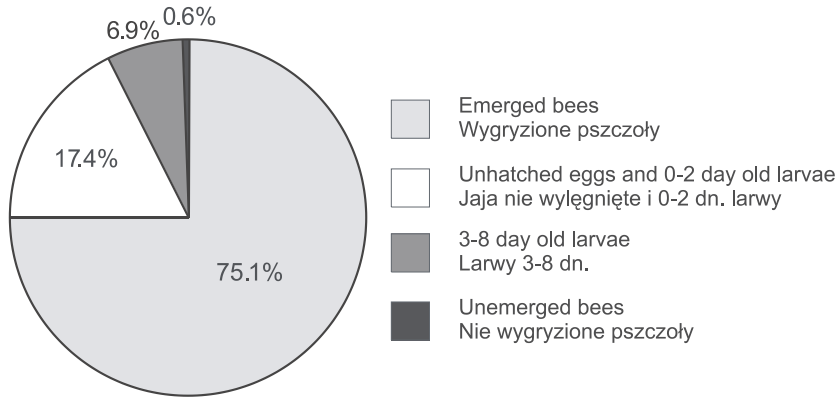

Fig. 1. Surviving rate of the brood deveoping stages in relation to the number of laid eggs. Przeżywalność czewiu w różnych stadiach rozwojowych w stosunku do liczby złożonych jaj.

There were no remarkable differences in brood survival rate between different breeds (Table 1). Although the observed survival rates for the brood hatched from the eggs laid by Caucasian queens were consistently higher in all development stages the superiority was not statistically significant. It was due to a high variability of results within replications. It is reasonable to suppose that should the numbers of observations had been higher those differences could have proven to be significant.

Likewise, no significant differences were observed in the survival rate of the brood hatched from eggs laid by queens of different age (Table 2). Nevertheless, the differences in favour of the brood hatched from eggs laid by younger queens were noticeable being more than 4% at the stage immediately prior to emergence. As in the case of the breed-related differences there are reasons to believe that those differences might have been significant should the number of observations had been higher.

No significant differences were found for brood survival over different times of the season (Table 3). The differences in survival rate were slightly increased for young larvae. As the larval development progressed those differences levelled off.

Apart from the survival rate affecting factors that were included in the planning stage of the study other factors were noticed during the observations that clearly influenced brood survival. One of them was brood compactness. When the brood combs were grouped into those in which all cells carried the brood and those in which cells

Table 1

Brood survival rate as affected by breed.
Przeżywalność czerwiu u różnych ras pszczół.

Breed Rasa	N Liczba obserwacji	0-24 h eggs 0-24 h jaja (%)	2-3 day old larvae 2-3 dn. larwy (%)	7-8 day old larvae 7-8 dn. larwy (%)	Brood before emergency Czerw przed wygryzieniem (%)
Caucasian Kaukaska	51	100.0	84.9 a	79.9 a	79.3 a
Carniolan Kraińska	41	100.0	80.6 a	72.1 a	71.6 a

Significant differences at $P<0.05$

Table 2

Brood survival rate as affected by queen age.
Przeżywalność czerwiu w zależności od wieku matki.

Queen age Wiek matki	N Liczba obserwacji	0-24 h eggs 0-24 h jaja (%)	2-3 day old larvae 2-3 dn. larwy (%)	7-8 day old larvae 7-8 dn. larwy (%)	Brood before emergency Czerw przed wygryzieniem (%)
0ne-year old Jeden rok	51	100.0	83.7 a	77.6 a	77.0 a
Two-year old Dwa lata	41	100.0	81.2 a	73.3 a	72.8 a

Significant differences at $P<0.05$

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Table 3

Brood survival rate as affected by time of season.
Przeżywalność czerwiu w zależności od pory roku.

Time of sezon Pora sezonu	N Liczba obserwacji	0-24 h eggs 0-24 h jaja (%)	2-3 day old larvae 2-3 dn. larwy (%)	7-8 day old larvae 7-8 dn. larwy (%)	Brood before emergency Czerw przed wygryzieniem (%)
May Maj	31	100.0	81.7 a	75.9 a	75.4 a
June Czerwiec	30	100.0	79.7 a	74.6 a	74.4 a
July Lipiec	31	100.0	85.4 a	76.2 a	75.4 a

Significant differences at $P<0.05$

Table 4

Brood survival rate as affected by compactness of oviposition.
Przeżywalność czerwiu w zależności od zawartości zasiewu.

Compactness of the oviposition Zawartość zasiewu	N Liczba obserwacji	0-24 h eggs 0-24 h jaja (%)	2-3 day old larvae 2-3 dn. larwy (%)	7-8 day old larvae 7-8 dn. larwy (%)	Brood before emergency Czerw przed wygryzieniem (%)
Tight Zwarty	32	100.0	86.6 a	77.6 a	81.0 a
Loose Rozstrzelony	30	100.0	71.2 b	65.4 b	64.9 b

Significant differences at $P<0.05$

with eggs were interspersed with empty cells it became apparent that the compact brood showed a higher survival rate (Table 4). Assessed after cell capping the survival rate of the tightly spaced brood was more than 16% higher than that of the loose brood. The differences proved to be statistically valid over all development stages.

The other factor that was shown to influence brood survival rate was the number of eggs deposited by the queen on a separated comb. More eggs laid by the queen resulted in an increased survival of the brood whereas fewer eggs resulted in reduced survival. When all the combs were divided into high and low egg number combs the differences proved to be substantial and significant (Table 5). The greatest differences were for the survival of eggs and young larvae (9%) being only 7% at more advanced development stages.

The effect of the amount and the compactness of the brood on its survival rate can be explained by factors inherent in the queens themselves and by the conditions of brood development. A queen that ovipositions intensely and in all the cells (compact brood) is more likely to lay better developed eggs and to use more sperm cells to fertilize them. Supposedly, a larger surface of compact brood ensures better thermal conditions for the developing larvae and pupae.

Table 5

Brood survival rate as affected by amount of oviposition.
Przeżywalność czerwiu w zależności od wielkości powierzchni zasiewu.

Amount of oviposition Powierzchnia zasiewu	N Liczba obserwacji	0-24 h eggs 0-24 h jaja (%)	2-3 day old larvae 2-3 dn. larwy (%)	7-8 day old larvae 7-8 dn. larwy (%)	Brood before emergency Czerw przed wygryzieniem (%)
Small Mała	46	100.0	87.5 a	80.1 a	79.9 a
Large Duża	46	100.0	78.3 b	73.2 b	72.9 b

Significant differences at P<0.05

Table 6

Brood survival rate as affected by the wire passed trough a cell.
Przeżywalność czerwiu w komórkach plastra nad drutami wzmacniającymi węzę.

	N Liczba obserwacji	0-24 h eggs 0-24 h jaja (%)	2-3 day old larvae 2-3 dn. larwy (%)	7-8 day old larvae 7-8 dn. larwy (%)	Brood before emergency Czerw przed wygryzieniem (%)
With wire Nad drutem	92	100.0	84.6 a	77.7 a	77.1 a
Without wire Bez drutu	92	100.0	44.2 b	36.7 b	35.8 b

Significant differences at P<0.05

An explanation was provided for the mechanism of brood losses from those sites on the comb where a wire that supports the foundation embedded into the frames passes through the cells. Brood survival rates were compared in cells with and without the wire. The brood survival rates in the cells with the wire threaded underneath were consistently significantly lower. The fact is generally known but is believed to be caused by the reluctance on part of the queen to lay the eggs to the cells above the wire. However, it turned out that the queen lays the eggs to all the cells and it is the bees that removed a large part of the young brood from the cells under which a wire was threaded.

CONCLUSIONS

Of the individuals that hatched from the eggs laid by the queen in comb cells only 3/4 reached the stage of a fully developed pupa. The remaining 1/4 died at different development stages most frequently at the beginning of the larval stage.

Brood survival rate is little affected by time of season, breed involved, and age of the egg-laying queen. This notwithstanding, more brood was hatched from the eggs laid by young and Caucasian queens.

Brood compactness and number of eggs laid in the comb have a significant impact on brood survival. Brood survival rates were reduced on the combs with loose brood and with fewer eggs.

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The number of brood reared in the cells with a foundation supporting wire threaded underneath was significantly reduced. Rather than being the results of omission during oviposition as is generally believed the reduction was due to the removal of young brood by worker bees.

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WPŁYW NIEKTÓRYCH CZYNNIKÓW NA PRZEŻYWALNOŚĆ CZERWIU W RODZINIE PSZCZELEJ

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Streszczenie

Oceniano wpływ różnych czynników genetycznych i środowiskowych na przeżywalność czerwiu w rodzinach pszczelich. Przeżywalność oceniano po osiągnięciu przez larwy wieku 2-3 dni, następnie 7-8 dni i u poczwarek na około jeden dzień przed wygryzaniem się pszczół z komórek plastra.

Nie stwierdzono istotnego wpływu na przeżywalność czerwiu pory sezonu wychowu, rasy pszczół i wieku matki, aczkolwiek z jaj składanych przez matki młodsze i rasy kaukaskiej czerw rozwijał się w nieco wyższym procencie. Korzystnie, w istotnym stopniu wpływała na przeżywalność czerwiu jego zwartość oraz duża liczba jaj złożonych na plastrze. Komórki plastra, pod którymi przebiegał drut utrzymujący węzę były zaczerwiane przez matki jednak ponad połowa młodego czerwiu była usuwana z nich przez pszczoły robotnice.

Słowa kluczowe: wychów czerwiu, przeżywalność, warunki środowiska.

ASSESSMENT OF HONEYBEE COLONIES INFESTATION BY THE MITE *Varroa destruktor* BASED ON ITS NATURAL MORTALITY DURING THE SUMMER SEASON

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S u m m a r y

Starting from 1987 the magnitude of natural mite fall from May to September was investigated at the Apiculture division by making weekly counts of dead *Varroa destructor* females on bottom insert grids. The level of autumn colony infestation was assessed by counts of dead females following application of varroacides.

Mean counts of dead mites in colonies treated with chemicals point to the occurrence of statistically significant differences beween the years of different levels of *Varroa* incidence: very high (1990), high (1988), moderately high (1995 and 1992) and low (1998). It was also found that annual counts of mite females in the natural fall from the whole season and in the fall after chemical treatment could be sorted in the same order. It means that higher number of mites in the natural fall was associated with an increased number of mites in the chemical treatment-induced fall.

High correlation coefficient values between those parameters calculated by using regression and linear correlation analysis confirm the existence of a very close relationship between them (1987 r. - 0,894*; 1988 r. - 0,699**; 1989 r. - 0,401; 1990 r. - 0,778**; 1992 r. - 0,817**; 1995 r. - 0,731**; 1998 r. - 0,863**).

Even though patterns of mite population increase and regression coefficients varied from year to year the data from all the years of the study were analysed jointly. The regression equation thus obtained was used to compare the final actual colony infestation and that estimated from the natural fall. For the majority of years actual and estimated parameters were very similar if not near-identical. It shows that credible results can be obtained by the development of a single mathematical model that comprises all study years.

Keywords: *Varroa destructor*, infestation, infestation prediction.

INTRODUCTION

The mite *Varroa destructor* is synonymous to that once called *Varroa jacobsoni* (Anderson 2000, Anderson and Trueman 2000). It parasitizes and thrives on some species of the genus *Apis* by feeding on the host's haemolymph and breeding in capped brood cells.

Characteristic of the disease caused by *V. destructor* is its slow and latent development during the first two years of the attack. In that period the few females in the colony pass unnoticed as does the damage caused by them. It is only after 2 - 3 years that the parasite's population grows large enough to kill the colony. After nearly 20 years of *Varroa* endemic presence in Poland it has become irrelevant to speak about the 1st, the 2nd or even further invasion years. However, the problem is a momentous one and the beekeeper must strive continuously to keep the mite numbers down to those occuring in the 1st and the 2nd year of invasion.

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The necessity to use chemicals and fat-soluble synthetic pyrethroids in particular is linked to numerous adverse effects. Active substances make their way to wax, honey and pollen gathered by bees. In turn, it creates a hazard to bee product consumers and to bees themselves. Due to the persistent content of cumulated small amounts of acaricides wax combs acts as a sustained varroa-killing treatment and thus may contribute to the parasite developing resistance to the chemical.

Since there is a possibility that the parasite may develop resistance to varroa-killing chemicals and that bee products may become contaminated chemical treatments in apiaries should be kept down to indispensable minimum. They must not be done shortly before and during the main commercial nectar flow that in Poland takes place from mid-May to August or to mid-September as is the case of honeydew flow. Indeed, with a high incidence of the parasite towards the end of July and In September honeybee colonies may become infested so heavily that they are in danger of collapsing. In such colonies a timely chemical control is absolutely necessary even if it still coincides with nectar flow. Consequent to that is the necessity to give up the harvest of commercial honey.

All this having been said there arose a necessity to develop a simple method to assess honeybee colonies for the extent of infestation by *V. destructor* that would allow the beekeeper to make a timely decision about the management strategies in his apiary.

The objective of the study was to find out if:

1. there is a close relationship between the natural summer mortality of the mite and the level of the subsequent mite infestation
2. the natural fall can be a parameter to be used to estimate autumn mite infestation

LITERATURE REVIEW

Methods used to assay the infestation of bees and brood samples (Ritter and Ruttner 1980, Ritter et al. 1983, Maul 1984) were very useful for detection of mites in colonies but when used to estimate the degree of mite infestation they produced highly inaccurate estimates (Fuchs and Koeniger 1984; Fuchs 1985). Even though high correlation existed between the infestation of bee (r=0.62) and brood (r=0.57) samples substantial differences were found among samples from one colony as well as among different colonies (Fuchs 1985). Those discrepancies were to a large extent due to random bee and brood sampling. However, a strong correlation was found between the fall resulting from natural mortality and brood emergence rate (Liebig 1994, Boot et al. 1995, Loob and Martin 1997). In winter month the mites have few opportunities for development due to the absence or limited amount of brood in colonies. Consistent with that the resulting natural death rate is lower than that in summer months (Maul et al. 1988). The average natural death rate in winter months (November - March) accounts for an average of 13.6% (from 3.8% to 40.4%) of the total winter infestation rate as expressed by the number of females in the winter fall and it is correlated (r=0.84) with the number of females in late autumn fall and following autumn varroa-controlling treatments (Imdorf and Kilchenman 1990, Moosbeckhofer 1991). With autumn control of the mite by means of sustained-action varroa-killing chemicals it is difficult to say whether the dead females found on the hive bottom perished as the result of chemical treatment or whether they died a natural death.

Imdorf and Charriere (1998) and Liebig (1998) hold the opinion that the beekeepers can use the summer natural death rate of the mite to decide whether or

not to make earlier varroa-controlling treatments before or shortly after honey harvest. Omholt and Creilsheim (1991) by using Rademacher's (1985) data developed a statistical model and demonstrated that it is possible to predict the degree of mite infestation rate based on its natural death-rate in an earlier period provided the amount of brood reared in the colonies is included in the calculation.

According to Calis et al. (1999) apart from natural death rate a number of other factors that affect mite population increase in bee colonies must be included in developing prediction models for *Varroa destructor* populations: brood production, length of the life cycle of bee and drone brood, reproductive potential of the mite i.e. number of offspring in bee and drone cells, percentage of sterile females and other. Those models are very complex and hence they are too difficult to be applied by commercial beekeepers. However, they prove that the dynamics of mite development can be well defined provided a sufficient number of parameters is available.

MATERIAL AND METHODS

The research project was carried out in a stationary apiary of the Apiculture Division, Institute of Pomology and Floriculture, Puławy, Poland.

The study was conducted in bee colonies with naturally and artificially inseminated *Carniolan* and *Caucassian* queens in Danant hives. A total of 137 colonies were included. Each year different colonies in the same apiary were singled out for study. In order to check for the number of *V. destructor* females that died a natural death bottom grid inserts were used that prevented the bees from taking dead mites out of the hive. The inserts were slid out to count the parasites at weekly intervals and once the counts were made they were carefully cleaned and put back in.

In 1987 only 6 colonies were included in mite counting. Based on those observations mite counting protocols for succeeding years were laid down. Starting from 1988 natural fall records were taken from May to September. The exceptions were the years 1989 and 1990. In 1989 record taking was terminated earlier (27.07) due to a dramatic increase in the natural fall of *Varroa*

Table 1

Schedule of mite counting in the natural fall of *V. destructor* females and chemicals used for control treatments. - Harmonogram obserwacji naturalnego osypu samic *V. destructor* oraz preparaty użyte do zabiegów chemicznych.

Year Rok badań	Number of colonies Liczba rodzin	Start of natural fall counts Termin rozpoczęcia kontroli osypu naturalnego	End of natural fall counts Termin zakończenia kontroli osypu naturalnego	*Varroa* - killing chemical applied (a.i.) Zastosowany środek warroabójczy
1987	6	15.07	22.09	5 x Apiwarol A + Mitac
1988	22	3.05	22.09	5 x Apiwarol A + Mitac
1989	21	3.05	27.07	Apiwarol AS; 2 x Perizin
1990	21	3.07	13.09	4 x Apiwarol AS; 2 x Perizin
1992	24	3.05	29.09	Fluwarol; 2 x Perizin
1995	26	3.05	14.09	Apifos - experiment series 4 x Apiwarol AS
1998	30	3.05	15.09	Apifos

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females. It raised the concern for the survival of the colonies and chemical control of the mite was initiated immediately. In 1990 counts of natural fall were started as late as July 3. Based on previous years' data it was thought that the July natural death rate would be a good clue to predict autumn infestation. In the remaining years the study was conducted as originally planned. Breaks in mite counts of several years' duration were caused by the appearance of chalkbrood disease. The disease brought about substantial brood losses in the colonies that more than once reached 58%. Mite counting schedules and chemicals used in chemical treatments are given in Table 1.

V. destructor females killed by the action of chemicals and falling onto the bottom grid inserts were sampled once a week (regardless of type of varroacide used).

Chemical treatments were done using I, II and III generation formulas available in a given year. The treatments were repeated several times and the chemicals were changed to make sure that all the mites were killed and deposited on the bottom inserts. Only Apifos, a formula in stripe presentation, was used in 1998. In an earlier study (Konopacka, Bieńkowska, Gerula, 2000) the formula's average efficacy was 99.9%.

The total number of the mites killed by the chemical treatments was taken as descriptive of the degree of mite infestation during the autumn season.

The data were analysed statistically by means of the following methods:

1. The mean number of *V. destructor* females was calculated over the successive months of the study
2. The „from-to" range was used as the measure of the variability within bee colony samples (for numbers see Table 1)
3. The relationship between the death rate induced by chemical treatments and the natural death rate was assessed by means of regression analysis and linear correlation. Those relationships were assessed separately for each study year. The closeness of those relationships was assessed by means of correlation coefficient between investigated variables.

The relationship between the natural mite death rate (expressed as the number of dead mites on bottom inserts counted during spring and summer) and the autumn infestation rate of bee colonies (expressed as the number of mite females dead as a result of chemical treatments) was established based on the results obtained.

RESULTS

Death rate - natural and induced by chemical treatments - of *Varroa destructor* females and the relationship between them

In all years of the study the average number of dead mites per colony fallen onto bottom inserts increased from May to September. In May and June it continued almost unchanged only to rise sharply in July and August (Table 2).

The natural death rate varied substantially from year to year (Table 2) from the lowest in 1998 to the highest in 1990. Each year the increase of dead mite females showed a different pattern.

Full data sets were available for the years 1988, 1992, 1995 and 1998 and they were analysed statistically to determine whether there were significant differences between study years. The data from 1989 and 1998 were not included in the study for reasons discussed in detail in the Material and methods section. In table 3 the means significantly differing from each other were marked with different characters.

Even though the death rate after chemical treatments showed a substantial colony-to-colony variability within a given

year the statistical analysis did show significant differences between some years for *Varroa* incidence: very high (1990), high (1988), medium high (1995 and 1992) and low (1998) (Table 3). Statistical differences between study years were found not only for the death rate after chemical treatments but also for the natural mite fall.

When analysed over the whole study period (1987 and 1989 not included) the annual counts of mite females in the natural fall over the whole mite counting period and those in the fall after chemical treat-

Table 2

Changes in mortality rate of *V. destructor* females in consecutive months. - Zmiany śmiertelności naturalnej samic *V. destructor* w następujących po sobie miesiącach.

Rok Year	Natural fall of female mites mean No / colony. - Osyp naturalny samic pasożyta średnio szt./rodzinę/miesiąc.			
	V	VI	VII	VIII
1987	-	-	37.2	29.5
1988	26.4	24.4	41.1	187.4
1989	43.2	46.9	87.1	-
1990	-	-	136.2	288.9
1992	10.4	20.3	54.7	72.6
1995	46.9	62.5	65.1	118.4
1998	4.3	3.8	4.8	7.9

Table 3

Infestation of bee colonies based on the natural fall of mites and on the number of mites dead after chemical treatment. - Porażenie rodzin pszczelich przez *V. destructor* na podstawie naturalnego osypu samic pasożyta i osypu po zabiegach chemicznych.

Year Rok badań	n	Mite fall of *Varroa destructor* females - Osyp samic *Varroa destructor*			
		Natural mite fall - Average (range) naturalny - średnie (zakres)			Total mite fall of females after chemical treatments Average (range). - po zabiegach chemicznych, średnie (zakres)
		M1 - L4*	L1 - W2*	for whole mite counting period - z całego okresu obserwacji	
1987	6	-	-	113.7 ** (25 - 267)	424 ** (38 - 1186)
1988	22	78.9 b	394.4 c	541 c (117 - 1579)	1150.4 c (266 - 2463)
1989	21	177.2 c	-	177.2 ** (23 - 440)	1220.8 ** (363 - 4560)
1990	21	-	559.9 d	559.9 ** (105 - 1363)	1848.6 d (510 - 3350)
1992	24	68.9 ab	172.2 ab	214.9 ab (15 - 848)	652.7 b (104 - 2288)
1995	26	151.2 c	261.0 bc	384.5 bc (19 - 1963)	909.7 bc (146 - 2986)
1998	30	12.8 a	23.4 a	31.5 a (2 - 213)	145.6 a (2 - 953)

* M1-L4 - from first week of May through fourth week of May - Okres od pierwszego tygodnia maja do czwartego tygodnia włącznie
L1-W2 - from first week of May through second week of September - okres od pierwszego tygodnia lipca do drugiego tygodnia września włącznie

** data not included in statistical analysis - lata których nie brano pod uwagę przy porównaniu średnich

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Table 4

Correlation coefficients between the number of *V. destructor* females in the natural mite fall and that following chemical treatment. - Współczynniki korelacji r_{xy} między naturalnym osypem samic *V. destructor* z całego okresu obserwacji, a osypem samic po zabiegach chemicznych.

Year Rok	r_{xy} x - *V. destructor* females in the natural mite fall x - samice *V. destructor* w osypie naturalnym y - *V. destructor* females after chemical treatments y - samice *V. destructor* w osypie po zabiegach chemicznych
1987	0.894 *
1988	0.699 **
1989	0.401
1990	0.778 **
1992	0.816**
1995	0.731 **
1998	0.863 **

* - significance level 95% - poziom istotności 95%;
** - significance level 99% - poziom istotności 99%

ment matched the order of severity of invasion. Sorted in the descending order the annual values of the natural fall for the whole record taking period and of those of the fall after chemical treatment are as follows: 1990, 1988, 1995, 1992 ,1998 (Table 3). It can also be seen that there is a clear relationship between the two sets of records i.e. as the one is higher the other is higher, too.

It can bee seen from the data listed in Table 3 that in the first part of the season the natural death rate of mite females was lower than in the second. When sorted according to the death rate calculated for that period the years were in an order that did not match that obtained by sorting the years according to all-season natural death-rates and chemical treatment-induced death rates. It shows that the natural death rate in the second part of the season may be a good indicator of the degree of colony infestation towards the end of the season.

The correlation coefficients between the natural death-rate of *Varroa* females during the season and the number of mites infesting colonies in the autumn were found to be significant or highly significant over all study years with the exception of 1989. In that year the natural mite fall was recorded in the first part of the season from May 3 to July 27 (Table 4).

Estimation of the final infestation of honeybee colonies by *Varroa destructor* based on natural mite fall

Correlation coefficients between the natural death rate of *V. destructor* females and that after chemical treatment point to a strong linear relationship between the two traits. The regression lines were different from year to year. Analysed over different years of the study the increase in natural death rate by 1 female was associated with different female counts in the chemical treatment-induced mite fall. The counts were high in the years with low degree of mite infestation and low in years with high infestation (Fig. 1). It may be indicative of the existence of factors that inhibit mite population increase in high infestation years.

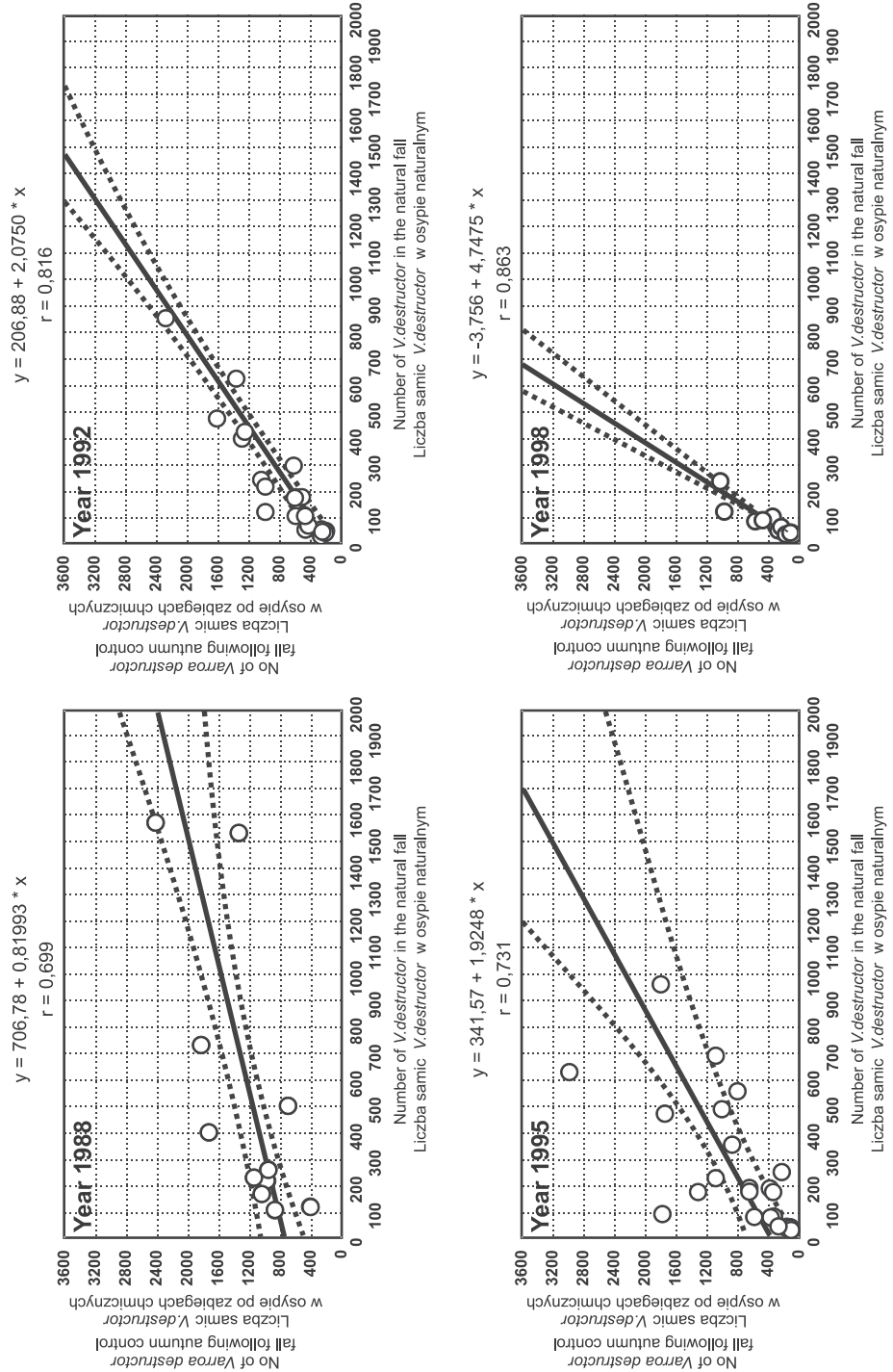

Fig. 1. Regression of the natural fall of *Varroa destructor* females on. the autumn infestation of the colonies. - Zależność między liczbą samic *Varroa destructor* z osypu po zabiegach chemicznych a liczbą samic z osypu naturalnego dla całego okresu obserwacji.

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Even though each study year was characterized with a different pattern of mite population increase and regression coefficients varied from year to year the data from individual years were pooled and analysed jointly. The regression equation was used to compare the final actual death rate with death rate estimates based on mite mortality records from the whole mite counting period (Table 5). Estimated autumn death rates were near-identical to actual death rates, the differences being from 0.3% to 3.5% save for 1998 with a very low infestation level and a death rate estimate (354 mites) more than twice as high as the actual figure (146 mites), 142% off the mark.

A similar regularity occurred when by using a single regression equation developed for all study years autumn colony infestation was estimated based on the natural mite fall in the second half of the season (from L1 to W2) (Table 6). Here also there was no match between the mean estimated values and their actual counterparts in the year of extremely low infestation (1998). On the other hand, the average estimated autumn infestation in the years 1990, 1988, 1995 and 1992 showed a good match with actual infestation, the difference between the two being fairly small (ca. 5 to 14%).

Estimates of autumn infestation of apiaries by *V. destructor* females based on mite counts in the natural summer fall can be looked on as being trustworthy also on the

Table 5

Estimated autumn infestation of colonies by *Varroa destructor* females (C) based on the natural female fall (A) as calculated from the regression equation common to the years of study in which survey was done from May (M1) to September (W2). - Szacowane jesienne porażenie pasiek przez samice *V. destructor* na podstawie ich letniego naturalnego osypu według równania regresji wspólnego dla lat badań, w których obserwacje prowadzono w okresie od maja (M1) do września (W2).

Rok Year	A	B	C **y = 302.48+1.64 x os.nat. M1W2** r = 0.823; D = 68%	Difference between Różnica pomiędzy B and C
1988	541 (117 - 1579)	1150 (260 - 2463)	1190 (494 - 2892)	-3.5%* (-90 - -17.4)
1995	385 (19 - 1936)	910 (146 - 2986)	933 (334 - 3478)	-2.5%* (-128 - -14.1)
1992	215 (15 - 848)	653 (104 - 2288)	655 (327 - 1693)	-0.3%* (-214 - +26)
1998	32 (2 -213)	146 (2 - 953)	354 (306 - 652)	+142%**

A- mean natural fall (range) - średni osyp naturalny (zakres);
B - actual mean fall of females following autumn control (range) - rzeczywisty, średni osyp po jesiennych zabiegach chemicznych (zakres);
C - estimated autumn infestation calculated upon regression equation - szacowane jesienne porażenie na podstawie letniego osypu pasożytów według równania regresji
* - signifies estimated autumn infestation higher than the actual mite fall after chemical treatments - oznacza przewidywane jesienne porażenie rodzin wyższe od rzeczywistego osypu pasożytów po zabiegach chemicznych;
** + signifies estimated autumn infestation lower than the actual mite fall after chemical treatments oznacza przewidywane jesienne porażenie rodzin niższe od rzeczywistego osypu pasożytów po zabiegach chemicznych.

Table 6

Estimated autumn infestation of colonies by *Varroa destructor* females (C) based on the natural female fall (A) as calculated from the regression equation common to the years of study in which survey was done from July (L1) to September (W2). - Szacowane jesienne porażenie pasiek przez samice *V. destructor* na podstawie ich osypu naturalnego z okresu obserwacji od lipca (L1) do września (W2) według jednego równania regresji dla wszystkich lat badań.

Rok Year	A	B	C **y = 320,33+2,44 x os.nat. L1W2** r = 0,861; D = 74%	Difference between Różnica pomiędzy B and C
1990	559,9 (105 -2215)	1849 (510 - 3350)	1686 (576 - 5725)	+8,8%** (-12,9 - -70,9)
1988	394,4 (86 - 1247)	1150 (260 - 2463)	1283 (530 - 3363)	-11,6%* (-103,8 - -36,5)
1995	261,0 (12 - 1657)	910 (146 - 2986)	957 (350 - 4363)	-5,2%* (-139,7 - -46,1)
1992	172,0 (5 - 738)	653 (104 - 2288)	740 (333 - 2121)	-13,8%* (-220,1 - +7,3)
1998	23,4 (0 - 178)	146 (2 - 953)	378 (320 - 755)	-159%*

A- mean natural fall (range) - średni osyp naturalny (zakres);
B - actual mean fall of females following autumn control (range) - rzeczywisty średni osyp po jesiennych zabiegach chemicznych (zakres);
C - estimated autumn infestation calculated upon regression equation - szacowane jesienne porażenie na podstawie letniego osypu pasożytów według równania regresji
* - signifies estimated autumn infestation higher than the actual mite fall after chemical treatments - oznacza przewidywane jesienne porażenie rodzin wyższe od rzeczywistego osypu pasożytów po zabiegach chemicznych;
** + signifies estimated autumn infestation lower than the actual mite fall after chemical treatments oznacza przewidywane jesienne porażenie rodzin niższe od rzeczywistego osypu pasożytów po zabiegach chemicznych.

account that the correlation coefficient values for the regression equations both for the whole season and for the L1 to W2 period are very high (r=0.826 and r=0.861, respectively). Determination coefficients are likewise high 68% and 74%, respectively (Table 5 and 6).

DISCUSSION

For a commercial beekeeper it is important to know the average level of infestation in his apiary. Even more important it is to be sure that no colony in the apiary is going to collapse. A prerequisite to keep colonies in good health is not to allow the number of mite females to rise above 3000 to 5000 at the end of season (Fuchs 1985, Moosbeckhofer 1991).

In this study the pattern and degree of the infestation of honeybee colonies by *V. destructor* were assessed by using the natural summertime death rate of the mite. The pattern of natural mite death rate from May to September was consistent with the data published by other investigators (Rademacher 1985, Maul 1984, Liebig et al. 1984). It rose abruptly in July and August. Consistent with the data presented by Boot et al. (1995), Martin and Kemp (1997) and Loob and Martin (1997) such a rapid increase could be explained by

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intensive brood rearing. In September however the mite death rate in this study dropped as compared to the data obtained by the above mentioned investigators. Undoubtedly, it was associated with the declining number of brood in that period in our local climatic conditions.

The assessment of the natural mite fall revealed significant differences between study years. Those differences can be explained not only by differences in the mite's reproductive potential as emphasized by Maul et al. (1983) and Ruttner and Marx (1984) or by different rates of mite population increase during the season (Schulz 1984, Bögel et al. 1986). They are due primarily to differences in the degree of mite infestation at the beginning of the season. These in turn can be accounted for by different efficacies of chemicals applied in the preceding autumn (Bieńkowska M., Konopacka Z. 1990, Konopacka Z., Bieńkowska M., Gerula D. 2000).

In this study it was also demonstrated that along with the increase of the natural mite fall there was an increase of the level of infestation in colonies expressed as mite counts in the fall following autumn treatment. Sorted according to mite counts in different kinds of fall the study years could be arranged in the same order. It shows that there is a good match between the studied traits and that the natural mite fall can be useful in prediciting infestation levels at the end of the season. Such conclusions are supported by the highly significant correlation coefficients between the counts of of *V. destructor* females in the natural summer fall (over the whole season from L1 to W2) and those in the fall resulting from the autumn chemical treatment. It contradicts the opinions of Rademacher (1985) and Maul (1984). The chemical treatment--induced fall in high infestation years seems to be lower than as could be expected from the magnitude of the natural fall. One reason for this may be reduced reproduction in a severe mite infestation situation as pointed out by Moosbeckhofer et al. (1988). Another reason may be a substantial mite loss as some of the mites die outside the hive together with their weakened hosts and thereby are not included in the natural fall which is consistent with the opinions of Omholt and Crailsheim (1991) and with research results of Kutscher and Fuchs (1999). They found that mite-infested bees frequently fail to return to the hive or lose their parasites outside.

Even though each year of the study was characterized by a different pattern of mite population increase and regression coefficients varied from year to year the data from individual years were pooled and analyzed jointly. A regression equation obtained in that manner was used to compare the final mite infestation levels - actual and estimated based on the natural mite fall. For the majority of years the actual infestation data and the corresponding estimates matched very closely or were nearly identical (Tables 5 and 6). It shows that credible results can be obtained by the development of a single mathematical model that comprises all study years.

CONCLUSIONS

1. Counts of *V. destructor* females in the natural fall increase as the season advances. They stay at approximately the same level in May and June and rapidly increase in July and August to reach the maximum values at the end of August and the beginning of September.
2. There is a substantial variability for the magnitude of natural fall among colonies in the apiary. Significant year-to-year differences in natural fall also occurred.
3. Honeybee colonies differed for the level of mite infestation as expressed

by mite counts in the fall resulting from chemical treatments. Significant year-to-year differences in infestation levels also occur.

4. There is a close match between the magnitude of natural fall and the level of colony infestation in the autumn as assayed by means of dead mite counts after chemical treatments. It is shown by the same order of apiaries when sorted either according to natural fall or chemical treatment-induced fall and also by high correlation coefficient values.
5. The natural fall in the later part of the season (mite counts from mid-June) correlates better with chemical treatment-induced fall than does the fall based on earlier mite counts. Hence the former is more suitable to predict levels of autumn infestation by the mite.
6. The increase of mite population over the season is lower in high infestation years compared to low infestation years.
7. Each individual year is characterized by a different and peculiar pattern of the increase of mite population. In spite of that the regression equation calculated for all years jointly can be used to estimate the level of autumn infestation of colonies with a fair degree of accuracy.
8. Based on natural fall figures it is possible to make correct estimates of autumn mite infestation in colonies.

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OCENA PORAŻENIA RODZIN PSZCZELICH PRZEZ PASOŻYTA *Varroa destructor* (Anderson Trueman 2000) NA PODSTAWIE JEGO NATURALNEJ ŚMIERTELNOŚCI W SEZONIE LETNIM

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

Przy dużym nasileniu inwazji pasożyta *V. destructor,* rodziny pszczele w końcu lipca i w sierpniu mogą być tak silnie porażone, że zagraża to ich egzystencji. W takich rodzinach wczesne rozpoczęcie zwalczania chemicznego jest absolutnie niezbędne nawet jeszcze w czasie trwania pożytku, co wiąże się z koniecznością rezygnacji z odwirowania miodu towarowego.

Imdorf i Charriere (1998) oraz Liebig (1998) uważają, że pszczelarze wykorzystując letnią, naturalną śmiertelność pasożyta mogą decydować o konieczności prowadzenia wcześniejszych zabiegów warroabójczych przed lub tuż po miodobraniu. Omholt i Creilsheim (1991) wykorzystując dane Rademacher (1985) opracowali model matematyczny i wykazali, że możliwe jest prognozowanie jesiennego stopnia porażenia rodziny przez pasożyty w oparciu o naturalną ich śmiertelność w okresie wcześniejszym jeśli uwzględni się ilość czerwiu wychowywanego w rodzinach.

Według Calisa i in.(1999) przy modelowaniu populacji *Varroa destructor* oprócz naturalnej śmiertelności pasożyta uwzględnić należy cały szereg czynników, które mają duży wpływ na przyrost liczby pasożytów w rodzinie pszczelej: produkcję czerwiu, okres rozwoju czerwiu pszczelego i trutowego, rozrodczość pasożyta tj. liczba potomstwa w komórkach pszczelich i trutowych, procent samic niepłodnych i inne. Modele te są bardzo skomplikowane, dlatego zbyt trudne do stosowania przez pszczelarzy praktyków.

W Oddziale Pszczelnictwa w Puławach badano od 1987 roku wielkość naturalnego osypu pasożyta w okresie od maja do września, licząc raz w tygodniu martwe samice *Varroa destructor* spadające na osiatkowane wkładki dennicowe. Dla ustalenia jesiennego porażenia rodzin, zastosowano warroabójcze środki chemiczne i również liczono martwe samice.

Wartości określające średnią liczbę samic osypujących się w rodzinach po zabiegach chemicznych wskazują na występowanie istotnych statystycznie różnic między latami o nasileniu warrozy bardzo dużym (1990), dużym (1988), średnim (1995 i 1992) i małym (1998). Stwierdzono również, że liczba samic w osypie naturalnym z całego okresu obserwacji i w osypie po zabiegach chemicznych pozwala na uszeregowanie lat w tej samej kolejności pod względem nasilenia inwazji co oznacza, że większa liczba pasożytów w osypie naturalnym wiązała się z większa liczbą pasożytów w osypie po zabiegach chemicznych.

Obliczone metodą analizy regresji i korelacji liniowej wysokie wartości współczynników korelacji między tymi wielkościami potwierdzają istnienie silnego związku między nimi (1987 r. - 0,894*; 1988 r. - 0,699**; 1989 r. - 0,401; 1990 r. - 0,778**; 1992 r. - 0,817**; 1995 r. - 0,731**; 1998 r. - 0,863**).

Jakkolwiek każdy rok badań wykazywał odrębny przebieg przyrostu populacji pasożyta i współczynniki regresji różniły się w poszczególnych latach, to jednak opracowano łącznie dane ze wszystkich lat. Tak uzyskane równanie regresji wykorzystano do porównania końcowego porażenia rodzin rzeczywistego i szacowanego na podstawie osypu naturalnego. W większości lat liczby charakteryzujące porażenie rodzin szacowane i rzeczywiste były zbliżone a nawet niemal identyczne. Świadczy to, że opracowanie jednego wspólnego dla wszystkich lat modelu matematycznego, pozwala na uzyskanie wiarygodnych wyników

Słowa kluczowe: *Varroa destructor*, porażenie, prognozowanie porażenia.

DAILY SUMMER FALL OF *Varroa destructor* (Andersen Treuman 2000) CALCULATED FROM SHORT (1, 2, 3, and 4-week) SAMPLING PERIODS TO BE USED AS AN INDICATOR OF AUTUMN MITE INFESTATION OF HONEYBEE COLONIES

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S u m m a r y

Investigations and statistical analysis suggest that the daily natural fall assayed for short periods - from 1 to 4 weeks - faithfully reflects the level of infestation of honeybee colonies by *Varroa destructor*.

The results from the study demonstrated that the increase of the number of mites in a colony and, consequently, of the natural fall varied from year to year. It is confirmed by a close relationship between daily natural fall and autumn infestation measured by the number of dead mites upon chemical treatments, a relationship expressed by high correlation coefficient values between the two parameters. A stronger relationship between natural vs. chemical-induced fall was found when the daily natural fall is calculated from sampling periods longer than 1 week.

The results showed that the increase of the mite number in a colony and hence the natural fall varied from year to year. Natural fall can be monitored from May to September, but the natural fall in the later part of the season (mite sampling from mid-June) lends itself better to make predictions about autumn mite infestation.

By using regression equations developed for all years of study a prediction table was compiled that lists espected values of mite infestation of colonies (mite counts) against mean daily mite death rates of 1, 2, 3, ...and 12 *Varroa* females recorded in different sampling periods. According to table the critical autumn infestation level that may cause the collapse of a colony occurs in colonies in which the daily natural fall is:

— 6 or more *V. destructor* females at the end of June and beginning of September, 7-8 and more females in June, more than 10 females at the end of July and beginning of August.

Keywords: *Varroa destructor*, infestation, infestation prediction.

INTRODUCTION

The screening of hive debris for the presence of dead females of *V. destructor* is based upon natural mortality of the mite. The total longevity of the mite female varies over the year. In the summer females live ca. 2 to 3.5 months, in winter 4 to 6 months (Shabanov et al. 1978, Ruijter 1987, Grobov et al. 1987). The ones that die a natural death fall onto the hive bottom.

The natural mite fall that can be observed on bottom insert grids or on hive bottoms is the measure of population development of *V. destructor* and it changes depending on the level of colony infestation and with the season of the year. According to Imdorf and Kilchenman (1990) and Moosbeckhofer (1991b) the late autumn daily death rate of 1 female corresponds to 500 females in an wintering colony.

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However, for the summer season mite population not to exceed 3000 - 4000 females (given its approximate tenfold growth during intensive egg-laying by queens from March to August) there must not be more than 250 females left in an overwintering colony which is equivalent to a natural mite death rate of 0.3 to 0.5 females per day in October. In the recent years it has been difficult to predict the level of colony infestation by taking counts of natural winter fall. With autumn mite control treatments using sustained-action varroacides it is difficult to say whether the dead females found in the winter among the bottom debris were there because they were killed by the chemical or because they died a natural death.

Analysis of summer natural fall was the subject of many studies (Ritter and Ruttner 1980, Liebig 1994, Rademacher 1985, Maul 1984, Konopacka and Bieńkowska 1990, Bieńkowska 1992, Bieńkowska and Konopacka 2000, Bieńkowska and Konopacka 2001). In all those studies the pattern of natural fall was very similar. In the period from May to July its values stayed at a low unchanged level. In July and in August the natural death-rate rose significantly only to drop down to spring months figures in October.

In the studies by Konopacka and Bieńkowska (1990), Bieńkowska and Konopacka (2000, 2001) Calatayuda and Verdu (1993) and Baggio (1994) a significant correlation was found between the female counts in natural fall and the final colony infestation as expressed by the number of females found on bottom inserts before and after chemical treatment.

Based on the rate of natural fall the level of infestation in colonies was predicted and the decisions were taken about treating colonies infested by the mite. Liebig et al. (1984) and Fremuth (1984) recognized natural mite death rate as being the tool to measure population growth. According to those authors the daily female death rate calculated from 4 to 10-day periods and multiplied by the cofactor of 120 (calculated from the relationship of female counts on brood vs. bottom inserts and on bees vs. bottom inserts) allows the whole female population present in the colony to be estimated with an accuracy of ca. 300 individuals. In a more recent study Liebig (1991) put the value of that cofactor at between 100 and 150. However, the valididy of colony infestation estimates by using that method holds good only for certain apiaries i.e those which were monitored for that trait. If applied to other apiaries the method failed to yield credible data which may indicate that apiaries differ from one another with the growth rate of mite population.

There is a difference of opinion among investigators about the possibility to estimate accurately the level of infestation by *V. destructor*. Many authors failed to find a close relationship between mite counts in the natural fall and those in the colony later in the season e.g. after the last honey harvest (Maul 1984, Fuchs and Koeniger 1984, Rademacher 1985, Milani 1990). However, many investigators hold the opinion that checking for natural fall allows honeybee colonies to be monitored for infestation level until the main curative treatment is carried out after honey harvest (Moosbeckhofer 1998, Pechhacker and Kern 1999, Imdorf and Charriere 1998, Liebig 1998, Bieńkowska and Konopacka 2001). It is advisable that monitoring of natural fall should become an inseparable element of Varroa management.

Imdorf and Charriere (1998) believe that using the assay of natural mite death-rate as the basis beekeepers may take decisions regarding the timing of Varroa-controlling treatments necessary to keep down the mite populations within the safe limits. According to them the natural

death rate of the mite of more than 5 females per day at the turn of August and September is indicative of the necessity of a prompt chemical treatment. On the other hand, a mite fall that is lower than 1 female per day from the beginning to mid-September allows the beekeeper to give up autumn treatments. According to Liebig (1998), though, the presence of as few as 3 females on the bottom inserts provides a clue to initiate chemical treatments. According to Moosbeckhofer (1998) and Pechhacker and Kern (1999) a natural mite fall of more than 10 females per day at the end of June/beginning of July and July and August is tantamount to critical infestation that threatens the death of a colony even before entering the wintering period.

The objective of the study was to explain:

1. is it warranted, based on daily death rates of the mite calculated from short sampling periods, to take decisions regarding the timing of chemical control treatments of *Varroa* disease?
2. in which period does natural death rate of the mite best characterize the level of incidence of *Varroa* disease in individual colonies and in the entire apiary?

MATERIAL AND METHODS

The study was carried out in the stationary apiary of the Apiculture Division, Institute of Pomology and Floriculture, Puławy.

The study was conducted in honeybee colonies with naturally and artificially inseminated *Carniolan* and *Caucassian* queens put in Dadant hives. A total of 137 honeybee colonies were investigated.

The rate of natural summer death-rate of the mite *Varroa destructor* further referred to also as natural fall was determined by weekly counts of dead mite females - dark and light-bodied - that dropped over the week onto the bottom grid inserts that prevented bees from taking dead mites out of the hive.

At the end of the season in order to assess the level of colony infestation varroacides were applied. After the treatment death *V. destructor* females falling onto grid inserts were also counted. Varroa-killing chemicals available in a given year were used for the treatments and the treatments were repeated several times to make sure that nearly all mites have been killed and deposited on bottom inserts. The total count of females on bottom inserts after chemical treatment was assumed as descriptive of the level of colony infestation in the autumn.

The data were analyzed statistically by means of the following methods:

1. The mobile mean method for 2- 3- and 4-week periods was used to characterize the dynamics of natural fall by converting to mean daily mite death-rates. The mobile mean method allowed random fluctuations in daily natural fall values to be eliminated.
2. Regression analysis and linear correlation were used to evaluate relationship between daily natural vs. chemical treatment-induced fall of *V. destructor*. The relationship was assessed by taking into account daily natural death rates calculated from sampling periods of different length: 1, 2, 3, 4 weeks. To examine the closeness of those relationships correlation coefficients between the variables were calculated.

Mite infestation of honeybee colonies was also assessed for its impact on colony development and strength and on wintering performance. Colony strength was assessed at the height of the season, before wintering and in the following spring. Number of combs settled by bees and number of brood combs provided the basis for evaluation. However, since those values varied over the season the following evaluation scale from 1 to 5 was used:

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1- very weak colonies
2 - weak colonies
3 - medium strong colonies
4 - strong colonies
5 - very strong colonies

Colonies were scored by 1 person from July 7 to 14, August 20 to 27 and September 25 to October 5.

Based on the data obtained guidelines were developed that allowed the post--season level of colony infestation to be predicted from natural death rates recorded during the season and their implications for *Varroa* control strategies were discussed.

RESULTS

Dynamics of natural fall of *V. destructor* females

There was a year to year variation among colonies both in natural death rate and in numbers of dead mite females after chemical treatments. Mean values of those traits were also different in different years.

The pattern of daily natural fall over the season is similar regardless of whether it is calculated on 1, 2, 3 or 4-week sampling period basis. However, the shorter the sampling period is the longer point-to-point distances on the graph are (Figure 1).

Daily summer natural death-rates differed considerably over the years. They were lowest in 1998 and highest in 1990. Each year the counts of *V. destructor* females in the daily natural fall calculated from sampling periods of different length increased in the summer season. Apart from that it was found that the minimum--maximum range for the counts of dead females increased as the season advanced which is indicative not only of differences in colony infestation level but also of dissimilar pattern of mite population growth in different colonies. Each year there were colonies in which not a single dead female of *V. destructor* was found on the bottom insert in some of the sampling periods. Such colonies were much more numerous in low (1992 and 1998) than in high (1988 - 1990) natural fall years. It was particularly conspicuous with daily death rates calculated on 1-week sampling basis, the number of zero dead mite counts being 71%. When the daily natural fall was calculated on the 2-week sampling basis zero counts were rarer accounting for 50% of the total. If calculated from 3- or 4-week sampling periods they became even rarer, accounting for 37 and 30%, respectively. Thus the longer the sampling period is the more reliable the results become.

Regression and linear correlation analysis showed a relationship between natural and chemical treatment-induced mite fall. It was found that two periods could be distinguished that differed from each other for mite fall values and correlation coefficients (Tables 1 - 4). In the first period, from the sampling starting date to mid-June low and insignificant coefficients prevailed, many of them being negative (the exception was the year 1988 for which significant correlation coefficients were found as early as in the first sampling week). In the second period, from mid-June to mid-September the majority of coefficients were highly significant and significant. The exception was 1987 for which the data obtained were poorly representative due to a small number of replicates (6 colonies). Thus it can be clearly seen that natural fall assessed after mid-June lends itself better to predict colony infestation at the end of the season.

It was also found that dates can be distinguished on which the correlation coefficients between the natural and chemical-treatment induced death-rates of *V. destructor* females were significant or highly significant across all study years (with the exception of 1987). With natural death rates calculated on 1-week sampling basis (Table 1) those dates were: the 3rd week of June (C3), the 2nd week of July (L2), the 3rd week of August (S3) and all

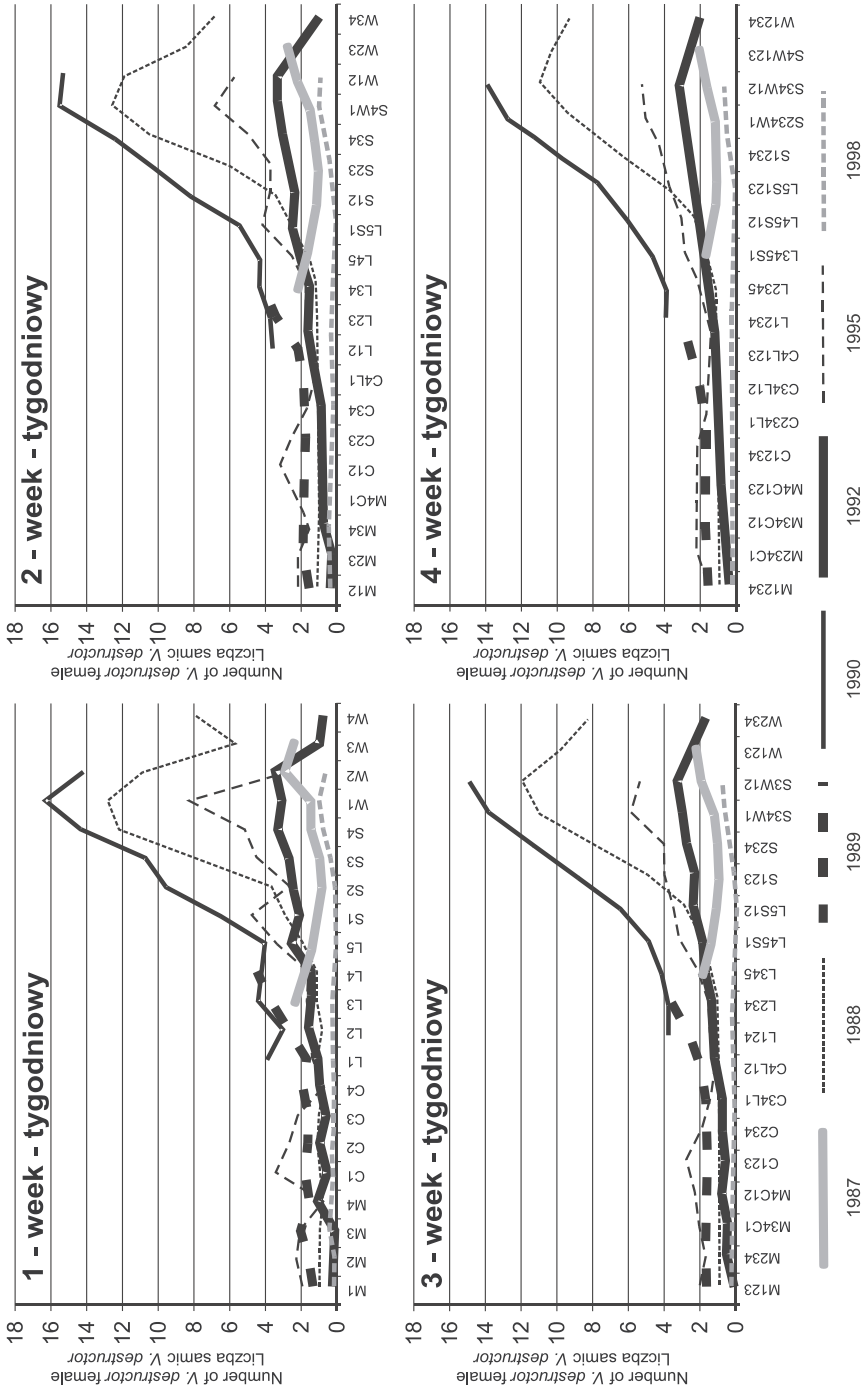

Fig. 1. Dynamics of natural fall of *Varroa destructor* females calculated from 1, 2, 3 and 4 weks count periods. - Dynamika osypu samic *Varroa destructor* wyliczona na podstawie średniej 1 tygodniowej i średnich ruchomych 2, 3, i 4 tygodniowych.

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Table 1

Correlation coefficients between the number *V. destructor* females following chemical treatment and the number of *V. destructor* females in the natural mite fall from 1- week sampling periods. - Współczynniki korelacji liniowej między liczbą samic *V. destructor* w osypie po zabiegach chemicznych a liczbą samic *V.destructor* w osypie naturalnym wyliczonym z okresów 1- tygodniowych.

Period Termin	Years - Rok						
	1987	1988	1989	1990	1992	1995	1998
M1	-	0.610**	0.187	-	-0.068	0.675**	0.590**
M2	-	0.426*	-0.075	-	-0.129	0.101	0.403
M3	-	0.714**	-0.107	-	0.212	0.297	0.502**
M4	-	0.610**	-0.000	-	-0.224	0.214	0.558**
C1	-	0.686**	0.183	-	-0.047	0.237	0.103
C2	-	0.718**	0.156	-	0.125	0.458*	0.359
C3	-	0.896**	0.525*	-	0.492*	0.545**	0.636**
C4	-	0.896**	0.464*	-	-0.077	0.537**	0.045
L1	-	0.624**	0.498*	0.195	0.469	0.647**	0.355
L2	-	0.629**	0.485*	0.647**	0.597**	0.664**	0.681**
L3	0.892*	0.796**	0.618**	0.102	0.752**	0.584**	0.595**
L4	0.085	0.388	0.354	0.573**	0.793**	0.358	0.458**
L5	0.166	0.352	-	0.507*	0.698**	0.589**	0.559**
S1	0.817*	0.688**	-	0.578**	0.855**	0.642**	-0.147
S2	0.954**	0.405	-	0.599**	0.831**	0.660**	0.468**
S3	0.962**	0.647**	-	0.683**	0.592**	0.660**	0.745**
S4	0.534	0.602**	-	0.764**	0.756**	0.625**	0.915**
W1	0.534	0.571**	-	0.509*	0.623**	0.739**	0.893**
W2	0.963**	0.496*	-	0.647**	0.599**	0.629**	0.823**

* - poziom istotności 95%; significance level of 95%;
** - significance level of 99%; poziom istotności 99%;
M - maj; C-czerwiec - June; L-lipiec -July; S-sierpień -August; W-wrzesień -September;
1, 2, 3, 4, 5 - kolejne tygodnie -succeessive weeks

the subsequent dates right to the end of mite counting. With calculations based on 2-week sampling periods (Table 2) the relevant dates were L1,2; L5S1 and all the subsequent ones until W1,2. With long 3-week periods of sampling dead mites all significant correlation coefficients were found for C4L1,2, and from L2,3,4 until the end of mite sampling (Table 3). Similar results were obtained from natural fall data calculated on the 4-week sampling basis (Table 4). In addition to that it was found that the percentage of significant and highly significant correlations increased with the length of sampling period. Depending on whether the natural fall assessment was based on 1-, 2- 3- or 4-week mite sampling periods significant and highly significant correlation coefficients accounted for 71, 75, 84 and 85% of the total, respectively.

Table 2

Correlation coefficients between the *V. destructor* females following chemical treatment and the number of *V. destructor* females in the natural mite fall from 2 - week sampling periods. - Współczynniki korelacji liniowej między liczbą samic *V. destructor* w osypie po zabiegach chemicznych a liczbą samic *V. destructor* w osypie naturalnym wyliczonym z okresów 2- tygodniowych.

Period Termin	Years - Rok						
	1987	1988	1989	1990	1992	1995	1998
M1,2	-	0,550**	0,037	-	-0,109	0,377	0,571**
M2,3	-	0,708**	-0,096	-	0,098	0,217	0,528**
M3,4	-	0,704**	-0,071	-	-0,212	0,312	0,607**
M4C1	-	0,661**	0,117	-	-0,202	0,242	0,355
C1,2	-	0,711**	0,193	-	0,077	0,367	0,255
C2,3	-	0,894**	0,384	-	0,309	0,548**	0,544**
C3,4	-	0,909**	0,570**	-	0,170	0,549**	0,395*
C4L1	-	0,823**	0,511*	-	0,242	0,727**	0,318
L1,2	-	0,631**	0,558**	0,459*	0,572**	0,705**	0,615**
L2,3	-	0,812**	0,648**	0,323	0,720**	0,670**	0,671**
L3,4	0,573	0,751**	0,531*	0,339	0,789**	0,501**	0,564**
L4,5	0,131	0,394	-	0,601**	0,762**	0,624**	0,483**
L5S1	0,390	0,564**	-	0,580**	0,794**	0,631**	0,345
S1,2	0,946**	0,576**	-	0,619**	0,875**	0,661**	0,423*
S2,3	0,959**	0,578**	-	0,662**	0,762**	0,695**	0,763**
S3,4	0,803	0,624**	-	0,748**	0,758**	0,663**	0,886**
S4W1	0,534	0,592**	-	0,720**	0,750**	0,697**	0,926**
W1,2	0,931**	0,550**	-	0,676**	0,656**	0,772**	0,919**

* - poziom istotności 95%; significance level of 95%;
** - significance level of 99%; poziom istotności 99%;
M - maj; C-czerwiec - June; L-lipiec -July; S-sierpień -August; W-wrzesień -September;
1, 2, 3, 4, 5 - kolejne tygodnie -succeessive weeks

It shows that calculations of daily mite fall based on sampling periods greater than 1-week long allowed random fluctuations of mite natural death-rate to be eliminated.

Both the percentage of high-value correlation coefficients and the low percentage of colonies with zero females in natural fall indicate that the daily natural fall calculated from 2-week and, preferably, from 3-week sampling periods could provide an indicator of autumn colony infestation by the mite. Hence the regression analysis (common to all study years) was performed for the mobile mean of daily natural death-rate. The mean was based on 3-week sampling for *V. destructor* in the second half of the season (L1 to W2), the relationships in that part of the season being shown to be closer than in the first half (from M1 to C4).

Values of natural fall based on three--week sampling in July (L1, 2, 3) and in August (S1, 2, 3) were substituted in regres-

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Table 3

Correlation coefficients between the *V. destructor* females following chemical treatment and the number of *V. destructor* females in the natural mite fall from 3- week sampling periods. - Współczynniki korelacji liniowej między liczbą samic *V. destructor* w osypie po zabiegach chemicznych a liczbą samic *V. destructor* w osypie naturalnym wyliczonym z okresów 3- tygodniowych.

Period Termin	Years - Rok						
	1987	1988	1989	1990	1992	1995	1998
M1,2,3	-	0.678**	-0.026	-	0.028	0.385	0.602**
M2,3,4	-	0.685**	-0.076	-	-0.214	0.230	0.608**
M3,4C1	-	0.698**	0.016	-	-0.192	0.282	0.528**
M4C1,2	-	0.691**	0.143	-	-0.114	0.354	0.408*
C1,2,3	-	0.844**	0.337	-	0.237	0.492*	0.399*
C2,3,4	-	0.914**	0.480*	-	0.158	0.555**	0.444*
C3,4L1	-	0.872**	0.581**	-	0.323	0.701**	0.475**
C4L1,2	-	0.777**	0.601**	-	0.420*	0.737**	0.558**
L1,2,3	-	0.778**	0.644**	0.318	0.680**	0.691**	0.626**
L2,3,4	-	0.751**	0.546*	0.446*	0.768**	0.585**	0.639**
L3,4,5	0.378	0.658**	-	0.458*	0.767**	0.648**	0.567**
L4,5S1	0.277	0.556**	-	0.643**	0.806**	0.643**	0.463**
L5S1,2	0.607	0.525*	-	0.621**	0.832**	0.643**	0.535**
S1,2,3	0.968**	0.619**	-	0.662**	0.859**	0.681**	0.760**
S2,3,4	0.860*	0.597**	-	0.720**	0.852**	0.670**	0.889**
S3,4W1	0.715	0.609**	-	0.724**	0.754**	0.704**	0.920**
S4W1,2	0.870*	0.581**	-	0.784**	0.720**	0.721**	0.931**

* - poziom istotności 95%; significance level of 95%;
** - significance level of 99%; poziom istotności 99%;
M - maj; C-czerwiec - June; L-lipiec -July; S-sierpień -August; W-wrzesień -September;
1, 2, 3, 4, 5 - kolejne tygodnie -succeessive weeks

sion equations developed for each of those periods but common to all years of the study to estimate autumn infestation of bee colonies and to compare it to real infestation (Table 5).

The data listed in Table 5 indicate that autumn infestation estimates based on the daily natural fall of *V. destructor* females are almost every year higher than the real infestation level (by 8 to 351 mites in July and by 102 to 223 mites in August). The exception was the date L1,2, 1988, where the estimate based on daily natural fall in July was lower than the actual infestation. High correlation and determination coefficients testified to a close relationship between the traits.

Relatively small departures of autumn infestation estimates from actual infestation i.e. from the number of dead females upon chemical treatments furnish evidence that by using a regression equation common to all years it is possible to predict the level of autumn infestation with a fair degree of accuracy.

Table 4

Correlation coefficients between the *V. destructor* females following chemical treatment and the number of *V. destructor* females in the natural mite fall from 4- week sampling periods. - Współczynniki korelacji liniowej między liczbą samic *V. destructor* w osypie po zabiegach chemicznych a liczbą samic *V. destructor* w osypie naturalnym wyliczonym z okresów 4- tygodniowych.

Period Termin	Years - Rok						
	1987	1988	1989	1990	1992	1995	1998
M1,2,3,4	-	0.667**	-0.021	-	-0.218	0.380	0.644**
M2,3,4C1	-	0.689**	-0.012	-	-0.194	0.248	0.544**
M3,4C1,2	-	0.712**	0.054	-	-0.106	0.371	0.546**
M4C1,2,3	-	0.810**	0.263	-	-0.026	0.475*	0.492**
C1,2,3,4	-	0.873**	0.421	-	0.130	0.510**	0.369*
C2,3,4L1	-	0.896**	0.498*	-	0.279	0.656**	0.489**
C3,4L1,2	-	0.839**	0.631**	-	0.443*	0.724**	0.619**
C4L1,2,3	-	0.832**	0.647**	-	0.581**	0.707**	0.616**
L1,2,3,4	-	0.723**	0.559**	0.430	0.733**	0.632**	0.613**
L2,3,4,5	-	0.685**	-	0.520*	0.769**	0.676**	0.638**
L3,4,5S1	0.476	0.687**	-	0.592**	0.800**	0.653**	0.559**
L4,5S1,2	0.449	0.517*	-	0.663**	0.834**	0.652**	0.577**
L5S1,2,3	0.763	0.590**	-	0.660**	0.851**	0.665**	0.751**
S1,2,3,4	0.877*	0.616**	-	0.715**	0.908**	0.667**	0.888**
S2,3,4W1	0.781	0.592**	-	0.714**	0.835**	0.705**	0.920**
S3,4W1,2	0.895**	0.599**	-	0.785**	0.718**	0.723**	0.918**

* - poziom istotności 95%; significance level of 95%;
** - significance level of 99%; poziom istotności 99%;
M - maj; C-czerwiec - June; L-lipiec -July; S-sierpień -August; W-wrzesień -September;
1, 2, 3, 4, 5 - kolejne tygodnie -succeessive weeks

Effect of *Varroa* disease on the condtion of honeybee colonies and their wintering performance

The condition of honeybee colonies over the years was distinctly related to the incidence of *Varroa* disease in the apiary. In low infestation years i.e. when *V. destructor* female counts upon chemical treatments were low (1987, 1992 and 1998) the average strength of colonies was but slightly reduced - by 0.1 to 0.5 point (Table 6). The colonies overwintered fairly well, the losses being from 0 to 12% and equal to overwintering losses in the same apiary before the mite *V. destructor* made its appearance.

In high infestation years a certain reduction in colony strength was seen in August and in September. In 1988 the score went down by 1 degree compared to that of July and 36% colonies failed to make it through the winter. As can be seen, many colonies collapsed in that year even though the mite female counts upon chemical treatments did not exceed 2500 thousand individuals thus falling short of 3,000 - 4,000 mites - a value recognized as critical under European conditions. In 1990 there was a particular dete-

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Tabela 5

Estimated autumn infestation of colonies by *Varroa destructor* based on the natural fall of females as calculated from the regression equation common to the years of study in which survey was done in July (L1, 2, 3) and in August (S1, 2, 3). - Szacowane jesienne porażenie rodzin przez *V. destructor* na podstawie osypu naturalnego z okresu obserwacji w lipcu (L1, 2, 3) i w sierpniu (S1, 2, 3) według modeli dla obserwacji prowadzonych w tych terminach.

Year Rok	A	Estimated autumn infestation calculated by regression equation. - Szacowane jesienne porażenie na podstawie dobowego osypu naturalnego L1,2,3 wg równania regresji **y = 431,89 + 381,11 (natural fall) L1,2,3** r = 0,743; D = 55%			Estimated autumn infestation calculated by regression equation. - Szacowane jesienne porażenie na podstawie dobowego osypu naturalnego S1,2,3 wg równania regresji **y = 344,71 + 187,11 x os. nat. (nat. fall) S1,2,3** r = 0,818; D = 67%		
		A1 L123 (zakres) (range)	**B1** (zakres) (range)	**C1**	**A2** (zakres) (range)	**B2** (zakres) (range)	**C2**
1990	1849 (510 - 3350)	3.74 (0.10-17.57)	1857 (470-7128)	-8*	8.97 (0.62-29.48)	2023 (461-5861)	-174*
1988	1150 (260 - 2463)	0.97 (0.05-3.48)	801 (451-1758)	+349**	4.85 (0.86-13.86)	1252 (506-2938)	-102*
1995	910 (146 - 2986)	1.49 (0-11.91)	1000 (432-4971)	-90*	3.92 (0.1-27)	1078 (363-5397)	-168*
1992	653 (104 - 2288)	1.30 (0.05-8.57)	927 (451-3698)	-274*	2.36 (0-10.90)	786 (345-2384)	-133*
1998	146 (2 - 953)	0.17 (0-1.19)	497 (432-885)	-351*	0.13 (0-1.48)	369 (345-622)	-223*

* - indicates estimated autumn infestation higher than the actual mite fall after chemical treatments;
** + indicates estimated autumn infestation lower than the actual mite fall after chemical treatments
* - oznacza przewidywane jesienne porażenie rodzin wyższe od rzeczywistego osypu pasożytów po zabiegach chemicznych;
** + oznacza przewidywane jesienne porażenie rodzin niższe od rzeczywiste go osypu pasożytów po zabiegach chemicznych
A - actual mean fall of females folloving autumn control (range)
- rzeczywisty średni osyp po jesiennych zabiegach chemicznych (zakres)
A1 - natural mite fall in L1,2,3 (range)- średni osyp naturalny w okresie L123; L -lipiec-July
A2 - natural mite fall in S1,2,3 (range) - średni osyp naturalny w okresie S123
B1 - estimated autumn infestation - szacowana liczba pasożytów jesienią
B2 - estimated autumn infestation - szacowana liczba pasożytów jesienią
C1 - Difference between A and B1 No - Różnica między A i B1 (szt.)
C2 - Difference between A and B2 No - Różnica między A i B2 (szt.)

rioration of colony strength. By the end of pre-winter feeding the colonies scored less than the average (2.9 points). Three colonies collapsed upon termination of chemical treatment and further three died before the winter cluster was formed. By the next spring colony losses totaled 70%.

In 1989 honeybee colonies were also heavily infested by *Varroa* just as they were in 1990 (Table 6). Consequently, the condition of honeybee colonies and wintering-related losses should have been similar. However, just because of the heavy natural fall in 1989 the chemical treatment was commenced as early as July 27. It allowed

Table 6

Condition of experiment colonies over the season and wintering performance.
Kondycja rodzin doświadczalnych w sezonie i wyniki zimowania.

Year - Rok		1987	1988	1989	1990	1992	1998
Numer of colonies Liczba rodzin		6	22	21	21	24	30
Colony strength rating Średnia siła pasiek w stopniach bonitacyjnych	July Lipiec	4,0	4,5	5,0	4,0	5,0	5,0
	August Sierpień	4,0	3,8	5,0	3,6	4,5	5,0
	Before winter Przed zimą	3,9	3,5	4,3	2,7	4,5	4,5
Percentage of non-surviving colonies % - Udział rodzin, które nie przeżyły zimy %		0	36	5	70*	12	6,6
Strength rating of surviving colonies - Siła rodzin które przeżyły zimę stopnie bonitacyjne		3,3	3,7	4,0	2,9	3,3	4,3
Chemical treatment-induced death rate of *V. destructor* (Mean and range) Osyp *V. destructor* po zab. chem. (średnia i zakres) [szt.]		424 (38-2463)	1150,4 (266-2463)	1220,8* (363-4560)	1848,6 (510-3350)	652,7 (104-2288)	145,6 (2-953)

* - 1/3 of colonies collapsed before final cluster formation
* - 1/3 rodzin osypała się przed ostatecznym utworzeniem kłębu zimowego

the apiary to be protected against the consequences of heavy *Varroa* attack and to keep the losses down to 5%.

In Table 6 the year 1995 was omitted. The winter of 1995/1996 was very severe and prolonged and the losses in the experiment apiary were heavy just as they were in many apiaries across the country. The *Varroa*-related losses could not be told from those brought about by extremely harsh wintering conditions. In heavy infestation years losses of honeybee colonies were recorded even though the mite counts after chemical treatments did not always reach values assumed as critical.

Daily natural death rate as an indicator of autumn colony infestation

Small deviations of autumn colony infestation estimates from actual mite counts following chemical treatments (Tables 5 and 6) give evidence that by using regression equations common to all study years it is possible to develop prediction tables of the level of autumn infestation by *V. destructor*. Such a table was compiled based on daily natural mite death rates calculated on the 3-week sampling basis (Table 7). The table lists predicted values of autumn infestation (mite numbers) against mean daily counts of 1, 2, 3, ...12 dead *V. destructor* females in different sampling periods. The table lists average values.

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Table 7

Estimated autumn infestation of bee colonies by *V.destructor* based on summer daily natural mite fall counts from 3 - week sampling periods and calculated from regession equations common to all study years. - Szacowane jesienne porażenie rodzin pszczelich przez *V. destructor* na podstawie dobowego letniego osypu naturalnego pasożyta wyliczonego z okresów trzytygodniowych, obliczone według wspólnych dla wszystkich lat badań równań regresji.

Sampling period Okres obserwacji	Estimated number of *V. destructor* females present in colonies in autumn Przewidywana liczba samic *V. destructor* obecnych w rodzinach jesienią obliczona na podstawie dobowego osypu naturalnego z kolejnych trzytygodniowych okresów obserwacji											
	1	2	3	4	5	6	7	8	9	10	11	12
C4L1,2	840	1337	1834	2331	2827	3324	3821	4318	4814	5311	5808	6305
L1,2,3	813	1194	1575	1956	2337	2719	3100	3481	3862	4243	4624	5005
L2,3,4	828	1224	1620	2015	2411	2807	3202	3598	3994	4389	4785	5181
L3,4,5	757	1103	1449	1795	2141	2487	2833	3179	3524	3870	4216	4562
L4,5S1	690	963	1235	1507	1780	2052	2324	2597	2869	3142	3414	3686
L5S1,2	619	870	1121	1372	1622	1873	2124	2375	2625	2876	3127	3378
S1,2,3	532	719	906	1093	1280	1467	1654	1842	2029	2216	2403	2590
S2,3,4	497	629	760	891	1023	1154	1285	1417	1548	1679	1811	1942
S3,4W1	448	552	657	761	886	971	1075	1180	1284	1389	1493	1598
S4W1,2	441	545	629	723	817	911	1005	1099	1193	1287	1381	1475

M-May; C- -June; L- -July; S- -August; W -September 1,2,3,4,5- -succeessive weeks
M-maj; C-czerwiec; L-lipiec; S-sierpień; W-wrzesień; 1,2,3,4,5- kolejne tygodnie

Obviously, estimates of the level of autumn infestation thus obtained must be burdened with a certain error. Because of that the difference was calculated between the infestation level estimate (expressed as the mean predicted number of mites in a colony) and the actual number of dead mites after chemical treatment. The differences were found to vary from year to year and from sampling period to sampling period. The variation was due to different infestation levels and to the dynamics of natural mite fall on different sampling dates and in different years (Table 8). The differences ranged from -374 to +384 mites. Thus it can be assumed that the predictions of mite numbers present in a colony in autumn made by means of a single regression equation common to all years may involve an error of +/- 400 *Varroa destructor* females. However, based on the differences listed in Table 9 and on correlation coefficients and determination coefficients derived from analysis of regression and linear corelation it can be assumed that according to the prediction chart (Table 7) autumn infestation of honeybee colonies by the mite can be estimated with a fair degree of likelihood. Practically, in order to determine the level of colony infestation i.e. to assess the hazard it poses to colony survival or to dispel the concerns about winter preparation it is not necessary to calculate the degree of autumn infestation. The mean daily natural fall provides a sufficient clue to go by.

Table 8

Differences between the estimated autumn infestation of colonies by light and dark-bodied *V. destructor* females and the actual mite fall following chemical treatment over the sampling periods and years of study. - Różnica między szacowanym jesiennym porażeniem pasiek przez *V. destructor* a rzeczywistym osypem po zabiegach chemicznych w poszczególnych terminach i latach badań.

Period Termin badań	Year - Rok badań				
	1990	1988	1995	1992	1998
C4L12	-	+384**	-20	-262*	-267
L123	-8	+349	-90	-274	-351
L234	-103	+334	-131	-337	-354
L345	-12	+278	-245	-363	-303
L45S1	+85	+206	-374	-301	-287
L5S12	-204	+86	-348	-297	-230
S123	-174	-102	-168	-133	-223
S234	-35	-261	+11	-78	-268
S34W1	+64	-346	-54	-1	-264
S4W12	+95	-321	+56	+4	-270

* - indicates estimated autumn infestation **higher** than the actual mite fall after chemical treatments;
** + indicates estimated autumn infestation **lower** than the actual mite fall after chemical treatments
M - May; C - June; L - July; S - August; W - September 1,2,3,4,5 - succeessive weeks
* - oznacza przewidywane jesienne porażenie rodzin **wyższe** od rzeczywistego osypu pasożytów po zabiegach chemicznych;
** + oznacza przewidywane jesienne porażenie rodzin **niższe** od rzeczywiste go osypu pasożytów po zabiegach chemicznych
M - Maj; C - Czerwiec; L - Lipiec; S - Sierpień; W - Wrzesień.

DISCUSSION

For a commercial beekeeper it is important to know the average infestation of colonies in his apiary. It is even more important to be sure that no colony will collapse because of the disease. The observations made by German investigators (Bretschko 1985, Maul 1984, Ritter and Ruttner 1981, Liebig 1994) indicate that the survival-threatening infestation threshold is ca. 8,000 to 1000 *V. destructor* females per colony. However, the prerequisite to keep the colony in good health is not to allow the number of mite females to rise above 3,000 to 5,000 in late summer (Fuchs 1985, Moosbeckhofer 1991a, 1991b).

In the literature it is recommended to sample the natural fall for 5 days (Anonymous 1999a), and for 7 days (Büchler 1994, Anonymous 1999b). The data presented in this study give evidence that such a short sampling period is not sufficient as in some of the colonies not a single dead female was found in weekly checks for natural fall. It gave the impression that mites were absent from those colonies. It turned out to be a false assumption at the end of the season upon the application of chemicals to determine the number of mites present in colonies before winter. With longer sampling periods those random fluctuations in the counts of daily natural mite fall were avoided. Data from 3-week sampling peri-

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ods were chosen to be included in linear correlation and regression analysis as they were more trustworthy than those based on 1- and 2-week periods and less labour-consuming than those derived from 4-week periods. However, correlation coefficients give evidence that the daily natural fall counts from 2-week sampling periods could also be useful in the estimation of autumn infestation.

The chemical treatment-induced fall in high infestation years seems to be lower as could be judged based upon natural fall values. On the one hand, the reason for the discrepancy could be lower propagation in a high mite density situation as pointed out by Moosbeckhofer et al. (1988). Another reason may be a substantial mite loss as some of the mites die outside the hive together with their weakened hosts and thereby are not included in the natural fall which is consistent with the opinions of Omholt and Crailsheim (1991) and investigations of Kutscher and Fuchs (1999). They found that mite-infested bees frequently fail to return to the hive or lose their parasites outside.

The results from this study indicate that the counts of daily natural mite fall based on 3-week sampling periods (Fig. 1) reflect the level of infestation rather faithfully. The actual natural fall in the periods L1,2,3 differed from year to year and the minimum-maximum value range also varied. In 1990, with an average daily fall of 3.74 (from 0.10 to 17.57) the maximum predicted level of colony infestation would be 7128 *V. destructor* females in the autumn (Table 5) thereby exceeding the critical infestation as established for European conditions. In that year lost colonies accounted for 70% of which 1/3 collapsed before the winter cluster was finally formed (Table 6). In 1995, the average daily fall in July was 1.49 females but the maximum death rate was as high as 11.91. The maximum predicted infestation (4971 mites) would therefore also constitute a critical value (Table 5). In 1992, with a lower average natural fall in that period (1.30 mites) and a range from 0.05 to 8.57 *V. destructor* females the mean predicted number of females in the autumn (927 individuals) would not pose a threat to colonies but the maximum estimated level of infestation (3698 individuals) could affect the condition of some of them. It is only with a very low natural fall in 1998, averaging 0.17, that the estimated lower than actual autumn infestation was not a cause for concern.

The exception was the year 1988 when based on a relatively low natural fall in July the estimated infestation level would fall within acceptable limits. However, the actual maximum fall upon chemical treatments was higher and colony losses were 36%. The substantial level of infestation is further borne out by the reduction in colony strength in August relative to that in July (Table 6). The pattern of natural fall was not typical in that year. Mite death rates stayed at almost the same level until mid-July only to rise abruptly towards the end of July reaching an average of 11.96 females per day - only a small reduction on the 1990 figures, the year of highest infestation of honeybee colonies.

It ensues from the above deliberations that the death rate of 7-8 females per day in July is indicative of a high level of colony infestation (Table 7) that should prompt beekeepers to treat their colonies as soon as possible. Those results are to some extent discordant with the opinion by Rademacher (1986) according to whom the death rate of 1 female per day in that period may already signify high infestation. The existence of correlation between the summer natural mite fall and the treatment-induced autumn fall was demonstrated by the author but in her study the correlation coefficients were very low and the mean number of females after chemical treatments did not exceed 600 individuals per colony. Moreover, there

were no year-to-year significant differences in infestation level. The above discrepancies could be caused by the fact that the investigator conducted her study for too short a time and it was difficult to make out the relationships that were demonstrated later by other authors.

It was also found in this study that the daily natural fall of 10 females at the end of July/beginning of August signifies critical infestation which may result in colony collapse already before the winter cluster is formed (Tables 5 and 7). The years 1988, 1989 and 1990 bear out that assertion. In 1989 natural fall counts were made until the end of July. Relative to that in 1988 the natural fall was very high so that heavy colony losses could be expected during the winter. Because of that as early as July 27 fumigation was initiated using Apiwarol, the only readily available varroa-controlling agent at that time. In 1990 sampling for *Varroa* was started in the beginning of July. Thus mite counting in July was common to the three years. The natural fall in that period in the years 1989 and 1990 was very similar regardless of the length of the sampling period on which the daily means were based. However, in 1989, owing to the early administration of chemicals after which there was a drop of 1220 mites on average (Table 6) the colonies were not weakened survived the winter in very good shape and wintering losses were only 5%. On the other hand, in 1990 with chemical treatments performed as late as mid-September the colonies started to become weak in August and wintering losses accounted for more than 2/3 of the total number of colonies.

The results are in agreement with those of Imdorf and Charriere (1998) according to whom a daily natural fall of up to 10 females calculated on the 2-week sampling basis after the last honey harvest is indicative of the presence of ca. 2400 *V. destructor* females, the number which still does not compromise the colony survival. However, given the usual date of the last honey harvest as the second half of July the number indicates already a critical infestation level. The opinion is shared by Moosbeckhofer (1998) and Pechhacker and Kern (1999) who maintain that a natural fall of more than 10 *V. destructor* females per day at the end of July/beginning of August spells critical infestation. A similar *Varroa* control strategy in Germany (Anonymous 1991a, b) stipulates that the natural mite fall should be checked twice: at the beginning of June and in the autumn.

Likewise, the results from this study demonstrated that the monitoring of natural fall could be conducted in either of two periods: an earlier or a later one. However, the assessment of natural fall in June is less suitable for autumn infestation predictions that the assessment done in July and in August (lower correlation coefficient values - Tables 1 to 4).

CONCLUSIONS

1. Based on the daily natural death rate of *V. destructor* females the level of colony infestation in the autumn can be predicted. The proof of that is the close relationship of the daily natural fall in the spring and summer season vs. the autumn level of colony infestation as measured by the count of dead mites upon chemical treatments and expressed by high correlation coefficients between the two parameters.
2. A closer relationship between daily natural vs. treatment-induced mite fall occurs when daily natural fall calculations are based on sampling periods longer than 1 week.
3. Natural fall in the later part of the season (sampling from mid-June) correlates better with treatment-induced fall than natural fall in earlier periods and lends itself better to predict autumn colony infestation by the mite.

4. Daily natural fall based on mite counts from 3-week sampling periods gives the best estimates of autumn colony infestation levels.
5. Autumn infestation estimates based on mite counts from earlier 3-week sampling periods are burdened with an error of +/-400 mites.
6. The condition of honeybee colonies in individual years is related to *Varroa* incidence.
7. Critical levels of autumn infestation that may result in colony collapse can be expected when daily rates of natural fall are:
   - at the turn of June and August - 6 *V. destructor* females and more
   - in July - 7-8 *V. destructor* females and more
   - towards the end of July and the beginning of August - more than 10 *V. destructor* females

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LETNI, DOBOWY OSYP NATURALNY SAMIC *Varroa destructor* (Anderson Treuman 2000) WYLICZONY Z KRÓTKICH (1, 2, 3 i 4-ro tygodniowych) OKRESÓW OBSERWACJI JAKO WSKAŹNIK JESIENNEGO PORAŻENIA RODZIN PSZCZELICH PRZEZ PASOŻYTA

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Streszczenie

Wśród badaczy zdania na temat możliwości dokładnego szacowania stopnia porażenia rodzin przez *V. destructor* są podzielone. Wielu autorów nie znalazło ścisłego związku między liczbą pasożytów w osypie naturalnym, a ich liczbą w rodzinie w okresie późniejszym, np. po ostatnim miodobraniu (Maul 1984, Fuchs i Koeniger 1984, Rademacher 1985, Milani 1990). Wielu autorów uważa jednak, że badanie osypu naturalnego pozwala na kontrolowanie porażenia rodzin pszczelich do głównego zabiegu leczniczego prowadzonego po zbiorach miodu (Moosbeckhofer 1998, Pechhacker i Kern 1999, Imdorf i Charriere 1998, Liebig 1998, Bieńkowska i Konopacka 2001). Według Imdorfa i Charriere (1998), naturalny osyp pasożyta wyższy niż 5 samic na dobę na przełomie sierpnia i września wskazuje na konieczność szybkiego wykonania zabiegów chemicznych, ale osyp niższy od 1 samicy na dobę w okresie od początku września do połowy września pozwala pszczelarzowi zrezygnować z jesiennych zabiegów chemicznych. Według Liebiga (1998) natomiast, obecność na wkładkach dennicowych już 3 samic na dzień późną jesienią, stanowi wskazówkę do przeprowadzenia zabiegów leczniczych. Według Moosbeckhofera (1998) oraz Pechhackera i Kerna (1999) naturalny osyp powyżej 10 samic na dobę na przełomie czerwca i lipca oraz lipca i sierpnia oznacza porażenie krytyczne grożące śmiercią rodziny często jeszcze przed zazimowaniem. Celem badań było wyjaśnienie:

1. czy na podstawie dobowego osypu pasożyta wyliczonego z krótkich okresów obserwacji można podejmować decyzje dotyczące terminu walki chemicznej z warrozą.
2. w jakim okresie naturalna letnia śmiertelność najlepiej charakteryzuje nasilenie warrozy w poszczególnych rodzinach i w całej pasiece.

Przedstawione wyniki badań i obliczenia statystyczne sugerują, że dobowy osyp naturalny oceniany przez krótkie okresy - od 1 do 4 tygodni, wiernie odzwierciedla stopień porażenia rodzin pszczelich przez *Varroa destructor*. Świadczy o tym ścisła zależność między dobowym osypem naturalnym a jesiennym porażeniem rodzin mierzonym liczbą martwych pasożytów po zabiegach chemicznych, wyrażona wysokimi wartościami współczynników korelacji między tymi wielkościami. Ściślejszy związek między dobowym osypem naturalnym pasożyta a osypem po zabiegach chemicznych stwierdzono wtedy, gdy dobowy osyp naturalny wylicza się z okresów obserwacji dłuższych niż 1-tygodniowe

Uzyskane wyniki wykazały, że przyrost liczby pasożytów w rodzinie (a więc i osyp naturalny) jest w różnych latach różny. Kontrolę osypu naturalnego można prowadzić od maja do września, jednak osyp naturalny w późniejszym okresie sezonu (pomiary od połowy czerwca) lepiej nadaje się do prognozowania jesiennego porażenia rodzin pszczelich przez pasożyta.

Posługując się równaniami regresji wspólnymi dla wszystkich lat badań opracowano tabelę prognostyczną, która podaje przewidywane jesienne porażenie rodzin pszczelich (liczbę pasożytów), gdy średni dobowy osyp naturalny w różnych okresach obserwacji wynosi 1, 2, 3 .....i 12 samic *Varroa*. Według tabeli, porażenie krytyczne jesienią (mogące spowodować śmierć rodzin) występuje w rodzinach w których dobowy osyp naturalny wynosi:
- na przełomie czerwca i lipca - 6 samic *V. destructor* i powyżej; - w lipcu - 7-8 samic *V. destructor* i powyżej; - na przełomie lipca i sierpnia - powyżej 10 samic *V. destructor*.

Słowa kluczowe: *Varroa destructor*, porażenie, prognozowanie porażenia.

EFFECT OF HONEYDEW HONEY-CONTAINING FOOD ON THE CONDITION OF CAGED BEES

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S u m m a r y

The study was conducted in laboratory in 1997. It involved three experiment series run from June to October. Each series comprised four treatments in which the bees were fed: 1) sugar syrup (SS) alone in concentration 3:2, 2) SS enriched with 10% of honeydew honey, 3) SS enriched with 40% of honeydew honey 4) 80% honeydew honey, 20% SS. Fir honeydew honey was used.

The objective of the study was to determine the effect of food formulations containing different proportions of honeydew honey on the degree of filling of the digestive tract, on Nosema disease incidence and on the condition of pharyngeal glands and of the fat body in worker bees.

The results from the study indicate that honeydew honey-enriched food may affect the changes in intestines of caged bees. Furthermore, a relationship was demonstrated between winter vs. summer generation of the bees and their reaction to honeydew honey in the food.

Keywords: honeydew honey, bees, caging.

INTRODUCTION

The presence of honeydew honey is commonly known to have a negative impact on wintering performance of bee colonies (Imdorf et al. 1985). Among the causes were cited rapid crystallization of stores (Haragsim 1970) and the negative impact of honeydew honey on individual worker bees, the latter being observed both in winter and during the honey-producing season (Gliński, Chmielewski 1994). Crailshaim and Pabst (1988) demonstrated that caged bees developed damage of midgut epithelium and peritrophic membrane already after six days of ingesting honeydew honey. According to Imdorf and associates (1985) it is caused by high concentration of mineral salts in that food. Matras and associates (1998) demonstrated that the presence of honeydew honey in the food of caged bees induces significant changes in their intestinal bacterial flora. It is not known, though, whether the presence of honeydew honey in the food of caged bees affects the degree of filling of their intestines as well as water content and pH of the intestinal content. The physiological condition of bees fed honeydew honey-containing food has not been studied, either.

The objective of this study was to determine the effect of foods with different additions of honeydew honey on the degree of filling of the digestive tract, on Nosema disease incidence and on the condition of pharyngeal glands and the fat body in caged bees.

MATERIAL AND METHODS

The study was conducted in the laboratory of the Division of Apiculture, Institute of Pomology and Floriculture in Puławy in

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1997. The experiment was run in three series set up on June 27, July 17 and October 20. Each series comprised the following feeding treatments:

Bees were fed throughout the study:

1) sugar syrup at 3:2 concentration,
2) sugar syrup at 3:2 concentration enriched with 10% of honeydew honey,
3) sugar syrup at 3:2 concentration enriched with 40% of honeydew honey,
4) sugar syrup at 3:2 concentration enriched with 80% of honeydew honey.

Fir honeydew honey was used in the experiments.

The sugar syrup prepared as 3 parts of sugar, 2 parts of water, the water content was 40%. Meanwhile, the water content of honeydew honey fed to bees did not exceed 16%. Therefore, in order to keep similar water content in each food preparation water was added to food preparations in treatments 3 and 4 - 7.6 g/100 g and 20 g/100 g, respectively.

Each treatment within each series was replicated three times (three cages). Each cage was settled with ca. 150 worker bees collected from the last comb in the nest. Over the entire duration of the study (10 days) the bees clustered under the feeder, did not build the comb and remained little active. This behaviour resembled that of bees forming a cluster during the wintering period.

Ambient temperature of the room in which the cages were kept was within a strict limit of 16 - 18°C. As was demonstrated in an earlier preliminary study (Muszyńska - unpublished data) bees kept in cages such as those used in this study at a temperature below 16°C are unable to form a cluster and to take food and die quickly. At a temperature above 18°C they also fail to form a cluster but this is because they are over-active. They ingest food intensively and set about building a comb in which they deposit stores. Their behaviour resembles that of bees during the honey-producing season.

In each series, once the cages were settled, four food treatment groups were formed at random and the respective food formulations were started to be fed ad libitum. After 10 days bees assembled in the cluster were killed by freezing. Subsequently, the bees were dissected to examine their digestive tracts and physiological condition. The fresh and dry weight of the entire intestine was measured and their water content was determined. The incidence of Nosema disease was also determined as well as the development of pharyngeal glands and of the fat body. In the first experiment series the pH of the rectum was also measured.

Each series also involved examinations of non-caged bees collected directly from the nest (zero treatment).

Fresh and dry intestinal weight was determined immediately after dissecting.Water content was calculated based on the difference between dry vs. fresh intestinal weight. Based on those data water percentage of fresh intestine weight was calculated. Each weighed sample was made up of three digestive tracts undamaged along their entire length. In each series and in each treatment three weighed samples corresponded to one cage. The data were calculated per intestine per treatment.

The pH of the rectum was determined for series I only using the method described earlier (Muszyńska, Leźnicka 1992). The assessment was done comprising 10 individuals in each cage. The results were presented as means per bee per treatment.

The condition of pharyngeal glands and of the fat body was scored using a scale from 1 to 6 where point 1 represented nearly total degeneration of the organ involved. In each series pharyngeal glands and the fat body were assessed based on individual scores of at ten least bees per

treatment, several individuals being collected from each cage within a treatment.

The samples were viewed using a binocular microscope at 100x magnification. A fragment of the dissected pharyngeal gland was placed on a slide in a drop of water. Fat body-containing sternites 4 - 6 were handled in a similar way.

At least 20 individuals per treatment in treatments 1 to 4 (several from each cage) and 10 individuals in the zero treatment were examined for spore infestation. Spore counts were made in individual intestines. The intestines were ground in 1 ml of distilled water and the spores were counted in 25 haemocytometer fields at 12x40 magnification.

The results were analyzed statistically by means of the Duncan test and the significance of differences was measured at $\alpha \leq 0.05$.

RESULTS

1. The condition of digestive tracts of worker bees fed food preparations with different honeydew honey content after 10 days of caging

Table 1

Condition of intestines of worker bees fed syrup containing different additions of honeydew honey. - Stan przewodów pokarmowych pszczół robotnic otrzymujących pokarm o różnej zawartości miodu spadziowego.

Trait Badana cecha	Series (set up date) Seria (data założenia)	Treatment - Kombinacja				
		0	1	2	3	4
Mean fresh intestine weight per bee (mg) - Średnia świeża masa przewodu pokarmowego jednej pszczoły (mg)	I (27.06)	45.4 b	25.4 a	42.0 b	34.9 ab	40.1 ab
	II (17.07)	42.0 b	21.6 a	38.8 b	35.1 b	38.2 b
	III (20.10)	30.1 a	33.2 a	29.9 a	35.2 a	25.0 a
Mean dry intestine weight per bee (mg) - Średnia sucha masa przewodu pokarmowego jednej pszczoły (mg)	I (27.06)	13.4 c	3.0 a	6.5 b	7.8 b	6.4 b
	II (17.07)	16.2 c	3.6 a	8.6 b	6.3 ab	6.6 ab
	III (20.10)	4.8 a	3.7 a	6.2 a	5.0 a	3.7 a
Mean water content of one intestine (mg) -Średnia zawartość wody w jednym przewodzie pokarmowym (mg)	I (27.06)	32.1 ab	22.4 a	35.5 b	28.1 ab	27.7 ab
	II (17.07)	25.7 b	14.7 a	28.3 b	28.3 b	31.5 b
	III(20.10)	25.2 a	28.0 a	23.6 a	30.2 a	21.4 a
Percent water content of one intestine - Procent wody w świeżej masie jednego przewodu pokarmowego	I (27.06)	71.0 a	89.2 c	63.6 a	78.7 b	80.1 b
	II (17.07)	62.2 a	83.7 c	75.8 b	80.1 bc	80.7 bc
	III (20.10)	83.6 a	84.9 a	84.5 a	85.0 a	84.9 a

Note: values followed by different letters are significantly different - odmienne litery oznaczają różnice istotne między wartościami.
Treatment: **0** - bees examined directly upon sampling from the nest - pszczoły badane bezpośrednio po pobraniu z gniazda; **1** - bees examined after 10 days of ingesting pure sugar syrup - pszczoły badane po 10 dniach otrzymywania czystego syropu cukrowego; **2** - bees examined after 10 days of ingesting sugar syrup with 10% addition of honeydew honey - pszczoły badane po 10 dniach otrzymywania syropu cukrowego z 10% dodatkiem miodu spadziowego; **3** - bees examined after 10 days of ingesting sugar syrup with 40% addition of honeydew honey pszczoły badane po 10 dniach otrzymywania syropu cukrowego z 40% dodatkiem miodu spadziowego; **4** - bees examined after 10 days of ingesting sugar syrup with 80% addition of honeydew hone - pszczoły badane po 10 dniach otrzymywania pokarmu zawierającego 80% miodu spadziowego i 20% syropu cukrowego.

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The most conspicuous differences for all traits under examination were found to occur between the zero treatment (bees collected directly from the nest) and treatment 1 (bees fed honeydew honey-free syrup for 10 days). The differences were more pronounced than those for bees from treatments involving the addition of honeydew honey (Table 1). In treatment 1 bees there was a significant reduction in fresh and dry matter content of the intestine and in their water content as well as the increase in the percent water content of the fresh matter. It must be stressed that the above regularity was valid for experiment series I and II. Instead, in series III caging bees for ten days did not affect the examined traits to any significant degree.

Table 2

pH of rectum content of worker bees fed food formulations with different honeydew honey contents (series I) - Odczyn jelita prostego robotnic otrzymujących pokarm o różnej zawartości miodu spadziowego (seria I).

Trait Cecha	Treatment - Kombinacja				
	0	1	2	3	4
pH	6,0 b	6,3 b	6,3 b	5,8 b	5,2 a

Note: for treatment designations see Table 1; values followed by different letters are significantly different.

Uwaga - kombinacje opisano przy tabeli 1 odmienne litery oznaczają różnice istotne między wartościami.

2. Rectum content pH of worker bees fed food formulations with different honeydew honey contents after 10 days of caging

Table 3

Mean infestation level by *Nosema apis* and the percentage of diseased individuals in bee samples fed food formulations with different honeydew honey contents.
Średnie porażenie przez *N. apis* oraz procentowy udział osobników chorych w próbach pszczół otrzymujących pokarm o różnej zawartości miodu spadziowego

Treatment Kombinacja	Series (set up date) - Seria					
	I		II		III	
	A	**B**	**A**	**B**	**A**	**B**
0	0	0	0	0	0	0
1	3,5 x10^6 a	40	0,1 x106 a	10	2,3 x106 a	16
2	0,2 x10^6 a	25	0,2 x106 a	30	1,0 x106 a	5
3	0,5 x10^6 a	25	2,7 x106 a	25	0,7 x106 a	10
4	2,4 x10^6 a	90	3,1 x106 a	30	2,0 x106 a	16

Note: for treatment designations see Table 1; values followed by different letters are significantly different

Uwaga - kombinacje opisano przy tab. 1 odmienne litery oznaczają różnice istotne między wartościami.

A Mean spore number/ sampled bee. - Średnia liczba spor/1 pszczołę z próby.

B Proportion of infested bees/sample (%). - Udział pszczół porażonych w próbie (%).

3. Nosema disease incidence in worker bees fed food formulations with different additions of honeydew honey after 10 days of caging

No Nosema spores were found in bees in any of the series involving bees sampled directly from the nest (zero-treatment). Instead, after 10 days of caging in each series and in each treatment Nosema apis -infested individuals were found. However, no significant treatment vs. treatment differences were found regarding mean infestation level (Table 3).

Noteworthy in series I is the high Nosema incidence among treatment 4 individuals that were fed syrup enriched with 80% of honeydew honey (Table 3). Such a regularity did not occur in the other series.

Table 4

Condition of pharyngeal glands and fat body in bees fed syrup with different honeydew honey contents. - Stan gruczołów gardzielowych i ciała tłuszczowego pszczół otrzymujących pokarm o różnej zawartości miodu spadziowego.

Trait Badana cecha	Series (set up date) Seria data założenia	Treatment - Kombinacja				
		0	1	2	3	4
Pharyngeal glands - Gruczoły gardzielowe	I 27.06	2,9 b	2,1 a	2,8 b	2,5 b	2,7 b
	II 17.07	1,0 a	1,6 b	1,4 b	1,5 b	1,7 b
	III 20.10	-	2,7 a	2,5 a	3,5 b	2,7 a
Fat body Ciało tłuszczowe	I 27.06	2,9 b	2,2 a	2,1 a	2, 5 ab	2,5 ab
	II 17.07	3,0 b	2,3 a	2,2a	2, 3 a	2,9 b
	III 20.10	-	2,7 ab	2,6 a	3, 0 b	2,9 b

Note: for treatment designations see Table 1; values followed by different letters are significantly different

Uwaga - kombinacje opisano przy tab. 1 odmienne litery oznaczają różnice istotne między wartościami.

4. Comparison of pharyngeal glands and the fat body of bees fed syrup with different honeydew honey contents after 10 days of caging

The data obtained are listed in Table 4.

The changes occurring in pharyngeal glands and in the fat body of worker bees caged during 10 days are in no clear relationship to the proportion of honeydew honey in the food supplied.

DISCUSSION AND CONCLUSIONS

The colony sampled for laboratory tests was infested by the Nosema but to a small extent during the whole season. In bees sampled directly from the nest (zero-treatment) no presence of Nosema spores was found even though in the sampling site (the last comb in the nest) there could be foraging bees susceptible to that disease (El-Shemy, Pickard 1989). The

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data obtained give evidence that no opportunity to make foraging flights favours propagation of the parasite and results in a heavier infestation rate among bees. Consistent with the data from literature (Lotmar 1943, Kellner 1980 cited after Pohorecka 1999) the duration of one full development cycle of the parasite may be from several dozen hours to several days. Therefore, it was possible to demonstrate the effect of 10 day-long caging on nosema disease incidence in bees (Table 3). On the other hand, no relationship was demonstrated between the proportion of honeydew honey in the syrup fed to bees and nosema disease incidence. The data obtained in this study are in agreement with those reported by Bailey (1967) who stated that it is by no means the rule that the colonies fed honeydew honey containing food develop high Nosema infestation in the spring. Similar observations were also made by Skubida (personal communication).

Demonstrated in this study significant differences between rectum pH of the bees from treatment 4 (80% of honeydew honey in food) and those from the remaining treatments (Table 2) may account for the occurrence of essential differences in intestinal bacterial flora among the different food treatments (Matras et al. 1998). However, the results do not warrant a generalization since pH in that intestinal segment was measured in the first experiment series only.

The study furnished evidence that under the conditions of the experiment the proportion of honeydew honey in the food fed to bees does not produce any clear impact on the development of pharyngeal glands or the fat body.

The study did not reveal significant differences between fresh and dry intestine weight among bees caged for 10 days fed syrup containing different proportions of honeydew honey: 10% (treatment 2), 40% (treatment 3), 80% (treatment 4). Likewise, no differences were found for water content of fresh intestine matter among those treatments. It is only in the first series that the bees of treatment 2 (food containing 10% of honeydew honey) were characterized by values for percentage of water in fresh intestine weight significantly different from those in treatments 3 or 4. However, in the remaining series the difference did not occur. Supposedly, the duration of 10 days is too short to produce differences among the treatments for the traits studied.

The period of 10 days in which bees ingested honeydew honey-containing food proved to be long enough to restrict the caging-related changes in intestinal characteristics under the conditions that resembled wintering period. The differences between bees sampled directly from the nest (treatment 0) and those confined in cages and receiving honeydew honey-free syrup for 10 days (treatment 1) were consistently more pronounced that those between the zero treatment and treatments 2, 3, and 4 (Table 1). At this stage it is difficult to judge if those changes are beneficial to them or not. Other investigations on changes in the intestines of bees collected from wintering colonies indicate that as the wintering season advances towards the first spring foraging flight the water percentage of the fresh intestine weight increases (Muszyńska, Bornus 1981). However, the increase is much slower than that observed in treatment 1 of this study. Moreover, it was shown that in the successive experiment series the experiment conditions affected bee intestines to different degrees in different treatments. In series 1 and 2 there were differences between bees from treatment 0 and those from the remaining treatments. On the other hand, in series 3 the experiment conditions did not bring about any significant differences between the treatments (Table 1). It can be conjectured that the difference between series 1 and 2 vs. series 3 in the response to the treatments to which the bees were exposed

was related to the fact that the bees were of different generations. In series 1 and 2 the bees were of summer generation whereas in series 3 of winter generation. The differences between bees of those two generations were pointed out earlier (Merz and associates 1979; Fluri and associates 1977; Konopacka and associates 1975; Maurizio 1953). The cited reports were not concerned with the description of bee intestines. It can be also relevant to this study that the winter generation bees sampled on October 20 had already stayed in the cluster for some time and had become adjusted to confinement and to lowered temperature. Conversely, the bees sampled in June and July had not been through that period.

In conclusion it should be stated:

1. The presence of honeydew honey in the food fed to caged bees mitigates the changes in their intestines related to the lack of flight opportunities.
2. Bee response to honeydew honey-containing food bears a relationship to the generation from which the bees are derived.
3. The 10-day period proved to be too short to demonstrate a significant relationship between the proportion of honeydew honey in food fed to caged bees and the traits investigated in the study.

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WPŁYW POKARMU ZAWIERAJĄCEGO DOMIESZKĘ MIODU SPADZIOWEGO NA STAN PSZCZÓŁ POZOSTAJĄCYCH W ZAMKNIĘCIU

J. Muszyńska

Streszczenie

Badania prowadzono w roku 1997 w warunkach laboratoryjnych w trzech seriach w okresie od czerwca do października. W każdej serii utworzono cztery kombinacje pokarmowe: 1- gęsty syrop cukrowy (3:2); 2 - gęsty syrop cukrowy z 10% dodatkiem miodu spadziowego; 3 - gęsty syrop cukrowy z zawartością 40% miodu spadziowego oraz; 4 - w której pszczoły otrzymywały miód spadziowy z 20% dodatkiem gęstego syropu cukrowego. W każdej serii wyżej wymieniony pokarm pszczoły otrzymywały przez okres 10 dni. Jedną kombinację pokarmową stanowiły trzy klateczki nasiedlone około 150 pszczołami pobranymi z produkcyjnej rodziny pszczelej. Klateczki te pozostawały w temperaturze 16-18°C, a obecne w nich pszczoły tworzyły kłąb wokół podkarmiaczki, z której ad libitum pobierały podawany pokarm.

Po zakończeniu każdej serii badań z klateczek pobierano żywe pszczoły, które zabijano przez zamrożenie a następnie określano stan ich przewodów pokarmowych (świeżą i suchą masę oraz procentowy udział wody w świeżej masie) stopień porażenia sporami *N. apis* oraz rozwój gruczołów gardzielowych i ciała tłuszczowego. Uzyskane wyniki porównano z danymi dotyczącymi pszczół robotnic pochodzących z tej samej rodziny badanych w dniu założenia serii, bezpośrednio po pobraniu z gniazda, nie przetrzymywanych w klateczkach.

W oparciu o przeprowadzone badania można stwierdzić, że w warunkach prezentowanego doświadczenia dodatek miodu spadziowego do pokarmu pszczół pozostających w zamknięciu zmniejsza nasilenie zmian zachodzących w ich przewodach pokarmowych w związku z brakiem możliwości oblotu. Równocześnie należy podkreślić, że w warunkach prezentowanego doświadczenia reakcja pszczół na pokarm zawierający miód spadziowy pozostaje w związku z pokoleniem do którego one należą. Okres dziesięciu dni poddawania pokarmu zawierającego różny udział miodu spadziowego pszczołom pozostającym w zamknięciu okazał się zbyt krótki na to by wystąpiły różnice między porównywanymi kombinacjami, w których pokarm zawierał różny udział miodu spadziowego.

Słowa kluczowe: miód spadziowy, pszczoły, zamknięcie.

JOURNAL OF APICULTURAL SCIENCE (notes for authors)

Journal of Apicultural Science is a scientific journal intended to publish original papers in the field of the broad-sense beekeeping science.

Manuscripts submitted in English and Polish (authors from Poland) should be concise and to the point without unnecessary circumlocutions and stylistic figures, written in a simple language. They must not exceed 40,000 characters.

The manuscripts should be sent to: Research Institute of Pomology and Floriculture, Apiculture Division, 24-100 Puławy, Kazimierska 2 Str., Poland

Each submitted manuscript is reviewed. The editor reserves the right to choose the reviewer/s and to make stylistic changes and other minor corrections that do not alter the scientific content of the paper. The authors are obliged to make the first proof reading of the manuscript. Once the corrected proofs are returned the authors bear the full responsibility for the content and the language of the paper.

The following arrangement of the paper is required:

The **title** of the paper should be as short as possible, clear and consistent with the subject. No abbreviations are allowed in the title save for the authors of taxonomic descriptions.

The **surnames of authors** are preceded by full first names. If there is more than one first name an initial of the second name is given. Below are full names and addresses of institutions at which the work was done. If authors' affiliations do not clearly follow from the order of names and institutions the names and the institutions should be marked by the same character e.g a numeral.

A **brief summary** with keywords - not exceeding half a page. It should give a short account of the paper's content.

The **introduction** gives an account of the subject with a synthetic discussion of the relevant literature. Citations of literature in the text are made by quoting the author's name followed by year of publication. If more than two co-authors are cited only the first author is quoted followed by "et al.". An exaggerated number of literature references should be avoided. The objective of the study should be defined at the end of introduction.

Methods - briefly states the protocols used and describes the experiment materials clearly enough for the reader to be able to reproduce faithfully a similar study.

Results should be reported very concisely without detailed discussion of data contained in the tables. An emphasis should be put on the significance of differences or the phenomena described. The same data must not be simultaneously contained in tables and figures (graphs).

The **discussion** is welcome only when it is necessary to confront one's own data with those of other authors. It is redundant in other papers.

The **conclusions** should generalize the results obtained. They should not be the summary of the paper or the recommendations for commercial beekeeping.

The **list of references** comprises original publications directly relevant to the subject of paper quoted in the main text body. The references should be arranged according to names of authors, the same authors should be placed chronologically. They should be written in the following style: Sakofski F., Koeniger N., Fuchs S. (1990) - Seasonality of honey bee colony invasion by Varroa jacobsoni Oud. Apidologie, 21(6):547-550.

The **extended summary** with paper title, initials and full surnames of authors and with keywords - not to exceed one and a half page of text. It gives a brief account of methods, results and conclusions. It is necessary to prepare this summary in Polish.

Keywords derived either from the title or from the main text ought to be informative of the subject. They should be placed at the end of summaries. The keywords, not to exceed five, should be in the first case and in singular if possible.

Tables presented in paper ought to be self-explanatory i.e. it should be comprehensible without making references to the main text. The headings must not contain symbols which are not generally known and the wording of headings must be concise. Tables and figures should be accompanied with descriptions. Tables - should be designed in such a way as to stand reduction to publication size (125 x 200 mm) without compromising legibility.

Figures (drawings, graphs) should be prepared on white paper and have a size of 125 mm x 200 mm. Larger illustrations will be reduced to publication size of which the due allowance should be made (if scaled down to required size small print may become illegible). The figures must be identified by placing a number on the back e.g. Fig. 1, Fig. 2 etc. Graphs can also be submitted as MS Excel 97 files or in the TIF format scanned at 300 dpi resolution with a hardcopy attached

Photos should be submitted with the number in the reverse and transparencies (slides) should have the number on the frame. If the original photo cannot be supplied a grayscale TIF file scanned at 300 dpi resolution is acceptable.

The manuscripts should be submitted in the MS Word 97 format. Should other word processing package be used they ought to be exported to the RTF or TXT formats and accompanied with one hardcopy containing legible formatting details (bold, italics, special characters). Hand-made corrections on the printout are allowed; they should be highlighted in red.