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Content

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ARTYKUŁY PRZEGLĄDOWE

EXPERIMENTS ON HYGIENIC BEHAVIOUR OF HONEY BEES

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Summary

It has been a long-debated question, if the colony is able to get rid of any agent of disease by themselves, without the help from outside factors. This is the reason, why the researchers follow with keen attention the hereditary hygienic behaviour of immune-mechanism among the bee colonies. Many experts are of the opinion that this form of behaviour has an important role in the defence against infectious and parasitic diseases.

We performed our experiments in the apiary of the Szent Istvan University, Gödöllo, (apiary 1), in a private apiary (apiary 2), and in the apiary of the Institute for Small Animal Research (apiary 3).

The differences in hygienic behaviour between colonies of the three apiaries can be seen in Tables 1-3. The average cleaning success was 74% in the first, and 72% in the second apiary, after 24 hours. In the third apiary, where the cleaning instinct as breeding trait was evaluated, the cleaning success was the highest, 86%, with 19.7% coefficient of variation. The results in the first two apiaries are nearly the same, in the third apiary, due to the freezing method, were significantly lower in the same colonies than the result of the piercing method.

In these experiments the very close results of observation for 24 and 48 hours show that the time factor has a little role in this behavioural pattern. In the case of the freezing method, the hygienic behaviour of colonies was nearly the same.

On the base of our experiments, we recommend the application of the pin piercing method for testing the hygienic behaviour. The systematic testing would be advisable in colonies kept for breeding and for queen rearing.

Keywords: hygienic behaviour, cleaning success, frosty method, pin piercing method.

INTRODUCTION

The new scientific results and experiences in the bee management have elucidated the pathological and epidemic reasons of many honeybee diseases. In the earlier days general view was that one part of the agent, causing illness, was connected to one definite territory, (supposedly had endemic origin), but nowadays, due to the very intensive international traffic, much of them became wide spread. This perception has arisen anxiety throughout the world, and resulted in serious consequences. Defence against the infectious honeybee diseases is going on in many countries using treatment with medicines (chemotherapy), and in many countries there are very strict official prescriptions and blockades against the infected apiaries combined with colony extermination with official compensation.

This defence has resulted in a very different outcome. A perfect solution for preventing the spreading or for defence against the bee diseases have not been developed up to now. The growing number of health problems due to high concentration of

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apiaries in Hungary, as well as different methodical troubles, arising from the different types of hives, make the Hungarian apiarists unquiet and disturbed about the honey production in our country.

It has been a long-debated question if the colony is able to get rid of any agent of disease by themselves without the help from outside factors. This is the reason, why the researchers follow with keen attention the hereditary hygienic behaviour of immune-mechanisms among the bee colonies. Many experts are of the opinion that this form of behaviour has an important role in the defence against the infectious and parasitic diseases (Bailey and Ball 1991).

Kefuss et al. (1996) advice to apply a practical method for checking the hygienic behaviour in the colonies. Their work stimulated our research team to test the applicability of this well known, but in Hungary hardly tested method, in our apiaries.

Rothenbulher (1964) was the first who proved that selection could be a tool in breeding much more resistant colonies. He recognised that the hygienic behaviour is checked by two recessive alleles. By one allele the bee recognises the problem, and is able to open the sealed cell, the other allele controls the instinct of clearing away the dead larvae. Both alleles are necessary for a hygienic colony. In heterozygous populations, the appearance of dominant alleles diminishes the success of this character. In this case only one part of bees has hygienic instinct, or follows one or the other behavioural form.

Harbo (1995) found significant connection between hygienic behaviour of three weeks old workers, and the pest.

Spivak (1996) infected larvae of hygienic and non-hygienic colonies by Varroa mite and found differences only by heavier infection. There were differences in the number of chewed or scratched larvae at the bottom of the hives. Among these the hygienic colonies. have an advantage.

MATERIALS AND METHODS

We performed our experiments in the apiary of the Szent Istvan University, Gödöllo, (apiary 1), in a private apiary (apiary 2), and in the apiary of the Institute for Small Animal Research (apiary 3). In apiary 1, and 2. 10 and 11 colonies were chosen by random in July, and from each of them one sealed comb was carved out containing larvae. In an area probably not containing empty cells, a space of 5 5 cm was marked out by help of a model. This fragment contained about 100 cells. By using a sterile injection pin, in the marked fragment each cell was pierced in its centre to kill the larvae, then the comb fragments were marked and taken back to their original place. After 24 and 48 hours, the numbers of cells that were opened and cleaned, opened but not cleaned, and not opened were counted.

Thirty pollen collecting bees were taken from each colony to be tested for Nosema. The bees were homogenised in a mortar, adding previously a little water to them. Then they were allowed to dry on a slide and were fixed above flame. Dyeing with 0.4% methylene blue lasted 15 minutes, the counter-dyeing with 0.6% fuchsine solution only a few seconds. The assessment was based on the number of spores in three levels of infection.

Testing of the third apiary was in June and August. This testing was different from the previous ones, because 5 5 cm larvae containing, sealed comb fragments were carved out and frozen during 24 hours in a refrigerator. These fragments were subsequently put and fixed into the covered, larvae containing combs of 10 colonies. (Kefuss et al. 1996) The experiment was repeated in the same colonies by piercing with a pin.

Other data were also collected, e.g. of the number of honeycombs with larvae, type of hive, age of queens, signs of illness, etc.

For the comparison of the results, Staatgraf (Advanced Procedures) program analysis was used.

RESULTS

The results of the experiments can be seen in Table 1. This table also contains the results of Nosema testing. For statistical comparison the term of cleaning success was introduced, which was equal to 100 minus the number of the cleaned cells, counted after 24 and 48 hours.

The application of the *chi* test failed to reveal differences between the first and the second apiary in the 24 hour test but a significant difference was between colonies ($P<0.01$) for cleaning success. In the third apiary a significant difference was found ($P<0.05$) between the results from the methods piercing and freezing.

DISCUSSION

The differences in the hygienic behaviour between colonies of the three apiaries can be seen in Tables 1-3. The average cleaning success after 24 hours was 74% in the first, and 72% in the second apiary. In the third apiary, where the cleaning instinct as breeding trait was evaluated, the cleaning success was the highest, 86%, with 19.7% coefficient of variation. The results in the first two apiaries are nearly the same, in the third apiary, results due to the freezing method were significantly lower in the same colonies than the result of the piercing method. ($P<0.05$).

In these experiments the very close results of the observation for 24 and 48 hours show that the time factor has a little role in this behavioural pattern. Fewer hygienic cleaning bees can clean the dead cells but during longer time, and for this reason we accepted the 24 hours observation. Within apiaries the treatments show significant differences, but the differences between apiaries did not reach the level of significance.

In the least hygienic two colonies of one of the apiaries a very serious pest was discovered thus verifying the statement of Harbo (1995). Nosema infection seemed strong in apiaries, tested in the beginning of summer, but there seemed to be no connection between the cleaning instinct and the infection.

In the case of the frosty method, the hygienic behaviour of the colonies was nearly the same. Application of this method is more complicated, needs more time, and the result does not give more information in spite of the fact that the bees should discover the dead larvae in a covered not injured honeycomb. In this method the strange smell of the little fragment of the treated honeycomb could be disadvantageous, as could perhaps be the low temperature, as well as the arising vapour. All this means more work for the bees, because on both sides of the honeycomb dead cells can be found, the evaluation is difficult, cannot guarantee the same number of cells (100-100 cells) on both sides of the honeycomb fragment. At piercing a lot of larvae are injured, which also means work for the cleaning bees.

The number of the injured cells could be diminished by cutting or piercing the honeycomb according to the shape of the cells e.g. by following the shape of rhombus. A freshly disinfected model containing 100 pins can be prepared for piercing,. An important point for the comparison is that the test in an apiary should be performed on the same date because the number of the young bees changes in different periods of the year. The place of testing should also be the same, because the instinct of cleaning is stronger in the middle of the honeycomb than farther away from it.

Based on our experiments we recommend the application of the pin piercing method for testing the hygienic behaviour. The systematic testing would be advisable in colonies kept for breeding and for queen rearing.

Table 1

Result of hygienic behaviour test at 24 and 48 hours (number of cleaned/not cleaned, or not opened cells), and result of *Nosema* test in three apiaries

Apiary 1			
Serial number/ Hive number	After 24 hours	After 48 hours	*Nosema* infection
1	78/18 all OK	98/2	+
2	57/24	90/10	+++
3	58/32	95/5	++
4	98/2	97/3	+
5	56/11	99/1	+
6	86/8	98/2	+
7	85/11	93/7	+++
8	49/5	95/5	+
9	93/7	99/1	+++
10	85/12	99/1	+++

Apiary 2			
Serial number/ Hive number	After 24 hours	After 48 hours	*Nosema* infection
1	72/20 OK	100	+++
2	65/4	100	+++
3	92/7	100	++
4	37/56	99/1	+++
5	76/14	99/1	+++
6	27/31	93/7	+
7	85/3	98/2	+
8	74/21	100	++
9	89/3	100	++
10	98/2	100	+++

Apiary 3					
Refrigerated			**Pierced**		
Serial/ Hive number	After 24 hours	After 48 hours	After 24 hours	After 48 hours	*Nosema* infection
1	28/5	93/3	97/3	97/3	-
2	21/7	98/2	91/3	98/2	+
3	62/8	100	87/10	98/2	-
4	93/4	100	94/6	97/3	-
5	74/2	98/2	64/8	88/9	+
6	88/6	100	99/1	99/1	-
7	92/2	100	88/10	91/9	-
8	97/3	100	47/49	82/16	-
9	99/1	100	97/3	99/1	-
10	84/6	95/5	94/6	100	-

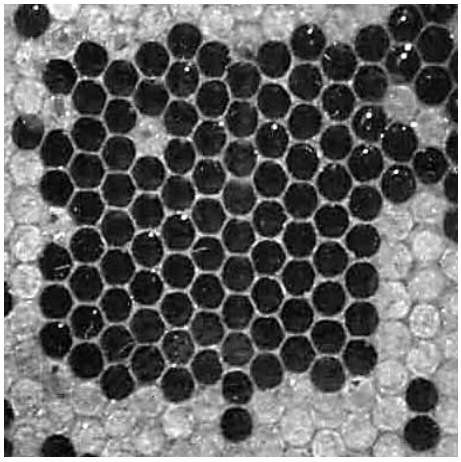

Fig. 1 Testing the hygienic behaviour of bee colonies by the method of pin piercing. The result of testing after 24 hours: in the middle area all cells are cleaned, a few pierced cells remained in the off-centre parts which were not recognised by bees

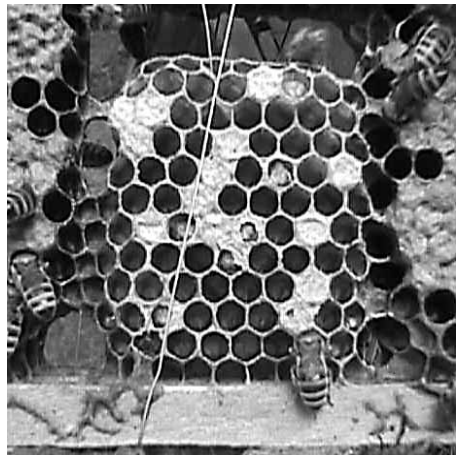

Fig. 2 Testing the hygienic behaviour of bee colonies, by the method of freezing a brood comb section. The piece of comb was fixed by wire. The bees cleaned only small cells after 24 hours. In the majority of the cells they did not recognize the dead larvae, or the cells had already been opened, but the dead larvae were not removed yet

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Thanks are extended to Dr. György Csaba Head of Dept. for his useful advice, Dr. Arpad Tóth and György Patocska for allowing the experiment in his apiary.

Without the help and stimulation of our already deceased colleague, this study could not have come into existence.

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BADANIA NAD ZACHOWANIEM HIGIENICZNYM PSZCZÓŁ

Békési L., Szalai E. M.

Streszczenie

Od dłuższego czasu prowadzone są rozważania czy rodzina pszczela jest w stanie przy pomocy czynników zewnętrznych chronić się przed czynnikami chorobotwórczymi. To jest powodem, dla którego badacze przywiązują wiele uwagi do dziedziczenia się zachowania higienicznego i odporności na choroby pszczół. Wielu ekspertów uważa, że to zachowanie

spełnia ważną rolę w obronie przed infekcją pszczół przez choroby pasożytnicze.

Nasze badania wykonaliśmy w pasiece Uniwersytetu Szent Istvan w Gödöllo, (pasieka 1), w prywatnej pasiece (pasieka 2) i w pasiece Instytutu Drobnego Inwentarza (pasieka 3).

Różnica w zachowaniu higienicznym między rodzinami trzech badanych pasiek (tabele 1-3). W pasiece 3, w której zachowanie higieniczne jest przedmiotem selekcji, częstotliwość oczyszczania komórek była najwyższa (86%). W dwóch pierwszych pasiekach wyniki były zbliżone ale zawsze niższe w przypadku czerwiu zabijanego przez zamrażanie niż przez przekłuwanie. W doświadczeniach tych podobne wyniki uzyskano po 24 i 48 godzinach wskazują, że czynnik czasu ma niewielki wpływ na wynik ostateczny.

Na podstawie naszych doświadczeń polecamy w badaniach behavioru higienicznego zabijanie czerwiu poprzez przekłucie szpilką. Ten sposób powinien być stosowany w pasiekach prowadzących selekcję pszczół.

Słowa kluczowe: zachowanie higieniczne, usuwanie czerwiu, zamrażanie, przekłuwanie szpilką.

EFFECT OF CHANGING OF COLONY STRUCTURE ON TRAPPED POLLEN

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S u m m a r y

The goal of the experiment was to establish the effect of the changing of the structure of bee colonies on the amount of trapped pollen. Colonies were divided into 5 groups: the group PTC (pollen trapping - control), the group PTF (pollen trapping and transferring colonies onto comb foundation), the group PTN (pollen trapping and nuclei building from brood and bees), the group PTI (pollen trapping and isolation of queens). The total production was established that consisted of harvested honey, trapped pollen and calculated honey (trapped pollen, wax, bees and brood taken from colonies expressed as kgs of honey).

Pollen trapping method applied in the group PTN (8.5 kg) was the most efficient during the experiment. The average amount of collected pollen was the largest by a highly dignificant degree in comparison with the other groups: PTC (3.6 kg), PTI (5.2 kg) and PTF (5.7 kg).

The highest honey yield was harvested from the control colonies (PTC - 16.3 kg) and the group transferred onto comb foundation (PTF - 14.4 kg), while the lowest one from the colonies where nuclei were created (PTN - 7.9 kg) and those differences were confirmed statistically ($p=0.01$).

Regulation of colony structure through taking brood and bees out in order to create nuclei (group PTN) as well as caging queens (group PTI) decreased significantly honey production in comparison with the control colonies (group PTC).

It can be said that the lower honey yield and the significant increase of pollen production in colonies of the group PTN were the result of the applied technology of regulation of colony structure.

Keywords: honey, pollen, pollen trapping, total production, brood, bees, queens.

INTRODUCTION

Pollen has been used for feeding honeybee colonies (Bobrzecki et al. 1994), bumblebees (Maciejewska, Wilkaniec 1998) and its therapeutic effect on the human body has been demonstrated as well (Drożdż, Gwizdek 1986, Stojko et al. 1997). The great interest in therapeutic properties (Taber 1984) of pollen as well as its usefulness for feeding honeybee colonies (Bobrzecki et al. 1994) recommend the aplication of pollen trapping in the apiaries. It has been proved, that this kind of bee management increases the gross income from an apiary (Cichoń, Wilde 2002, Marcinkowski 1994, Nelson et al. 1987, Wilde, Cichoń 1997).

Flow conditions, strength and condition of colony as well as applied pollen trapping technology are the main factors that decide about the amount of trapped pollen (Bobrzecki, Wilde 1991, Bratkowski et al. 1999, Bratkowski, Wilde 2002, Duff, Furgala 1986, Nelson et al. 1987). In foreign cuntries with a longer growing season and richer pollen flows ca 13 kg of pollen loads per one colony are obtained (Nelson et al. 1987), while under Polish climatic and weather conditions it is supposed to be about 2 kg (Bratkowski, Wilde 1998).

The installation of a pollen trap is the

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simplest pollen trapping method. A 10-day-cycle of pollen trapping installation has been recommended in order to avoid making pollen trapping difficult to bees: pollen is trapped for 5 days and after that period it should be stopped for the next 5 days (Poliščuk 1984). Duff and Furgala (1986) also applied a 14-day-cycle but they obtained a lower pollen production (from 1.4 to 2.9 kg) in comparison with traps application lasting the whole season. Better results in the amount of trapped pollen have been obtained by interference in the structure of the honeybee colony (Wilde et al. 1994a).

The goal of the experiment was to establish the effect of the changing of the structure of bee colonies on the amount of trapped pollen.

MATERIALS AND METHODS

The experiment was carried out in 1997-1999. Colonies of *Apis mellifera carnica* Pollm. were used in the experiment in 1997, 1998 and 1999, respectively: 48, 43 and 49, and were divided into 5 experimental groups. In the group PTC (pollen trapping, control) traditional bee management method were applied and pollen was trapped. In the group PTF (pollen trapping, foundation) all combs were replaced by frames with comb foundation as soon as as the eggs appeared in queen-cell cups. Honey and brood combs were given to the colonies of the same group that did not show symptoms of the swarm mood. In the group PTN (pollen trapping, nuclei) a part of combs with capped brood and some bees crowding its surface were used to make the nuclei. The nuclei were delivered with egg laying queens and pollen traps were installed. Their trapped pollen was included into productivity of the colonies from which they were built (Wilde et al. 1994b). In the group PTI (pollen trapping, isolation) queens were put into the Zander cages for 2 weeks as soon as they started laying eggs into queen cell cups. The queens got easy in touch with bees because of a peace of queen excluder installed on the cage sides.

Productivity of bee colonies

Top pollen traps were used in the experiments (Bobrzecki et al. 1989), that were installed on the bottom of the hives (Wilde, Bobrzecki 1989). Their functional parts were provided by plastic bars with round holes 5 mm in diameter. Pollen traps were installed at the hive bottom at the beginning of winter rape blooming, and taken off in the end of July on the following respective dates over the study years: 10th May and 30th July in 1997, 17th May and 27th July in 1998, and 20th May and 27th July in 1999. The weight of pollen loads was determined individually for every single colony in the each group.

The amount of harvested honey was measured twice in each year for two main honey flow periods, after blooming of winter rape and of buckwheat (Bornus et al. 1974, Wilde, Siuda 1996).

Converted production consisted of additional bee products expressed as kgs of honey which was evaluated as honey value units as follows: 1kg of bees = 2.5 kg of honey, 10 dm^2 of sealed brood = 4 kg honey, 1 kg of wax = 3.5 kg of honey (Bornus 1973, Bornus et al. 1974), 1 kg of pollen loads = 4 kg of honey (Wilde, Siuda 1996). The total production was expressed as kgs of honey and it consisted of centrifuged honey and converted production (Bornus et al. 1974, Wilde, Siuda 1996).

Average mean ($\bar{x}$) was the main numerical value for analized characters. The collected data were evaluated statistically using the computer programme STATISTICA. The estimation of significance of differences was made by variance analysis and the least significant differences (LSD) method.

RESULTS

Influence of method of bee colony regulation on pollen production

In 1997 the amount of pollen loads, concerning pollen trapped from nuclei, trapped in the group, where nuclei were created (PTN - 7.1 kg) was high significantly more in comparison with the control group (PTC - 3.0 kg - tab. 1), and significantly higher than amount of pollen collected from the group, in which queens were kept in cages (PTI - 3.6 kg). In 1998 the biggest amount of pollen loads was trapped again from the colonies of the groups PTN and PTI, 11.1 and 7.6 kg, respectively. The differences for the amount of trapped pollen in the group with nuclei vs. the control group (PTC - 3.5 kg) and vs. the colonies transferred onto comb foundation (PTF - 6.2 kg) were highly significant and they were significant compared with the group where queens were kept in cages (PTI - 7.6 kg). As in the previous years, in the season of 1999 the largest pollen yield was obtained from the nuclei group (PTN - 7.4 kg). This value was significantly higher compared with the groups where traditional bee management (PTC - 4.3 kg) was applied and queens were caged (PTI - 4.4 kg).

In the whole period of the experiment the creating of nuclei (PTN - 8.5 kg) was the most efficient pollen trapping method. The average amount of collected pollen was, to a highly significant degree, the largest of all the other groups. The pollen production of the rest of the colonies was very similar, and statistical differences appeared only between the colonies transfred onto comb foundation (PTF - 5.7 kg) and the control colonies (PTC - 3.6 kg).

In 1997, 1998 and 1999 nuclei were created in the group of PTN: 10, 11 and 18, respectively. In 1997, the average production obtained from one new colony was only 1.6 kg, whereas in the last year it increased to an average of 3.1 kg of pollen loads (fig. 1).

Influence of bee colony structure regulation on honey yield

In 1997, 13.9 kg of honey was harvested from control colonies, which was highly significantly more than from the colonies in which which nuclei were created (PTN - 4.4 kg - tab. 2). In 1998, the total honey yield ranged from 13.5 kg (group PTI) to 18.4 kg (group PTF) in the season, but the differences between all the groups came

Table 1

Pollen trapped in 1997, 1998 and 1999 as well as in the course of the experiment (averages in kg)

Grupa	1997	1998	1999	1997-1999
PTC - control, pollen trapping	3.0^{aA} n=11 d=1.7	3.5^{aA} n=12 sd=1.5	4.3^{aA} n=12 sd=2.4	3.6^{aA} n=35 sd=2.1
PTF - pollen trapping, foundation	5.4 n=12 sd=3.9	6.2^{abA} n=10 sd=3.0	5.4^{a} n=12 sd=2.1	5.7^{bA} n=34 sd=3.4
PTN - pollen trapping, nuclei	7.1^{bB*} n=13 sd=4.1	11.1^{cB*} n=10 sd=5.8	7.4^{bB*} n=13 sd=3.1	8.5^{bB} n=36 sd=5.1
PTI - pollen trapping, isolation of queen	3.6^{a} n=12 sd=1.2	7.6^{b} n=11 sd=4.0	4.4^{aA} n=12 sd=1.6	5.2^{abA} n=35 sd=3.2

* - including pollen trapped from nuclei,

Explanations: Different letters indicate significant differences at p=0.05 (small letters), and at p=0.01 (capitals)

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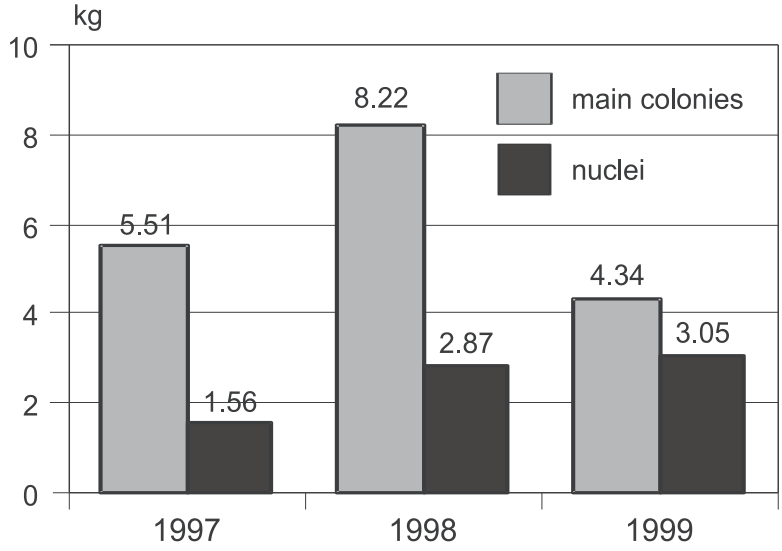

Fig. 1 Pollen trapped from main colonies and their nuclei in the group PTN in 1997, 1998 and 1999 (average in kg)

Table 2

Centrifuged honey in 1997, 1998 and 1999 (averages in kg)

Grupa	1997	1998	1999	1997-1999
PTC - control, pollen trapping	13.9^{B} n=11 sd=6.8	15.6 n=12 sd=18.1	19.5^{bcB} n=12 sd=9.5	16.3^{cB} n=35 n=13.6
PTF - pollen trapping, foundation	9.2 n=12 sd=8.2	18.4 n=10 sd=8.2	15,6^{bc} n=12 sd=5.5	14.4^{bcB} n=34 n=7.6
PTN - pollen trapping, nuclei	4.4^{A} n=13 sd=5.1	13.8 n=10 sd=9.1	5.5^{aA} n=13 sd=3.6	7.9^{aA} n=36 n=7.3-
PTI - pollen trapping, isolation of queen	8.4 n=12 sd=5.8	13.5 n=11 sd=6.4	11.9^{abA} n=12 sd=8.3	11.3^{ABab} n=35 sd=9.1

Explanations: Different letters indicate significant differences at p=0.05 (small letters), and at p=0.01 (capitals)

within the limits of the experiment error. In 1999 highly significantly more honey was centrifuged from the control group (PTC - 19.5 kg) in comparison with the PTN (5.5 kg) and PTI (11.9 kg) colonies, whereas the group PTF (15.6 kg) significantly outyielded the colonies of the group PTN.

During the whole experiment the highest honey yield was obtained from the control colonies (PTC - 16.3 kg) and from the group transferred onto comb foundation (PTF - 14.4 kg), while the lowest yield was from the colonies, where nuclei were created (PTN - 7.9) and these differences were confirmed statistically (p=0.01). Significance of differences was confirmed for the average efficiencies obtained from the PTC vs. the PTI colonies.

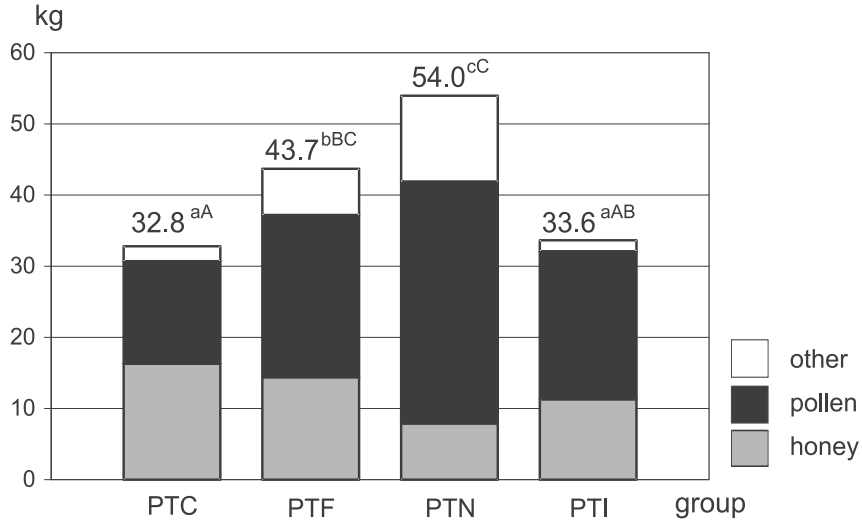

Fig. 2 Harvested and calculated honey expressed as the total production and contribution of particular components during the course of the experiment (in kg of honey)
Explanations: Different letters indicate significant differences at p=0.05 (small letters), and at p=0.01 (capitals)

Influence of structure changes of bee colonies on total production

Colonies, from which nuclei were created (PTN - 54.0 kg - expressed as kg of calculated honey as well as colonies transferred onto comb foundation (PTF - 43.7 kg) gave the highest total production (fig. 2). These groups highly significantly surpassed the control group (PTC - 32.8 kg). Highly significant differences also appeared between the PTN group and PTC (32.8 kg) and colonies with queens kept in cages (PTI - 33.6 kg). However, differences at p=0.05 were found between all the groups, along with PTC and PTI.

The obtained values expressed as the percentages of the total production show the importance of the particular components of the total production for shaping the productivity of the colonies. Trapped pollen decided about the total production of the colonies in all groups. In the control group (PTC) it had a lower importance and contributed 41% of the total production, whereas in the best group, where nuclei were created (PTN), pollen decided about the total production and its contribution was 63.2% of the total value. That is why honey had a marginal importance in that group. That value was similar in the group PTI (61.8%). It is characteristic of the best group PTN, that the high pollen contribution to the total production is also accompanied by the high calculated production which came from conversion to honey units of brood, bees and wax, having much more influence on productivity than honey. Such relationships only occurred in that one group. In the other groups the importance of production got from calculated honey and expressed as the percentage of the total production was not as important as the contribution of honey.

DISCUSSION

The differences in quantity of collected pollen in particular groups confirmed that changes of bee colony components decided about the volume of pollen production. In the case of the group PTN (creating of nuclei) the values were better than those achieved by Duff and Furgala (1986),

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the latter investigators having trapped 9.6 kg of pollen loads on average. However, Nelson et al. (1987) collected an average of 13 kg of pollen for 3 months, using, like the previous authors, a method based only on the installation of traps. Such a method applied in this study (pollen trapping without additional operations on bee colony - group PTC) did not allow the obtaining of comparable efficient results. However, the chances are that pollen production will be increased through making smoller the diameter of pollen bars wholes. Wilde et al. (1994a) produced on average 8.1 kg of pollen loads without changing the structure of bee colonies, using plate with meshes 4.8 mm in diameter. Bieńkowska and Pohorecka (1996) proved, that the wider diameter of the holes in the plate from 5.0 mm to 4.8 mm caused the 50% increase in pollen trapped. Taking into consideration that relationship, our result obtained in the group PTC (control) agrees with dates of Wilde et al. (1994a). However, pollen trapping and traditional management of bee colonies was less efficient as evaluated against the other methods, but it was statistically confirmed in comparison with the groups PTN ($p=0.01$) and PTF ($p=0.05$).

In 1998, in the group made up of nuclei (group PTN) 11.1 kg of pollen loads was trapped in the authors' experiment while Wilde et al. (1994a) obtained with that method only 3.9 kg of pollen. However, Wilde and Bratkowski (1996) increased the efficiency of that method to 7.4 kg, which is a value close to the average for the whole experiment of this study (PTN - 8.5 kg). Wilde et al. (1994b) stressed the influence of new nuclei on the production obtained, the amount of pollen trapped being similar to that in the colonies in which the nuclei were built. Such results can be explained by stimulation of egg laying by the queens and by the positive correlation between pollen collection by bees and reared brood (Crailsheim et al. 1992, 1996, Fewell, Bertram 1999), which was especially manifest in the last year of investigations.

The relationship between trapped pollen and honey yield showed that the applying of colony structure regulation methods may have caused a decrease in pollen production especially under unfavourable climatic and flow conditions. In 1999 the results confirmed such a finding and that the year was considered as cold and rainy. In the groups PTF, PTN and PTI not more that 1kg of pollen on average was trapped in the second part of season, while in the group PTC (control) that production was 2.7 kg. It was especially interesting that colonies transferred onto comb foundation (group PTF) responded like this in each year which suggested a very negative influence of this method on pollen collection. Comparing collection of honey and pollen in particular parts of the season it may be also noticed that high rate of pollen trapping was connected with a low honey yield, while low pollen production was associated with a high honey yield, which is consistent with results obtained by Poliščuk (1984).

On the basis of honey yield in investigated colonies it was found that applied technologies of pollen trapping decreased honey output which is consistent with the findings by Wild et al. (1994a). Silmilar relationship for pollen production vs. honey yield was found by Wilde and Bratkowski (1996). Alongside the group with nuclei, the group in which the queens were kept in cages also showed lower honey production in comparison with the group from which pollen was trapped and traditional bee manegement methods were applied, the relevant differences being statistically valid. Nelson et al. (1987) found only once in the course of 3 years a negative effect of pollen trapping on the honey yield. Wilde et al. (1994a) explained the decrease in honey production observed in the group, in which nuclei were made, by

the weakening of the colonies rather than by pollen trapping only. In our experiment significantly lower amounts of honey were centrifuged from the colonies from which brood and bees were taken out compared with honey yielded by the group PTC, which confirms a negative effect of removing brood and bees on honey yield. Thus, when trapping plenty of pollen and using nuclei we have to take into consideration a significant decrease in honey production. A increase from 9.5 kg (1997) to 14 kg (1999) in honey centrifuged was found in the group PTN in comparison with the group PTC (control), which is equivalent to the increase in honey yield, 0.74 and 1.89 kg, respectively, for every kg of trapped pollen. Caging queens (group PTI) also caused significant decrease in honey yield ($p=0.05$) in comparison with the group PTC (control), but the amount of trapped pollen was at one time the smallest during the experiment. The observed relationships between pollen and honey production in those two last groups in comparision with the control group allows the conclusion that such kind of regulation of colony structure decides about the efficiency of pollen trapping.

Pollen trapping increased to a large extent the total production in the group PTN - 63.2%, despite a significant decrease in centrifuged honey, compared with that in the control group. Our results were superior to those obtained by Pidek (1988), who noticed a decrease of only about 15.4%, Nelson et al. (1987), in whose study the decrease in output was about 26% and Poliščuk (1984), who got production more about 33%. It should be said, that they did not regulate structure of colonies, from which pollen was trapped, but they compared their productivity with colonies without pollen traps.

Biological conditions of bee colonies were very important and decided about different reaction of bees to applied methods (Page, Fondrk 1995). A permanent swarm mood was a very negative symptom in the group of PTF, which was observed in each colony transferred onto comb foundation. We could suppose that the reason for this phenomenon was removing of brood, which changed the biological relationships in colonies (Pankiw et al. 1998, Seeley 1989) and disturbed pheromons interaction between brood, queen and bees (Fewell, Bertram 1999, Hrassnigg, Crailsheim 1998, Winston et al. 1991). Pettis et al. (1997) demonstrated that brood pheromones and of queen in relation with queen's pheromones decreased about 50% the number of queen cells. Among colonies with caged queens (group PTI) bees made quen cells after queens were placed into cages. Such behaviour was observed by Muszyńska (1987). We may draw the conclusion, that queen cells were the result of the lack of a spread of the queen substance (Seeley 1989). Creating of nuclei was the only guarantee to eliminate the swarm mood, because in the group PTN there were no symptoms of swarming in any of the years (Wilde, Bratkowski 1996). Such behaviour caused a necessity for an additional inspection of bee colonies and for more frequent visits to the apiary to which increased costs.

CONCLUSIONS

Regulation of colony structure thrugh taking brood and bees out in order to create nuclei (group PTN) as well as caging queens (group PTI) decreased significantly honey production in comparison with that in the control colonies (group PTC). Lower honey yield and significant increase of pollen production in colonies of the group PTN were the result of the applied technology of regulation of colony structure.

Taking brood and bees out in order to create nuclei and pollen trapping in this group was the most efficient method of the

intensification of the production. Results of pollen production obtained from nuclei as well as elimination of swarm mood in colonies influenced the effectiveness of that method.

Pollen trapping in colonies transferred onto comb foundation (group PTF) and in colonies with caged queens (group PTI) have the limited practical application because of a high costs of labour resulting from additional colony inspections to develop removed brood, liquidation of swarm mood, destroying of queen cells and looking for the queen.

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WPŁYW ZMIANY STRUKTURY RODZIN PSZCZELICH NA ILOŚĆ ODEBRANYCH OBNÓŻY PYŁKOWYCH

Bratkowski J., Wilde J.

Streszczenie

Celem badań było ocenienie wpływu zmian struktury rodzin pszczelich na ilość odebranych obnóży pyłkowych. Doświadczenie przeprowadzono w latach 1997-1999. Rodziny podzielono na pięć grup doświadczalnych. W grupie PTC (kontrolna) stosowano tradycyjne metody gospodarki pasiecznej oraz odbierano obnóża pyłkowe. W grupie PTF wszystkie plastry zastępowano ramkami z węzą. W grupie PTN tworzono odkłady, i zakładano im również poławiacze pyłku. W grupie PTI matki umieszczano na 2 tygodnie w klateczkach Zandera

Produkcja całkowita rodzin została wyrażona w kilogramach miodu i składa się z miodu odwirowanego i miodu przeliczeniowego. W całym okresie doświadczenia tworzenie odkładów (PTN - 8,5 kg) było najbardziej wydajną metodą produkcji pyłku. Średnia masa pozyskanego pyłku była wysoko istotnie wyższa w porównaniu z pozostałymi grupami. Produkcja w pozostałych grupach była zbliżona, a różnice istotne stwierdzono jedynie pomiędzy rodzinami grupy przesiedlanej na węzę (PTF - 5,7 kg) a grupą kontrolną (PTC - 3,6 kg).

W tym czasie najwięcej miodu odwirowano od rodzin grupy kontrolnej (PTC - 16,3 kg) i grupy, w której wymieniano gniazdo na węzę (PTF - 14,4 kg), zaś najmniej od rodzin, od których tworzono odkłady (PTN - 7,9 kg) i różnice te potwierdzono statystycznie ($p=0,01$). Istotność różnic przy $p=0,05$ wystąpiła pomiędzy średnimi wydajnościami uzyskanymi od rodzin grup PTC i PTI.

W doświadczeniu najwyższą produkcję całkowitą uzyskano od rodzin, w których tworzono odkłady (grupa PTN - 54,0 kg) i przesiedlano na węzę (PTF - 43,7 kg). Grupy te wysoko istotnie przewyższyły grupę kontrolną (PTC - 32,8 kg). Wysoko istotne różnice wystąpiły również pomiędzy grupą PTN a PTC (32,8 kg) i rodzinami, w których matki izolowano w klateczkach (PTI - 33,6 kg).

Można, zatem stwierdzić, że regulowanie struktury rodzin pszczelich przez odbieranie czerwiu i pszczół, w celu wykonywania odkładów (grupa PTN) oraz izolowanie matek (grupa PTI) obniżało istotnie produkcję miodu w porównaniu z rodzinami grupy kontrolnej (grupa PTC). Obniżenie produkcji miodu a istotny wzrost produkcji pyłku w rodzinach grupy PTN były wynikiem zastosowanej metody regulowania struktury rodziny.

Odbieranie czerwiu i pszczół w celu tworzenia odkładów oraz pozyskiwanie od nich obnóży pyłkowych było najbardziej wydajną metodą intensyfikacji produkcji.

Słowa kluczowe: miód, pyłek, pozyskiwanie pyłku, produkcja całkowita, czerw pszczeli, pszczoły, matki pszczele.

BIOLOGICAL CRITERIA FOR ACCEPTING OF BEE BREAD AS A MITE FOOD BY *Tyrolichus casei* Oudemans (*Acarina: Acaridae*)

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Summary

The object of the study was to confirm the possibility of the development of *Tyrolichus casei* Oudemans on bee bread and the acceptance of this product by mites as evidence that these pests might threaten the provisions of honeybees.

Monocultures of the species were set up from specimens collected from hive debris and were kept in the laboratory at temperature ca. +20°C, RH - about 85%; bee bread was used as mite food. Biological experiments were conducted under the same conditions.

Some live parameters (average) of *T. casei* were as follows: embryonic development - 3.4 days, complete life history - 14.5 days, mortality of juvenile instars - 10.0%, frequency of females - 52.2%, longevity of adults - 101.8 days, oviposition period - 34.3 days, fecundity of females per lifespan - 426.3 eggs.

These results show a comparatively high biological potential for raising *T. casei* on bee bread and confirm a working hypothesis as concern the attractiveness and effectiveness of this kind of medium in rearing acarid mites. Evidence is presented that cheese mites are potential pests of stored hive products.

Keywords: *Acarina*, bee bread, biology, pests, *Tyrolichus casei*.

INTRODUCTION

Tyrolichus casei Oudemans is a cosmopolitan acarid species commonly known as a cheese mite and pest of various stored food products. Apart from cheese and other stored food it has been observed as a pest infesting grain, damp flour, dog-food, sometimes on ergot of rye and in insect collections. There are also records of this species under the bark of old tree stumps and in a mouse nest (Boczek 1980, Hughes 1976, Olsen et al. 1982, Robertson 1946, Smiley 1991, Türk, Türk 1957, Zakhvatkin 1941). It belongs to a group of allergenic mite species causing human dermatitis (Henschel 1929, Rosicky et al. 1979)

In apiaries *T. casei* was observed as an inhabitant of beehives colonizing hive debris, old honeycombs and as a pest infesting stored bee products and the provisions of bees, first of all bee bread and pollen (Chmielewski 1991 1992, 2001, Grobov 1981). As the results show from earlier studies on cheese mites fed bee- -collected pollen (pollen pellets), they accept this product as a suitable nourishment for population increase (Chmielewski 1994).

The purpose of this study was the experimental confirmation of the possibility of cheese mite development on bee bread and the acceptance of this product as evidence that these pests might threaten the provisions of honey bees.

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MATERIAL AND METHODS

Monocultures of the species were set up from live specimens collected from hive debris which were put into rearing cages and kept in the laboratory at temperature ca. +20°C, RH - about 85%; bee bread taken from honeycombs was used as mite food. Biological experiments were conducted under the same conditions.

Observations of mite development began on 100 newly deposited eggs (10 rearing cages x 10 one-day-old eggs) and finished after the eclosion of adults. Advances in the development cycle and mortality of specimens were recorded every 1-2 days.

Longevity and fecundity of mites were examined during observations of 25 pairs formed of freshly emerged adults (one pair = 1 female + 1 male), which were placed into rearing cages supplied with small pieces of bee bread (each pair was put into separate cage). Observations were made every 2-3 days until the natural death of all specimens.

More particulars and descriptions of procedures used in similar studies on some acaroid mite species fed bee bread and bee-collected pollen, were published in earlier papers (Chmielewski 1978, 1983, 1994, 1995, 1998, 2000, 2002).

RESULTS AND DISCUSSION

Life parameters of *T. casei* obtained in the present studies conducted on bee bread, showed that this kind of nourishment was acceptable by these mites.

Comparison of these results with the bionomics of cheese mite obtained on bee-collected pollen, i.e. pollen loads, under the same temperature and humidity (Chmielewski 1994) proved that both of these media are very effective and bring about a favourable population increase. However some biological data were differentiated.

Table 1

Comparison of live parameters of *Tyrolichus casei* Oudemans reared on bee bread taken from honey-combs (present results) with biological data obtained on bee-collected pollen (Chmielewski 1994), under the same laboratory conditions: temperature - ca. +20°C, RH - ca. 85%; n - 100 specimens (development, mortality), n - 25 pairs (1 pair - 1 ♀ + 1 ♂) (longevity, fecundity)

Character	Bee bread (present results)	Pollen loads (Chmielewski 1994)
Embryonic development (days)	3.4(3-7)	5.7(3-10)
Complete life history (days)	14.5(9-19)	18.5(14-22)
Mortality of juvenile stages (%)	10.0(0-30)	49.3(10-90)
Frequency of females (%)	52.2(40-62)	47.2(30-90)
Longevity of adults (days)	101.8(28-216)	85.6(30-205)
Oviposition period (days)	34.3(19-53)	63.9(16-129)
Nonfecundity general (days)	39.6(4-137)	19.2(3-123)
Fecundity of females per life-span (eggs)	426.3(236-570)	443.9(93-820)
Productivity of female per oviposition day (eggs)	12.4(1-35)	7.4(1-25)

Such parameters as embryonic development (3.4 days) and complete development cycle (14.5 days) of mites obtained on bee bread were shorter than on pollen (5.7 and 18.5 days, respectively). The oviposition period on bee bread (34.3 days) was also significantly shorter than on pollen (63.9 days), but productivity of females fed bee bread (average 426.3 eggs) was slightly lower than on pollen pellets (443.9 eggs per life-span). Viability of *T. casei* fed bee bread expressed as a percentage of specimens finishing their development cycle as imagines (90.0%) and their longevity (101.8 days) was significantly higher than viability of mites reared on pollen (eclosion of adults - 50.7% and their average longevity - 85.6 days) (Table 1).

A comparison of the bionomics of *T. casei* fed on bee bread with biological data of other mite species (*Acarus farris* (Oudemans), *Acarus immobilis* Griffiths, *Acarus siro* L., *Glycyphagus domesticus* (De Geer)) obtained earlier, on the same kind of food, and under similar conditions, shows that biological parameters of cheese mite are evidently greater than the same parameters of other examined acaroids (Chmielewski 1983, 2002).

CONCLUSIONS

The results of these experiments show a comparatively high biological potential for raising *T. casei* on bee bread and for its population increase on this medium.

Calculated biological parameters confirm a working hypothesis concerning the attractiveness of this kind of food for this species.

It also provides evidence that cheese mite is a potential pest of some stored hive products (bee bread, bee-collected pollen).

Bee bread might be recommended as an useful, effective medium for rearing acarid mites under laboratory conditions for experimental and educational purposes.

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BIOLOGICZNE KRYTERIA AKCEPTACJI PIERZGI PSZCZELEJ JAKO POKARMU ROZTOCZY PRZEZ *Tyrolichus casei* Oudemans (*Acarina*: *Acaridae*)

Chmielewski W.

Streszczenie

Rozkruszek serowiec, *Tyrolichus casei* Oudemans, powszechnie znany jako szkodnik wielu przechowywanych produktów spożywczych, jest także mieszkańcem uli pszczelich, gdzie zwykle kolonizuje osyp gromadzący się na dennicach i poraża zapasy pokarmu swych gospodarzy zgromadzone w plastrach (pierzgę, pyłek, miód); pojawia się też niekiedy w pracowniach pszczelarskich i magazynach pasiecznych na składowanych w nich produktach.

Czyste gatunkowo hodowle rozkruszka założono na bazie osobników dorosłych wybranych z osypu zimujących pszczół i prowadzono w laboratorium - w temperaturze ±20°C, w wilgotności względnej powietrza ok. 85%, w klateczkach hodowlanych używanych przez akarologów w tego typu badaniach biologicznych; jako pokarm podawano roztoczom pierzgę pszczelą.

Doświadczenia biologiczne prowadzono w tych samych warunkach. Obserwacje rozwoju roztoczy rozpoczynano na 100 świeżo złożonych jajach (10 jedno-dniowych jaj x 10 komórek hodowlanych) i kończono po wylęgu osobników dorosłych. Zaawansowanie cyklu rozwojowego sprawdzano co 1-2 dni. Długowieczność i płodność roztoczy były badane w toku obserwacji 25 par utworzonych ze świeżo wylęgniętych imagines (jedna para = 1 samica + 1 samiec), które umieszczano w klateczkach hodowlanych zaopatrzonych w małe kawałki pierzgi pszczelej (każdą parę trzymano w oddzielnej komórce hodowlanej). Kontrolę doświadczeń,

a także w miarę potrzeby uzupełnianie lub wymianę pokarmu, prowadzono co 2-3 dni aż do naturalnej śmierci wszystkich osobników.

Uzyskane parametry życiowe *T. casei* (średnie dane) przedstawiają się następująco:

Rozwój embrionalny (dni) - 3.4 (3-7)
Całkowity rozwój osobniczy (dni) - 14.5 (9-19)
Śmiertelność osobników w stadiach młodocianych (%) - 10.0 (0-30)
Frekwencja samic (%) - 52.2 (40-62)
Długość życia osobników dorosłych (dni) - 101.8 (28-216)
Okres składania jaj (dni) - 34.3 (19-53)
Bezpłodność ogółem (dni) - 39.6 (4-137)
Płodność samic w ciągu całego życia (jaja) - 426.3 (236-570)
Produktywność samic na dzień płodny (jaja) - 12.4 (1-35)

Wyniki te wskazują na stosunkowo wysoki potencjał biologiczny *T. casei* na pierzdze pszczelej i potwierdzają założenia hipotezy roboczej odnośnie do atrakcyjności i skuteczności tego rodzaju pożywki w hodowli rozkruszków. Są one także jednym z dowodów na to, że rozkruszek serowiec należy do potencjalnych szkodników przechowywanych produktów pasiecznych stanowiąc dla nich realne zagrożenie, w tym zwłaszcza dla pierzgi.

Słowa kluczowe: *Acarina*, biologia, pierzga, szkodniki, *Tyrolichus casei*.

ONSET OF OVIPOSITION IN HONEYBEE QUEENS KEPT IN BOXES WITH NON-FREE FLYING BEES

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Summary

The aim of the paper was to examine the start of egg lying by instrumentally inseminated queens kept both before and after insemination with non-free flying bees in closed boxes in a room without air-conditioning.

The experiment was carried out on queens reared from 1-day-old larvae in June and July. Sealed queen cells were introduced 1-2 days before queens emerging to boxes 130×115×70 mm settled with about 50 bees. Each box was provided with a comb, candy and a syringe with water. Queens were instrumentally inseminated twice at the age of the 7th and 9th day with 4 µl of semen. After the first insemination they were introduced to new boxes with about 250 bees and candy containing 5% pollen. Control group consisted of queens kept in trapezoid mating nuclei with about 400 bees. These queens were also instrumentally inseminated twice at the same age and with the same dose of semen.

It was found that queens kept in boxes started egg lying. However, insemination efficiency was worse than that in queens kept in nuclei. Queens in nuclei started egg lying on average 10 days after the first insemination, whereas those in boxes about 1.5 times later.

Keywords: honeybee queen, instrumental insemination, oviposition, box.

INTRODUCTION

The employment of instrumental insemination in the process of rearing honeybee queens has substantially contributed to the progress in beekeeping, i.e. to the introduction of highly productive and docile bee lines. By their appropriate crossbreeding, one obtains hybrids that produce significantly more honey then the starting material (Prabucki and Chuda-Mickiewicz 1998, 2000, 2002). A considerable portion of inseminated honeybee queens produced by breeding and reproductive apiaries consists of queens without verified oviposition (Troszkiewicz, personal communication). These queens are malevolently accepted by bees and their introduction into colonies requires the employment of suitable methods (Wilde et al. 2002). This situation indisposes beekeepers for introducing them to apiaries. Woyke and Jasiński (1979 1980) recommend to keep queens with non-verified oviposition for two days more, after insemination, in boxes (with a capacity of 793 ml each) settled with 250-350 bees. Studies of Chuda-Mickiewicz and Prabucki (1993) showed that inseminated queens kept in the apiary in boxes accompanied by about 250 free flying bees started egg lying in the same time as those kept in trapezoid mating nuclei.

The aim of the study was to examine the onset of oviposition by queens kept both before and after insemination with bees in closed boxes placed in a room.

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MATERIAL AND METHODS

Experiment was carried out in 2001 and 2002 on Carniolan (*Apis mellifera carnica*) queens bred in 2 rearing series. Queens were reared from 1-day-old larvae in a queenless colony, the first series was in June and the second in July. On 1-2 days prior to queens emerging, queen cells were introduced into boxes, 130x115x70 mm, settled with about 50 bees., A slice of comb was secured in the boxes to the ceiling, next to which a syringe with water was introduced through an opening in the ceiling, and a candy lying on a shelf above box bottom. Boxes were kept in a room without air-conditioning both before and after queen insemination at 18-20°C. Queens were instrumentally inseminated twice at the age of 7 and 9 days, each time with 4 µl of semen. After the first insemination, they were introduced into new boxes settled with about 250 bees and provided with candy containing 5% pollen addition. Control group consisted of queens kept in trapezoid mating nuclei with about 400 bees. These queens were also instrumentally inseminated twice on the 7th and the 9th day of life with 4 µl of semen. The obtained data were analysed statistically with the use of Student's t-test.

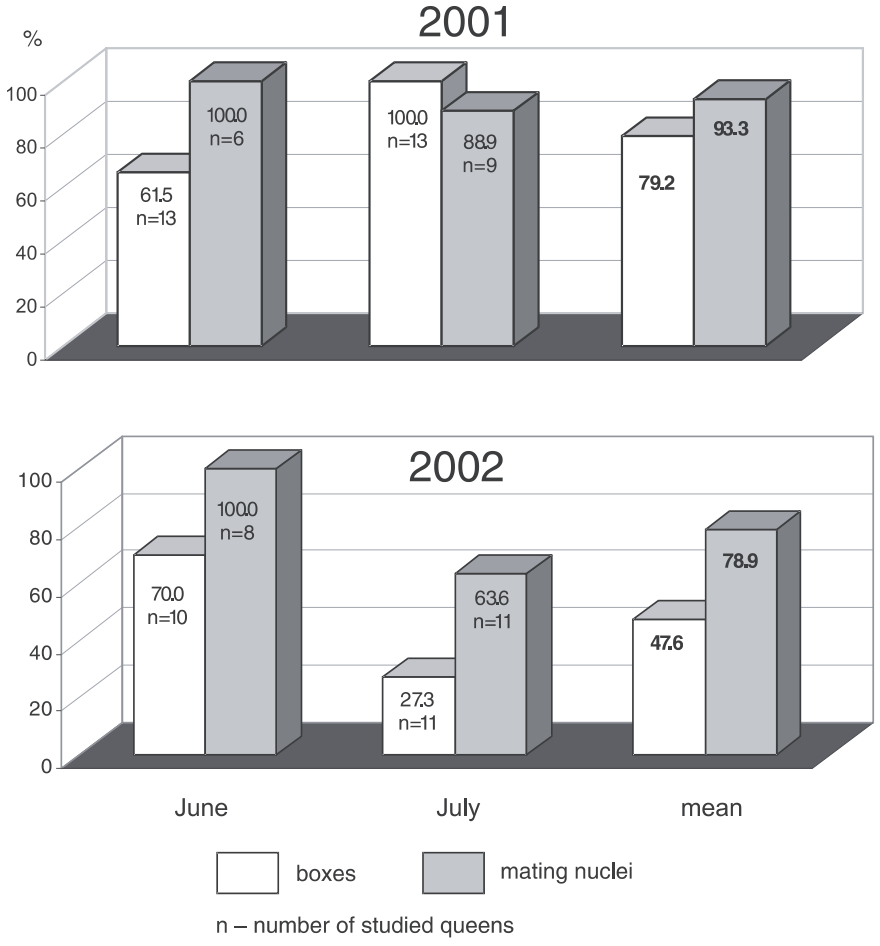

Fig. 1 Percentage of queens which started egg laying in boxes.. (in room) and mating nuclei

Table 1

Onset of oviposition in queens according to the method of their keeping before and after instrumental insemination

Year	Period of rearing	Method of keeping queens before and after insemination					
		Boxes			Mating nuclei		
		n	no. of days since 1 insemination to start of oviposition		n	no. of days since 1 insemination to start of oviposition	
			range	mean		range	mean
2001	June	8	13 - 21	14.2	6	8 - 10	8.5
	July	11	14 - 21	15.4	8	6 - 23	9.6
Mean				14.9 b*			9.1a
2002	June	7	16 - 28	19.4	8	8 - 20	12.4
	July	3	19 - 21	20.0	7	6 - 14	9.3
Mean				19.6b			10.9a
Overall mean				**16.5b**			**10.1a**

* numbers in rows followed by different letters differ significantly at $p=0.05$

RESULTS

Out of the total number of 79 instrumentally inseminated queens, 73.4% started egg lying. However, insemination efficiency (% of queens starting oviposition) differed in groups; from 64.4% in queens starting egg lying in boxes, to 85.3% laying eggs in nuclei (Fig. 1). Lower efficiency of insemination in queens kept in boxes was caused mainly by queens reared in July 2002. The same situation was found in queens kept in mating nuclei when the lowest number started egg laying. Thus, insemination efficiency depended upon the method of queen keeping and probably upon conditions during their rearing.

Time of starting the egg lying by queens depended upon the method of their keeping (Tab. 1). Queens kept in mating nuclei started egg laying significantly earlier than those kept in boxes. The former started oviposition on average 10.1 days after the first insemination. Queens kept in boxes started egg laying on average 6.4 days later. In the first study year, they started egg lying 14.9 days after insemination, and in the second one after 19.6 days. In 2002, queens kept in mating nuclei also started egg lying slightly later. Conditions prevailing during queen rearing influenced the results of queen insemination. This was noticed especially in the second study year, when insemination efficiency of queens kept in boxes was low and onset of oviposition was delayed.

CONCLUSIONS

The obtained results show, that the percentage of instrumentally inseminated queens which started oviposition in boxes is lower (ca. 20%) than that achieved in mating nuclei and the time of beginning of oviposition is over 1.5 times longer.

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ROZPOCZYNANIE CZERWIENIA PRZEZ MATKI PSZCZELE PRZETRZYMYWANE W SKRZYNKACH Z PSZCZOŁAMI BEZ MOŻLIWOŚCI OBLATYWANIA SIĘ PSZCZÓŁ

Chuda-Mickiewicz B., Prabucki J., Samborski J.

Streszczenie

Doświadczenie przeprowadzono w 2001 i 2002 roku na matkach wychowywanych z 1-dniowych larw w czerwcu i lipcu. Mateczniki na 1-2 dni przed wygryzieniem się matek poddawano do zasiedlonych ok. 50 pszczołami skrzynek o wymiarach 130x115x70 mm. W skrzynkach umieszczony był plasterek woszczyny, ciasto miodowo-cukrowe i strzykawka z wodą. Skrzynki do czasu inseminacji jak i po unasiennieniu matek przetrzymywano w nieklimatyzowanym pomieszczeniu. Matki inseminowano dwukrotnie w wieku 7 i 9 dni, 4µl nasienia. Po pierwszej inseminacji poddawano je do nowych skrzynek z ok. 250 pszczołami i ciastem miodowo-cukrowym z 5% dodatkiem pyłku kwiatowego. Grupę kontrolną stanowiły matki przetrzymywane w ulikach weselnych z ok. 400 pszczołami. Matki te również inseminowano dwukrotnie w 7 i 9 dniu ich życia, 4µl nasienia.

Stwierdzono, że matki przetrzymywane w skrzynkach podejmują czerwienie. Skuteczność unasieniania była jednak gorsza niż u matek przetrzymywanych w ulikach weselnych. Okres rozpoczęcia czerwienia był zróżnicowany. W ulikach matki przystępowały do składania jaj średnio po 10 dniach od pierwszego unasieniania, natomiast w skrzynkach ok. 1,5 raza później.

Słowa kluczowe: matka pszczela, unasienianie, rozpoczynanie czerwienia, skrzynka.

EXPERIENCE WITH USING BUMBLEBEES AS POLLINATORS IN THE REGENERATION OF THE GENETIC RESOURCES OF SOME FORAGE LEGUMES

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Summary

In our study the effectiveness of the bumble bees as pollinators of several species of the genera *Trifolium* L. (*T. rubens*, *T. ambiguum*, *T. hybridum*, *T. repens*, *T. pratense*, *T. medium*), *Medicago* L. (*M. romanica*, *M. polychroa*, *M. falcata*, *M. sativa*) and *Lotus corniculatus* was evaluated over a 5-year period at the Research Institute of Plant Production, Piestany. Those species were propagated in technical isolation separately or in combinations using *Bombus terrestris* for pollination. In the years 2000, 2001 *Bombus pascuorum* and *B. lapidarius* were used for pollination of *T. pratense* and *M. falcata*. The results showed that bumblebees could serve as effective pollinators of some perennial forage legumes with the aim to obtain sufficient amount of seeds for their maintenance in a gene bank. The existing differences in attractiveness between various plant genera may influence the amount of harvested seed in the case of their presence under the same coverage. Better seed yields per plant were obtained in *Medicago* in comparison to *Trifolium* species when grown together in the same enclosure. The effect of *Bombus* species on the seed weight of *T. pratense* was not significant. However, additional research is required to improve our knowledge in the reproductive biology and pollination requirements inclusive specific demands on the insect agent.

Keywords: forage legumes, multiplication, pollination, *Bombus* spp.

INTRODUCTION

Regeneration of germplasm is one of the most critical processes involved in the conservation of plant genetic resources and in the gene bank management. Even under optimum *ex situ* storage conditions, the viability of seeds declines and the genetic diversity is lost. Thus, the monitoring of viability and timely regeneration of seeds must be the prior activity of all gene banks (Richards 2001).

Maintenance of perennial forage legumes, which mostly need insect cross-pollination and are self-incompatible, is more difficult and costly than that of self-pollinated crops. The complexity and expense of regeneration are higher for species, which are insect pollinated.

Regeneration of sufficient number of plants in isolation is a possible solution to maintain the genetic integrity of increased perennial forage legumes. Principally, there are three possible ways of isolation to achieve it: technical, space isolation and manual pollination.

Manual pollination being thorough and precise it is also time consuming with high demand for labour. On the other hand, natural pollinators are more efficient and effective in producing quality germplasm than pollinating by hand.

Isolation in space may be possible, if

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specific areas for the multiplication of genetic resources are reserved. Risk arises in the case of forage crops as cross-pollination with wild-growing species and possibly with cultivated species grown on neighbouring fields may occur. Also the inter- as well as intra-specific competition for pollinating insects may play its negative role if space isolation is used.

The possible solution how to maintain the genetic integrity of increased forage legumes seems to be through the regeneration of sufficient number of plants in technical isolation. The use of screened cages with specially designed hives for honey, leafcutter or bumble bees for several insect-pollinated crop species is now common (Ellis et al. 1981, Ptacek 1987, 1999, Richards 1995). From several insect species bumble bees, *Bombus* spp. are recognized as efficient and effective pollinators of many crop species. Bumblebees have some obvious advantages over honeybees for pollinating plant germplasm in cages. Under limited space condition various *Bombus* species can pollinate successfully even those plant species which do not belong among their favourite food sources in the open. Ptacek (1983), Ptacek et al. (1984) obtained satisfactory results in cage pollination of alfalfa secured not only by *B. lucorum* and *B. terrestris*, but also by *B. lapidarius*, *B. pratorum* and *B. sylvarum*. To save on resources, different crop species may be planted under one enclosure (Engels and Rao 1995).

The aim of this study was (1) to obtain the basic information on agronomics and pollination requirements of some perennial forage legumes, (2) to compare the possibilities to pollinate various species of forage legumes in one enclosure, (3) to find out those *Bombus* species which are the most suitable for pollination of particular forage legumes, (4) to improve the regeneration procedures for the concerned crops.

METHODS

The effectiveness of bumble bees as pollinators of several species of genus *Trifolium* L. (*T. repens* L., *T. pratense* L., *T. medium* L., *T. rubens* L., *T. ambiguum* L., *T. hybridum* L.,), *Medicago* L. (*M. romanica* Prodan, *M. polychroa* Grossh., *M. falcata* L., *M. sativa* L.) and *Lotus corniculatus* L. was evaluated over a 5-year period at the Research Institute of Plant Production, Piestany. These species were multiplied in technical isolation separately or in combinations. In the years 1997, 1998, and 1999 *Bombus terrestris* colonies were used for pollination, in the following years, also *B. pascuorum* and *B. lapidarius* were used for pollination of *T. pratense* and *M. falcata*.

Plants were planted out individually on 2 m x 3 m beds and the first or the second cropping season year was used for seeds production. Isolators were assembled and installed in the time of flower bud formation, and hives with bumblebees were put into cages at the beginning of flowering. The flowering period lasted 6 to 8 weeks, depending on the particular plant species.

Colonies of bumblebees were prepared in laboratory conditions. Given the restricted space and consequently limited food sources from plants on beds, smaller units were prepared. In each of them a laying queen or workers and brood in larval stage were present. In order to minimise initial stress after release, the colonies was supplied with a portion of pollen dough. Inside the isolators sugar solution was supplied ad libitum.

RESULTS AND DISCUSSION

In 1997 seed production per plant of 10 white clover (*Trifolium repens* L.) varieties grown outside and in enclosures were compared. The varieties grown in isolators yielded more seed in 7 of all cases (Fig. 1),

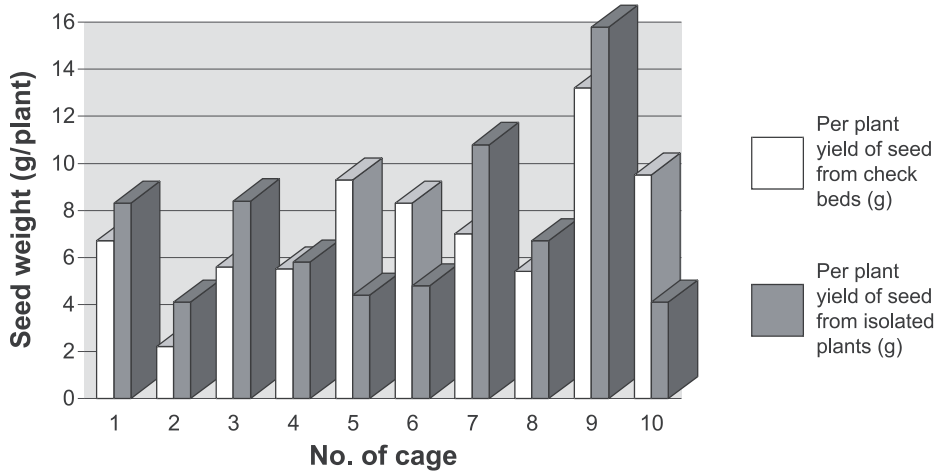

Fig. 1 Weight of white clover seeds (1997)

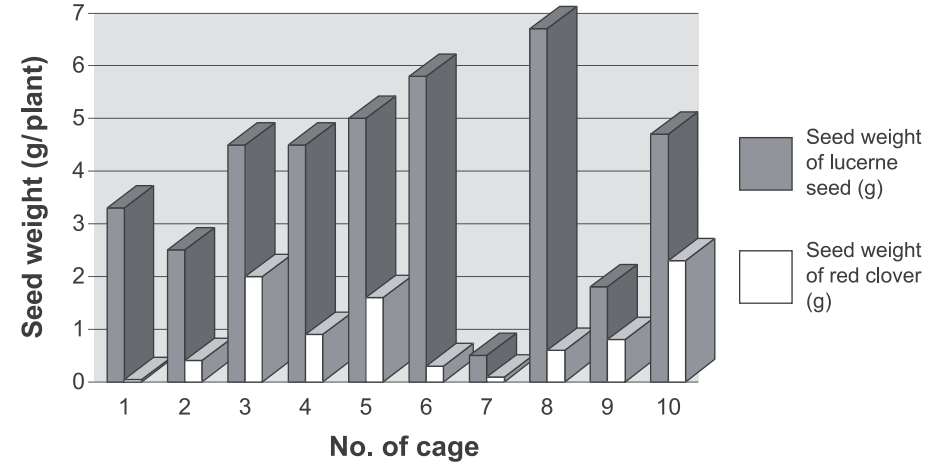

Fig. 2 Weight of lucerne and red clover seeds (1998)

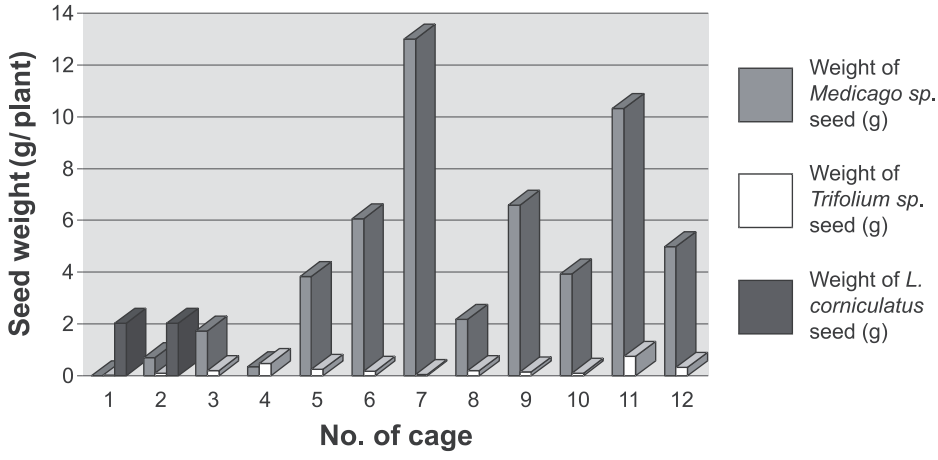

Fig. 3 Weight of *Medicago*, *Trifolium* species and *Lotus corniculatus* seeds (1999)

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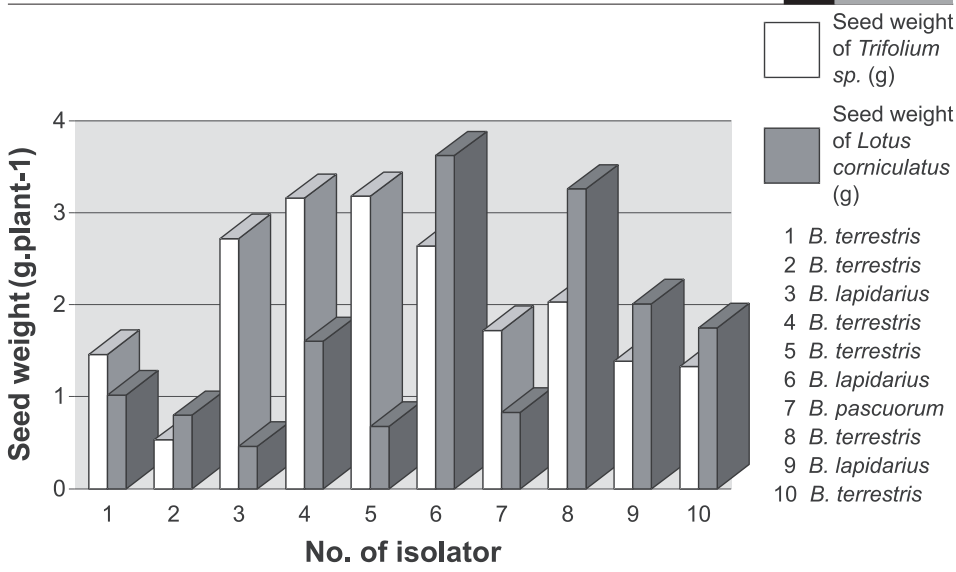

Fig. 4 Weight of *Trifolium* species and *Lotus corniculatus* seeds (2000)

Fig. 5 The arrangement of the trials

but generally, the yields under coverage were similar to those in the open air.

The next year alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) varieties were placed in ten enclosures. Better average results per plant were obtained in alfalfa in comparison with red clover (Fig. 2).

In 1999 combinations of several *Medicago* (*M. romanica* Prodan, *M. polychroa* Grossh., *M. falcata* L., *M. sativa* L.) and *Trifolium* (*T. rubens* L., *T. ambiguum* L., *T. hybridum* L., *T. repens* L., *T. pratense* L.) species (Fig. 3) grown in one enclosure have shown, that higher average seed yields per plant were obtained mostly in *Medicago* in comparison to *Trifolium* species. When alfalfa and red clover were grown in isolators together with *Lotus corniculatus*, bird's-foot trefoil gave the most satisfactory results. According to the results of both years *Bombus terrestris* preferred to forage on *Medicago* species rather than on clovers.

Some other studies with pollination of forage legumes showed similar results. Hofbauer and Pridal (1997) found that bumble bees, such as *B. terrestris* and

B. lucorum operate less effectively in isolators on red clover than *B. lapidarius*, *B. pascuorum* or *B. sylvarum* do. Brodie (1996) compared several species of bumblebees in the open and observed that *B. terrestris* never foraged on *T. pratense*. *T. pratense* was foraged in her study only by *B. pascuorum*. Also Ptacek (1987) found that *B. pascuorum* and *B. sylvarum* were more effective on *Trifolium pratense* and *B. terrestris* on alfalfa.

Basically on these statements, together with *B. terrestris* also *B. pascuorum* and *B. lapidarius* were used as pollinating agents in the cages with red clover next two years.

In 2000, combinations of *L. corniculatus* with *T. pratense* or *T. repens* were evaluated. There were no considerable differences between particular forage legumes in seed weight, but the higher average seed yield per plant was obtained in *Trifolium* species. In contrast to the previous findings, no negative effect of *Bombus* species on seed weight of *T. pratense* (Fig. 4) was observed. Only in some cases red clover pollinated by *B. lapidarius* and *B. pascuorum* gave better seed yield than by *B. terrestris*. According to the results both plant species seem to be similarly attractive for any of the bumblebee species used for pollination. In 2001 mainly *B. lapidarius* was used for pollination of *T. pratense* and *M. falcata* grown separately that time. In that year higher seed yield was obtained in red clover.

The simple evaluation of the results that we were able to do so far does not allow much definite conclusions. Obviously, the technical isolation can be used for regeneration of genetic sources because it brings at least as high seed yields as the pollination outside. The comparison of plants on the level of the genera, especially in the case of *Trifolium* and *Medicago*, hides the possible interspecific differences. Nevertheless, it can be concluded, that *B. terrestris* might not be a suitable pollinator in cases where *Medicago* together with *Trifolium* with long corolla tubes should be grown in one enclosure. *Trifolium* species can be grown with *Lotus* species regardless of the bumblebee species. Using of some "non terrestris" species may be convenient for enabling the pollination "made-to-measure".

CONCLUSIONS

According to the results from this study bumblebees can serve as pollinating agents of some perennial forage legumes in enclosures and thus ensure the sufficient amount of seeds for the maintenance of a given plant population in gene banks. Growing more than one plant species under the same coverage in order to lower the expenses is possible. However, remarkable differences in seed yields between *Trifolium* spp. and *Medicago spp*. grown together show that the process itself has to be studied more accurately and from the point of view both of the plants and the bees. Additional knowledge of the reproductive biology and pollination requirements of plants in the enclosures would be helpful, as well as the improvement of bumblebee management in order to allow the choice among several species.

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DOŚWIADCZENIE Z UŻYCIEM TRZMIELI JAKO ZAPYLACZY W ODNAWIANIU ZASOBÓW GENETYCZNYCH KILKU STRĄCZKOWYCH ROŚLIN PASTEWNYCH

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Streszczenie

W 5-letnich badaniach (1997-2001) prowadzonych w Naukowym Instytucie Produkcji Roślinnej w Piestanach (Czechy) oceniano efektywność trzmieli jako zapylaczy kilku gatunków koniczyny (*Trifolium rubens*, *T. ambiguum*, *T. hybridum*, *T. repens*, *T. pratense*, *T. medium*), lucerny (*Medicago romanica*, *M. polychroa*, *M. falcata*, *M. sativa*) i komonicy rożkowej - *Lotus corniculatus*. Rośliny uprawiano pod izolatorami oddzielnie lub w różnych kombinacjach używając do ich zapylania trzmiela ziemnego (*Bombus terrestris*). W latach 2000-2001 do zapylania *T. pratense* i *M. falcata* użyto dodatkowo trzmiela rudego (*Bombus pascuorum*) i kamiennika (*B. lapidarius*). Badania wykazały, że trzmiele mogą być wykorzystane jako efektywne zapylacze trwałych pastewnych roślin motylkowych dla uzyskania dostatecznej ilości nasion na potrzeby banku genów.

Słowa kluczowe: pastewne rośliny motylkowe, zapylanie, *Bombus* spp.

THE ANTIMICROBIAL ACTIVITY OF HONEY OF THE STINGLESS BEE *Trigona* spp.

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Summary

Stingless bees (*Trigona* spp.) produce a special honey which is less viscous and darker than honey of the honeybees (*Apis* spp.) and has a strong acid flavour. *Trigona* bee honey is highly prized in Ethiopia for medicinal use as a panacea for many ills. The antimicrobial activities of two samples of *Trigona* spp. honey obtained from Ethiopia and honeybee honey from Germany were tested *in vitro* against four fungal and six bacterial species.

Fungi were generally less sensitive than the bacteria or not sensitive at all to the treatments. Only *Aspergillus niger* and *Penicillium chrysogenum* responded slightly to one honey sample at a higher concentration. The bacteria reacted differently to the honey samples tested, with no relation to their Gram reaction. The minimal inhibitory concentrations of catalase treated honeys from stingless bees were higher than that of the non-treated samples for most bacterial species, indicating that hydrogen peroxide contributes to the antibacterial activity. *Apis* honey's hydrogen peroxide played no essential role in the antimicrobial activity against most bacteria tested. Concentrations below a certain value resulted in a microbial lawn of some bacteria around the inhibition zone indicating that the use of honey against infections at lower concentrations could be dangerous by encouraging the growth of bacteria.

Keywords: stingless bee, stingless bee honey, antimicrobial activity, non-peroxide activity, *Trigona* spp.

INTRODUCTION

Therapy with bee products (apitherapy) is a worldwide old tradition, used for thousands of years but replaced by antibiotics in modern times by all but a few individuals preferring traditional or natural remedies or those with no access to antibiotics (Molan et al. 1988). It is, however, reviving currently, apart from others, due to the increasing report of resistance of bacteria against antibiotics (Greenwood 1995, Molan and Brett 1998). In addition to the drug resistance of bacteria, the side-effects of some pharmaceutical products may give rise to an aversion to synthetic drugs (Thompson 1976, Kauffman 1991) and to an increasing interest in the use of alternative therapies. Although honey has been used as a medicine since ancient times, its effectiveness as a remedy has been revealed to be due to its antibacterial activity only a century ago (Molan 2001). The antibacterial activities of honey were considered to be due to: (I) osmotic effects - owing to the high osmolarity of honey whereby water is drawn away from microorganisms, reducing their ability to survive; II) acidity - as honey is acidic with a pH value between 3.2 and 4.9 which inhibits growth of many pathogens whose optimal pH of growth is not acidic; (III) hydrogen peroxide - which is produced in the honey due to conversion

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of glucose to gluconic acid by glucose oxidase, an enzyme produced by the hypopharyngeal gland of the bees; (IV) phytochemical factors - non peroxide antibacterial factors believed to be the various complex phenols and organic acids often referred to as flavonoids. These latter complex compounds do not breakdown easily under heat or light treatment and provide some types of honey with antibacterial activities even after these honeys were exposed to the mentioned factors, which usually destroy enzymes (Molan 2001).

The typical honey investigated by most researchers is produced by *Apis* spp. This honey is undoubtedly the most widely and massively collected and used by people for different purposes. But regionally, especially in the tropics and subtropics, there are other honeys made by different bee species, which are sometimes collected in substantial quantities (Krell 1996). One group of bees that produces a considerable amount of honey and hence can be used in beekeeping are the stingless bees.

Stingless bees are eusocial insects that belong to the family *Apidae* and the subfamily *Meliponinae* and lack a functional sting. Some species defend aggressively by biting the intruder - since they possess well developed mandibles, emitting a caustic liquid from the mouth, releasing unpleasant odours, and irritating by crawling in to the eyes, ears etc., of a predator (Ruttner 1992). Like the honeybees, stingless bees store honey and pollen (Michener 1974). The two important genera of *Meliponinae* that produce large amounts of honey and hence are used in stingless beekeeping are *Melipona* and *Trigona* (Crane 1992). The *Melipona* species are restricted to central and south America whereas *Trigona* species occur in all the tropical continental regions (Amano et al. 2000, Wille 1979).

Trigona spp. occur in Ethiopia at medium altitudes of up to 2300 m above sea level and are about 10 mm long (Fichtl and Adi 1994). They construct their nests in underground cavities of naturally abandoned ant nests, termite mounds, and cavities under plant roots at a depth of about 1m. The nest consists of thimble-sized oval shaped honey/pollen pots placed around the one cell thick brood area of the combs that are arranged horizontally, unlike the vertical combs of honeybees.

Stingless bees produce a special honey known in Ethiopia as Tazma honey. The Tazma honey is less viscous and darker than *Apis* honey and has a stronger acid flavour. In addition to that it also possesses a stronger bacteriostatic effect (Krell 1996). Ripened honey of the stingless bees contains 30 to 35% water unlike *Apis* honey, with about 17.1% (Ruttner 1992). Though the water content of stingless bee honey is high, it does not ferment in the nest presumably due to the abundant resin chemicals, which impart dark colour to it, and hydrogen peroxide (http://www.sibexlink.com.my/g15magazine/g15mag_vol3jan_science.html). This honey is often highly prized locally for medicinal use as a panacea for many ills, especially by the poor people of rural areas with less access to modern medicine. Due to its strong sour taste it is inedible and used only for medicinal purpose to treat cough, stomach disturbance, sore throat, tonsillitis, stomach and intestinal ulcers, cold, disease of the mouth and mucus membrane, and as wound dressing. Stingless bee honey is considered in folk medicine to be more powerful than honeybee honey for use as a “natural” cure for treating common diseases (Vit 2001).

The yield of honey from a colony rarely exceeds 1 to 2 litres. It is sold on market and is more expensive than *Apis* honey mainly due to its medicinal value and also the labour intensiveness of its harvesting process.

The purpose of our present *in vitro* investigation is to elucidate if the *Trigona*

honeys collected from Ethiopia have antimicrobial effects against different bacterial and fungal strains.

MATERIALS AND METHODS

Honey source

Two samples of *Trigona* spp. honey were obtained from two regions in Ethiopia, (I) Honey B from Bahir Dar (11° 35' N and 37° 28' E) Northwest Ethiopia, at an altitude of 1830 m above sea level; (II) Honey T from Temben (13° 53' N and 39° 53'E) Northern Ethiopia, at an altitude of 1500 m above sea level. The vegetation in both regions is categorized as Ethiopian undifferentiated woodland (White 1983). Honey samples were bought from Tazma experts and stored in a refrigerator, for one year, until needed for laboratory assay.

For comparison of the activity of *Trigona* honey with that of honeybee honey, a sample of honey was obtained from the research beehives of the Institute of Zoology, Free University of Berlin and named here as Honey D.

The pH of all the three honey samples was measured using a WTW Multi 340i pH meter (Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany) after diluting them 1:1 with distilled water.

Biological material

Bioassays of the antimicrobial activities of the different honey samples were performed using (I) four fungal species: the yeast *Saccharomyces cerevisiae* (DSM 211) and three filamentous fungi, *Aspergillus niger* (DSM 737), *Penicillium chrysogenum* (DSM 844) and *Trichoderma viride* (DSM 63065); (II) four species of Gram positive bacteria: *Bacillus subtilis* (DSM 347), *Micrococcus luteus* (DSM 348), *Bacillus megaterium* (DSM 90), *Bacillus brevis* (DSM 5609); and (III) two species of Gram negative bacteria: *Escherichia coli* (DSM 31), *Pseudomonas syringae* (DSM 5176).

Growth media

Cultivation of all bacterial cultures was done on Standard I nutrient broth (Merck Lot VL 630582) and/or Standard I nutrient agar (Merck, Lot VL 694681). The yeast was cultivated on a medium composed of 10 g Oxoid agar (Agar Bacteriological No. 1, Lot 817706-2), 1 g yeast extract (Sigma, CAS No. 8013-01-2), 2 g glucose (Merck), 0.5 g peptone (Sigma, Lot 128H0184), 10 ml Na-phosphate buffer (1M, pH 7) in 1 l distilled water. This same medium without agar was used as a nutrient broth for the yeast.

Trichoderma viride was cultivated on malt extract peptone agar (MEPA) composed of 30 g malt extract (Sigma, Lot 41k0181), 3 g soya peptone (Hy soy T, Sigma, Lot 128 H0184), 15 g Oxoid agar (Agar Bacteriological No. 1, Lot 817706-2) in 1 l distilled water. Aspergillus niger and Penicillium chrysogenum were cultivated on potato dextrose agar (PDA) composed of 20 g glucose (Merck), 15 g Oxoid agar (Agar Bacteriological No.1, Lot 817706-2), in 1 l potato infusion obtained by boiling 200 g scrubbed and sliced potato in 1 l water for 1h and passed through a fine sieve.

All types of media were sterilized by autoclaving at 121°C and 15 psi for 15 min.

Bioassay

Preparation of the honey samples

The different honey samples mentioned were serially diluted to 1:1 (50%), 1:5 (20%), 1:10 (10%), 1:20 (5%), 1:50 (2%), and 1:100 (1%), with distilled water and used for the determination of total antimicrobial activity. The total antimicrobial activity of the honey samples is assigned the letter **T** followed by a subscript of the letter for the name of the corresponding honey samples **B, T, D** for honeys from Bahir Dar, Temben, and Germany, respectively. Therefore the total antimicrobial activity of these honey samples is hereafter referred to as $\mathbf{T_B, T_T, T_D}$. By analogy the

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corresponding non-peroxide (NP) antimicrobial activities of these honey samples are hereafter referred to as NP_B, NP_T, NP_D, respectively.

The honey samples were treated with catalase to remove H_2O_2 and to elucidate their non-peroxide activity. Treatment was done by a 1:1 mixing of the honey sample with catalase solution of 20,000 units ml^{-1} modified slightly after Molan and Russell (1988). The catalase solution was prepared by dissolving a 13,300 units/mg solid catalase from bovine liver (Sigma, Lot 32k 7031, E.C. 1. 11. 1. 6) in distilled water. The catalase treated honey, which is diluted 1:1 due to the treatment, was diluted further to 1:5, 1:10, 1:20, and 1:50 and used in the Petridish bioassay.

Petridish Bioassay

Bacteria and yeast

An isolated pure colony of an overnight grown strain was picked carefully using a sterile transfer loop, inoculated to a nutrient broth in an Erlenmeyer flask and grown overnight at 30°C. About 50 µl of the overnight culture was inoculated to a 20 ml solution of nutrient broth and grown further for about 3 to 5 h until an O.D. of 0.6 (546 nm) was achieved (method slightly modified after Faye and Wyatt 1980). The suspension was then diluted 1:50 with the corresponding nutrient broth in order to prepare the standard inoculum.

The sterilized nutrient agar was cooled to 48°C, 5 ml of the standard inoculum was mixed with the 1 l nutrient agar and poured in to plastic Petridish of ∅ = 85 mm, 10 ml in each. When the agar was solidified 3 holes were bored per Petridish using a cork borer of ∅ = 9 mm. Each hole was then filled with 50 µl honey of a certain dilution and the Petridishes were placed in a refrigerator for 24 h, giving the honey enough time to diffuse. Finally, the plates were removed from the refrigerator, incubated at 30°C for 24 to 48 h and the inhibition zones were measured. Each concentration of all honey samples was investigated in triple Petridishes with three holes per Petridish, i.e., n = 9.

Filamentous fungi

An isolated pure colony of fungal culture which was grown for 72 h on solid medium and started to sporulate was scrubbed up using a sterile transfer loop and placed in 5 ml sterile distilled water in a test tube. The hyphae were disintegrated by adding sterile glass beads and shaking vigorously for 1 to 2 min, in order to get a uniform suspension. The 5 ml suspension was added to a 1 l agar solution at 48°C and the procedure above was followed further. The plates were taken out of the refrigerator and left at room temperature (*ca*. 25°C) for 72 h and the inhibition zones were measured.

The control experiments for both the bacterial and fungal cultures were done by inoculating the cultures with sugar syrup (Lyle's Golden syrup, UK) of dilutions similar to the honey samples. Sugar syrup has similar osmolarity to honey (Efem et al. 1992) and could reveal if the osmolarity of honey plays a significant role in the bacteriostatic/bactericidal activity of honey. The control experiments in the case of the non-peroxide activity tests were done using a catalase solution of 20,000 units ml^{-1} in distilled water. Each concentration of all honey samples was investigated in triple Petridishes with three holes per Petridish, i.e., n = 9.

Turbidimetric bioassay

Though the agar well diffusion technique is convenient to use, it is less sensitive than the turbidimetric method in displaying the activity of an antimicrobial agent. The main reasons for its insensitivity is that the honey sample is further diluted by the agar medium surrounding the well and that the extent of the clear zone obtained is not directly proportional to the activity of the sample, because outlying

colonies could grow before an inhibitory concentration diffuses to them (James et al. 1972, Molan et al. 1988). In contrast to the agar well diffusion technique that displays cumulative result of incubation after 24 or 48 h, the turbidimetric method allows for kinetic investigations and displays the activity of the antimicrobial agents with incubation period "live". It demonstrates the activity of the antimicrobial agents that may not completely inhibit growth but prolong the cell cycle time and hence could not be observed by the Petridish bioassay method. For this reason the antimicrobial activities of the two stingless bee honeys were additionally investigated using this method. Since the 1:1 and 1:5 diluted honey samples were too turbid, it was impossible to investigate their antibacterial activities spectrophotometrically.

An actively growing bacterial culture was diluted with nutrient broth to an O.D. = = 0.6 (546 nm) and 100 µl of the suspension was added to an Erlenmeyer flask with side nose (for O.D. measurement) that contains 10 ml nutrient broth with 1:10, 1:20, 1:50, or 1:100 diluted honey. The culture was then incubated at 30°C by measuring the O.D. at 2 h intervals for 6 to 8 h. Nutrient broth with the corresponding dilution of honey was used for blanking the spectrophotometer during each measurement. The control experiments were bacterial cultures with no honey and also bacterial cultures inoculated with sugar syrup/golden syrup with concentrations corresponding to that of the honey samples. Each concentration was investigated six times (n = 6).

Statistical analysis

Results are presented as mean ± S.D. values. Statistical tests were performed using the two-tailed student's test, Tukey's test, two-way ANOVA and $\alpha = 0.05$ was considered as the critical value.

RESULTS

The two honey samples from *Trigona* species were darker than the honey from *Apis mellifera* and also more acidic, with pH values of 3.2 and 3.45 for honeys B and T, respectively, compared to the higher pH of 4.9 for honey D.

The control experiments with sugar syrup did not show any inhibitory action on any bacterial or fungal species tested except for *B. brevis*, where a remarkable inhibition zone was observed at 100% and 50% concentration with inhibition zone diameters of 12.3±0.3 and 10.7±3.5 mm, respectively.

The responses of a specific microorganism to the different honey samples (Tukey's test) and to the two categories (total- and non-peroxide activities) of the same honey sample (two-tailed t-test) were significantly different in some cases and not in others, as seen in Table 1. The total activities of both honeys B and T (T_B and T_T) were superior to the non-peroxide activities (NP_B and NP_T) against the bacteria *B. brevis*, *M. luteus*, and *E. coli* at all concentrations tested, though the activities of only 50% concentrations of the different honey samples are displayed in Table 1. In addition to that 50% T_B exhibited stronger antibacterial activity against *B. megaterium* contrary to NP_B which encouraged lawn growth of this bacterium. There was no significant difference (two-tailed t-test) in the antibacterial activity of 50% T_T and NP_T against *B. megaterium*, both showing weak inhibitory activities. *Micrococcus luteus*, though inhibited with 50% T_B and T_T, showed a very dense microbial lawn due to incubation with the corresponding concentrations of hydrogen peroxide devoid samples, i.e., 50% NP_B and NP_T. *Escherichia coli* was inhibited strongly with 50% concentrations of hydrogen peroxide containing honeys B and T, but not with hydrogen peroxide free honey samples. Though T_B and NP_B showed slight differences in the strength of

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Table 1

Antimicrobial activity of 50% dilution of different honey samples elucidated by the inhibition zone diameter (mm), mean ±S.D. First letters denote the total activity (T) or non-peroxide activity (NP) and the subscripts indicate the origin of each sample; B: Bahir Dar, T: Temben (both from Ethiopia), D: Germany. One way ANOVA and Tukey's tests ($\alpha = 0.05$, n = 9) were performed across honey samples for each microbial species (along a row but not a column). Values within a row that possess the same letter do not have significant difference. l.g. denotes the promotion of lawn of growth

Organism	T_B	T_T	T_D	NP_B	NP_T	NP_D
B. brevis	21.1±0.7^a	16.2±1.7^{ab}	7.3±2.3^c	13.3±1.5^b	7.0±1.0^c	10.3±2.1^{cb}
B. megaterium	11.0±0.8^a	3.0±0.4^b	7.0±1.0^b	l.g.	5.3±1.2^b	6.0±1.0^b
B. subtilis	14.8±3.5^a	12.4±0.9^a	42.3±9.3^b	20.7±5.0^a	29.0±3.6^b	46.7±4.2^b
M. luteus	10.8±1.1^a	33.9±3.0^b	42.0±2.0^b	l.g.	l.g.	41.7±2.1^b
E. coli	13.1±0.8^a	27.6±7.7^c	21.7±2.9^c	0.0	0.0	20.3±0.6^c
P. syringae	12.5±0.5^a	4.1±0.0^b	7.2±0.8^c	15.7±6.4^a	7.3±2.3^c	10.0±2.0^{ac}
S. cerevisae	0.0	0.0	0.0	0.0	0.0	0.0
A. niger	5.0±1.0^a	0.0	0.0	4.7±1.2^a	0.0	0.0
P. chrysogenum	3.7±0.6^a	0.0	0.0	4.0±1.0^a	0.0	0.0
T. viride	0.0	0.0	0.0	0.0	0.0	0.0

Table 2

Comparison of responses of the different species of bacteria and fungi to treatment with 50% dilution of each honey sample represented by the mean diameter ± S.D. of the inhibition zone in mm. Statistical significance test was done across species (along rows) using One way ANOVA and the Tukey's test ($\alpha = 0.05$, n = 9) for each honey sample. Values with in a row that possess the same letter do not have significant differences. Identical letters at the values along columns do not designate any statistical relation. l. g. denotes the promotion of lawn of growth

	T_B	NP_b	T_T	NP_T	T_D	NP_D
B. brevis	21.1±0.7^a	13.3±1.5^a	16.2±1.7^a	7.0±1.0^a	7.3±2.3^a	10.3±2.1^a
B. megaterium	11.0±0.8^b	l.g.	3.0±0.4^b	5.3±1.2^a	7.0±1.0^a	6.0±1.0^a
B. subtilis	14.8±3.5^b	20.7±5.0^a	12.4±0.9^a	29.0±3.6^b	42.3±9.3^b	46.7±4.2^b
M. luteus	10.8±1.1^b	l.g.	33.9±3.0^c	l.g.	42.0±2.0^b	41.7±2.1^b
E. coli	13.1±0.8^b	0.0	27.6±7.7^c	0.0	21.7±2.9^c	20.3±0.6^c
P. syringae	12.5±0.5^b	15.7±6.4^a	4.1±0.0^b	7.3±2.3^a	7.2±0.8^a	10.0±2.0^a
S. cerevisae	0.0	0.0	0.0	0.0	0.0	0.0
A. niger	5.0±1.0^c	4.7±1.2^b	0.0	0.0	0.0	0.0
P. chrysogenum	3.7±0.6^c	4.0±1.0^b	0.0	0.0	0.0	0.0
T. viride	0.0	0.0	0.0	0.0	0.0	0.0

Table 3

Minimal inhibitory concentrations (MIC) of the different honey samples (in %) against the tested bacterial and fungal species. * = no inhibitory concentration, rather promotion of lawn of growth. ¤ = enhanced lawn of growth at the indicated and lower concentrations while inhibited by higher concentrations

	T_B	NP_b	T_T	NP_T	T_D	NP_D
B. brevis	>1	>10	>5	>20	>10	>5
B. megaterium	>5	*	>20	>20	>20	>20
B. subtilis	>5	>10	>20	>10	>10	>5
M. luteus	>20	*	>20	*	>20¤	>20¤
E. coli	>20	None	>20¤	None	>20	>20
P. syringae	>20	>5	>20	>20	>20	>10
S. cerevisae	None	None	None	None	None	None
A. niger	>20	>20	None	None	None	None
P. chrysogenum	>20	>20	None	None	None	None
T. viride	None	None	None	None	None	None

Table 4

Effect of sugar syrup (control) on the growth rate of different bacterial species. Growth determined spectrophotometrically (546 nm) and given as O.D factor (O.D. at t = 6 h divided by that at t = 0 h). At the 6th h of incubation period all the bacterial species were growing logarithmically

	Control	50%	10%	5%
B. megaterium	13.9	2.3	36.3	13.5
B. subtilis	27.5	2.2	22.8	6.5
E. coli	15.7	2.0	30.9	16.4
M. luteus	6.1	2.5	50.9	12.6
P. syringae	19.5	3.5	50.9	31.6

their inhibition on *B. subtilis*, this was not statistically significant (two-tailed t-test). However, NP_T paradoxically exhibited a superior inhibitory activity against *B. subtilis* compared to that of T_T. The total and non-peroxide preparations of the two stingless bee honeys did not show statistically significant difference in their inhibitory activities on *Pseudomonas syringae* (two-tailed t-test). Unlike in the case of the two honey samples from stingless bees whose strength of activity depended on the presence of hydrogen peroxide, the total- (T_D) and non-peroxide (NP_D) activities of *Apis* honey showed no difference in the strength of bacterial inhibition for each bacterial species tested (two-tailed t-test) (Table 1).

Fungi were generally less sensitive, or even insensitive, to the treatments. Only T_B and NP_B showed slight inhibitory effects on the two fungal species: *A. niger* and *P. chrysogenum*, but no statistically significant difference exists (two-way ANOVA) either across these two species or across the two honey preparations (Table 2). *S. cerevisae*

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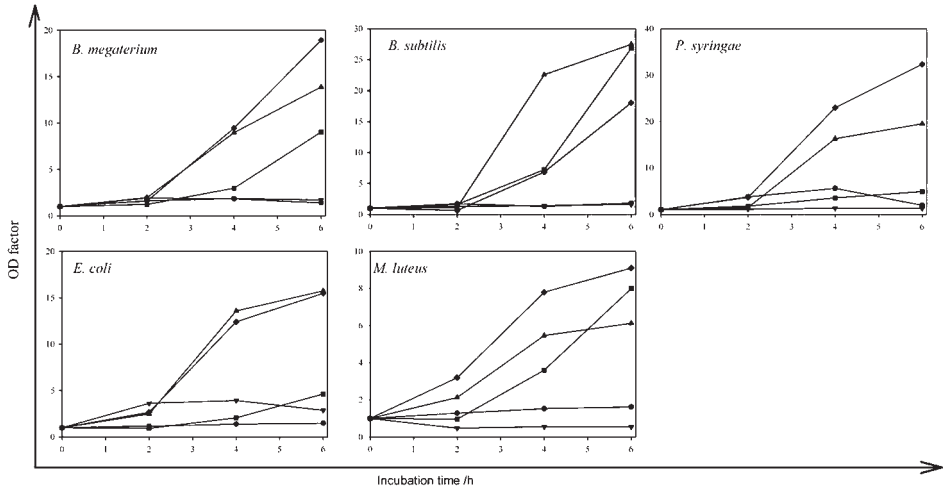

Fig. 1 The effect of stingless bee honey (Honey B) on the growth rate of different bacterial species determined spectrophotometrically. The OD factor is obtained by dividing the OD at t_t with the OD at t_0. The different symbols represent honey concentrations

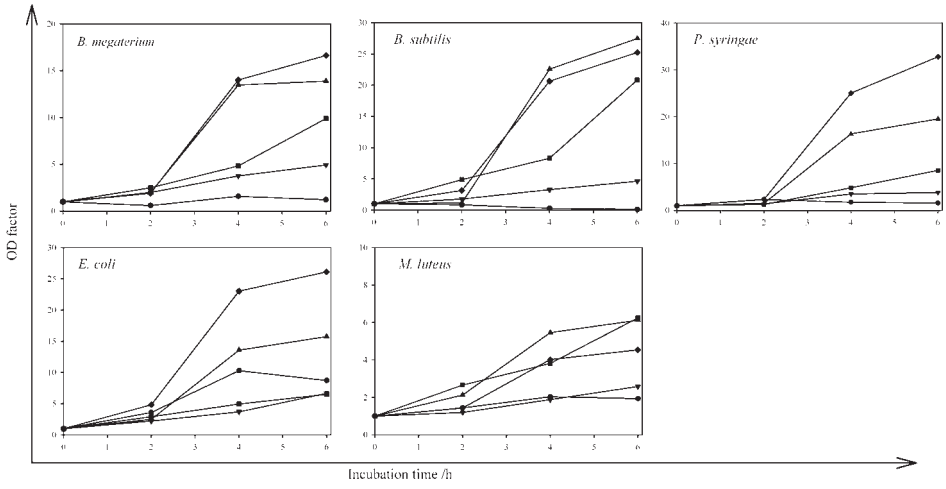

Fig. 2 The effect of stingless bee honey (Honey T) on the growth rate of different bacterial species determined spectrophotometrically. The OD factor is obtained by dividing the OD at t_t with the OD at t_0. The different symbols represent different concentrations of honey

and *T. viride* are insensitive to any of the honey samples. The response of the different bacterial species to the treatment with a certain honey sample is not dependent on the Gram reaction of the bacteria, both the Gram positive and Gram negative bacteria responding to the treatments. The responses of the bacterial species are rather dependent on the type of honey sample and individual organisms within a category, Gram positive or Gram negative, rather than the Gram reaction (Tables 1 and 2).

In case of the three species of *Bacillus* and *M. luteus* there were dense bacterial growth zones next to the inhibition zones with the honey samples B and T even at a

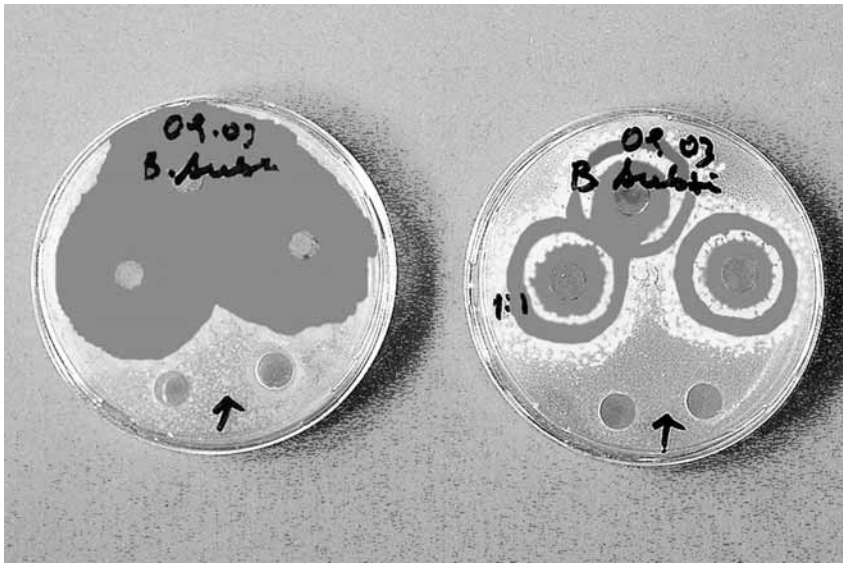

Fig. 3 Hormesis effect observed after treatment of *B. subtilis* with a 1:1 diluted Honey T. The arrows at the bottom side of the photo indicate the wells for the control treatment, i.e., sugar syrup of the corresponding concentrations

concentration of 100%. *M. luteus* and *B. subtilis* showed a unique response to the treatment with T_T where alternating rings of inhibition zones and dense growth zones were observed. The inner inhibition zones were clearer and larger, with complete inhibition of bacterial growth, and the outer inhibition zones were weaker and smaller, with the strength of inhibition decreasing outward. Up to three pairs of alternating rings were observed, with the number of rings declining with decreasing concentration of honey.

The minimal inhibitory concentration of catalase treated honey B was higher than that of the non-treated sample for most bacterial species, except for *P. syringae* where NP_B showed lower MIC than T_B. The MIC values of T_B and NP_B did not show much difference in the case of the two fungal species that were inhibited, *A. niger* and *P. chrysogenum*. The catalase treated honeys B and T failed to inhibit the growth of *E. coli* and also encouraged dense growth in case of *M. luteus*. Both T_D and NP_D resulted in microbial lawn of *M. luteus* at concentrations 20%. A dense growth of *B. megaterium* was also encouraged by 50% NP_B (Table 3).

MIC values of T_B and T_T obtained using the Petridish bioassay method were higher than those from the turbidimetric method. Lower concentrations of both T_B and T_T encouraged fast growth of most bacteria with the growth rates surpassing that of the control, i.e. without honey (Fig. 1 and 2).

The effect of sugar syrup on the growth rate of bacteria, though not detectable with the Petridish bioassay method, was revealed with the spectrophotometeric method (Table 4). The O.D. measurement showed that 50% sugar syrup retarded the growth rate of all bacterial species remarkably compared to the control. The 10% sugar syrup, however, encouraged growth of all bacteria tested. The 5% Sugar syrup displayed slight encouragement of growth in some bacteria.

DISCUSSION

The insensitivity of the different bacterial and fungal species to the control, sugar

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syrup, is an indication that the osmolarity of honey does not play an important role in the inhibition of growth of these microorganisms, but higher concentrations may prolong the cell cycle time. Hence, the other antibacterial factors of honey, i.e. hydrogen peroxide activity, non-peroxide activity mainly due to the flavonoids of honey, acidity, and lysozyme (Bogdanov and Blumer 2001) could be responsible for the inhibition of growth of these microorganisms. *Bacillus brevis*, however, is sensitive to and inhibited by the high osmolarity of sugar syrup indicating that, apart from others, the high osmolarity of honey could inhibit the growth of this bacterium.

The stronger total antibacterial activities of stingless bee honeys against some bacterial species compared to the non-peroxide activities are indications that hydrogen peroxide plays a significant role in the bactericidal/bacteriostatic action of these honey samples. This may be due to the fact that honey from stingless bees possesses considerable amount of hydrogen peroxide that could be, at least partially, responsible for preventing the fermentation of this honey which otherwise would have taken place due to its higher amount of water, 30 to 35%. Catalase treated honey B resulted in an enhancement of growth of *B. megaterium* and *M. luteus* though these bacteria were strongly inhibited by the non-treated honey samples. The most likely explanation for this could be that they are inhibited by hydrogen peroxide in honey in case of the total activity, and that the non-peroxide components are below inhibitory concentrations. Removal of hydrogen peroxide eliminates the inhibitory factor and hence bacterial growth could be encouraged either due to the higher sugar concentration, providing them with excess energy and nutritional sources, or the lower concentration of the phytochemical components below a certain critical level. The presence of certain bacteriostatic/bactericidal chemicals in the growth medium at a concentration lower than a critical inhibitory level enhances the growth of the organism that otherwise would have been inhibited by higher concentrations, a phenomenon known as hormesis (Edward et al. 1998). This effect of hormesis was also clearly observed in the case of treatment of *M. luteus* with 50% NP_T, though 50% T_T showed a very strong inhibitory action with an inhibition zone of nearly 34 mm diameter. The activities of both types of stingless bee honeys against *E. coli* were only due to the presence of hydrogen peroxide, whose removal resulted in the normal growth of this bacterium without any detectable inhibition zone.

The formation of concentric rings of inhibition- and dense growth zones could also be explained based on the phenomenon of hormesis, with the different components of honey (mainly the phytochemical components with varying diffusion potentials) acting as hormesis factors in the different zones.

Both stingless bee honeys showed a higher non-peroxide activity against the bacterium *B. subtilis* than total activity, though it was statistically insignificant for honey B. This is in contrast to most literature and difficult to explain. It was, however, pointed out by Burdon (1995) that hydrogen peroxide, at a concentration below a certain critical level, could stimulate the division and proliferation of many cell types in mammalian tissue and plays a role in wound healing. This chemical acts at various points in the mechanisms that control cell growth and differentiation probably by oxidising proteins involved, and thus causing a change in the conformation of the protein molecules. Apart from this evidence on mammalian cells, a positive role of hydrogen peroxide supporting the growth of bacteria was not yet reported.

The comparable antimicrobial activity of catalase treated and non-treated samples of honeys T and B on some bacteria

demonstrates that the activities are not due to hydrogen peroxide. The lack of hydrogen peroxide based activity and the insensitivity to sugar syrup demonstrates that the activity of stingless bee honeys could be mainly due to the phytochemical (flavonoids), lysozyme, organic acid components (Bogdanov and Blumer 2001) and probably due to its strong acidity. Conclusive evidence has been demonstrated by Molan and Russell (1988) that the antibacterial activity of some New Zealand honeys was not only due to hydrogen peroxide but mainly to the antibacterial agents of plant origin. The authors also concluded that in honeys of higher antibacterial activities, non-peroxide activity is by far the largest part of the total activity.

The *Apis* honey tested did not show hydrogen peroxide based activity. One possible reason could be that the honey was ripe since it was stored for one year. Storage of the honey for one year was important since its activity had to be compared with *Trigona* honey that was also stored for one year. The enzyme glucose oxidase is practically inactive in ripe honey, no more replenishing the degraded hydrogen peroxide and hence lower concentration of the latter which hardly inhibits bacteria (Bogdanov and Blumer 2001, http://privatewww.essex.ac.uk/~islamic/ilm/health-fit/honey_2.html). This is because the acidity produced in the action of the enzyme drops the pH to a value too low for the enzyme to work any more. In addition to that the activity of water (a_w) of a ripe honey is very low inhibiting the process (Bogdanov and Blumer 2001). The lack hydrogen peroxide activity demonstrates that the antibacterial activity of this honey is mainly due to the phytochemical components, or lysozyme. The acidity of this honey (pH = 4.9) is not strong enough to inhibit the growth of most bacteria as it is further diluted and weakened in the growth medium. Osmolarity also does not play a role since the bacteria tested did not respond to the osmolarity of sugar syrup, except *B. brevis*. The pH and osmolarity of different New Zealand honeys tested against various organisms did not inhibit bacterial growth (Molan and Russel 1988).

CONCLUSIONS

The traditional use of *Trigona* honey as a panacea against different illnesses is rational if the infection to be treated is caused by bacteria, not by fungi, since only one of the honey samples showed a minor antifungal effect against two of the four fungal species tested, though reports exist that honey inhibits the growth of fungi.

The enhancement of growth of some bacterial species by lower concentrations of honey indicates that the use of honey at lower concentrations as a means of apitherapy could be dangerous. However, if used undiluted, *Trigona* honey offers many possibilities as a broad spectrum-healing agent against both Gram positive and Gram negative bacteria. Especially the use of this honey as a wound dressing agent may be effective since its dilution by body fluid is less intensive than for internal administration.

Though it is premature to conclude at this level that stingless bee honey can be used as a panacea, as claimed by the local people, it can be ascertained that it has a broad spectrum of antimicrobial action inhibiting the growth of both Gram positive and Gram negative bacteria. Further research in this field may help to recognize and use indigenous knowledge to help alleviate the ever-increasing report of resistance of pathogenic bacteria to current antibiotics.

ACKNOWLEDGMENTS

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AKTYWNOŚĆ PRZECIWDROBNOUSTROJOWA MIODU PSZCZÓŁ BEZŻĄDŁYCH *Trigona* spp.

Garedew A., Schmolz E., Lamprecht I.

Streszczenie

Pszczoły bezżądłe (*Trigona* spp.) wytwarzają charakterystyczny miód o niskiej lepkości, zdecydowanie bardziej ciemny i kwaśny w stosunku do miodu pszczoły miodnej. W Etiopii miód *Trigona* spp. jest droższy ze względu na jego zastosowanie jako panaceum na wiele schorzeń.

W pracy oznaczono aktywność antymikrobową dwóch próbek miodu pszczół bezżądłych z Etiopii, i dla porównania, aktywność jednej próbki miodu pszczoły miodnej z Niemiec. Aktywność miodu określono testami *in vitro* w stosunku do czterech gatunków grzybów i sześciu gatunków bakterii.

Grzyby generalnie są dużo mniej wrażliwe na działanie miodu niż bakterie. Tylko *Aspergillus niger* i *Penicillium chrysogenum* w niewielkim stopniu reagowały na jedną z dwóch próbek miodu *Trigona* spp. i tylko przy jego wyższych stężeniach. Miód oddziaływał na bakterie bez związku z ich reakcją na test Grama. Minimalne stężenie miodu *Trigona* spp., które hamowało rozwój bakterii (MIC), było wyższe po zadziałaniu na miód katalazą, od tych próbek, które nie były traktowane katalazą (nie został rozłożony nadtlenek wodoru). Świadczy to o wpływie akumulacji nadtlenku wodoru na aktywność antymikrobową tego miodu. W przypadku miodu pszczoły miodnej (próbka porównawcza miodu niemieckiego) akumulacja nadtlenku wodoru nie odgrywała głównej roli w aktywności antybakteryjnej w reakcji przeciw większości użytych w doświadczeniu szczepów bakterii.

Słowa kluczowe: pszczoły bezżądłe, miód pszczół bezżądłych, aktywność przeciwdrobnoustrojowa, aktywność nie-nadtlenkowa *Trigona* spp.

FLOWERING PHENOLOGY AND FERTILITY OF SOUR CHERRY (*Prunus cerasus* L.) CULTIVARS SELECTED IN HUNGARY

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S u m m a r y

Experiments were conducted between 1972 and 2002 at three sites in Hungary. At Érd 97, Helvécia 10, and Újfehértó 3 cultivars of sour cherry were studied in variety collections. Observations were made on the flowering phenology (start, main time, end and length of the bloom period), on the flowering dynamics (the rate of the open flowers counted every day), on the fruit set following self- and open-pollination and on the effect of association of varieties in the orchards (choice, rate and placement of pollinisers).

Based on the results the rate of the overlap of the blooming times were calculated and varieties were assigned into five bloom time groups according to their main bloom. Self-fertility conditioned by natural self pollination was studied and good pollinisers were chosen (sweet, sour and duke cherry varieties) for the self-sterile and partially self-fertile varieties.

The necessity of bee pollination was proved by different pollination methods: natural self-pollination, artificial self-pollination, open pollination.

Keywords: fertility, flowering, pollination, sour cherry.

INTRODUCTION

Sour cherry production is an important industry and it is also an export item in Hungary. All except one of the registered and available 22 varieties are of Hungarian origin. The assortment is rather rich, moreover, regional selection and pedigree breeding promise currently new varieties.

Fertilisation in the *Pándy meggy*, then leading variety, has concerned the growers since the 19th century. Maliga (1942) surveyed the relevant literature. Self-fertility and its relation to fruit set obtained by open pollination was assessed in several Hungarian and foreign varieties by Nyéki (1974). All variants of *Pándy meggy* proved to be self-incompatible, and mutually inter-incompatible. Nyéki et al. (1992) called the attention to the fact that the self-incompatible Pándy meggy cannot be fertilised by several self-fertile varieties (Debreceni bőtermő, Kántorjánosi, Újfehértói fürtös), i.e. they are inter-incompatible.

Sugar content of nectaries is high in sour cherry flowers; thus honeybees are attracted. Earlier observations stated the beneficial effect of bee activity in both self--incompatible as well as self-fertile varieties. i.e. fruit set increased substantially (Benedek et al. 1990).

In the present paper first of all the variability of fruit set depending on self- or alternatively open pollination in sour cherry varieties was analysed.

The objectives of the studies were to determine the blooming time, self and open

pollination of Hungarian sour cherry varieties.

METHODS

Observations of blooming dynamics followed the method applied by Nyéki (1974) on flowers distributed to the four quarters of the heavens on the end of branches, a sample comprising 100-500 flowers per variety. Starting with the beginning of bloom, every day at the same time (between 10 and 12 a.m.) the number of flowers according to their stage of development from the bud phase, opening and petal shedding was registered.

Flowers are considered to be open if the anthers and the pistil are easily recognised from above, and the stigma is green or yellow. The stigma of fading flowers started browning. Those dynamic observation served as a basis of determining the start of blooming (with the first open flowers), the main blooming period (when more than 50% of flowers opened) and the day of main bloom (when the number of open flowers reached the maximum) and the end of bloom (when the last flower faded).

According to the start of blooming varieties are grouped into 5 categories (early, medium early, intermediate, medium late, late).

Studies of fertilisation were done according to the method of Rudloff & Schanderl (1950).

Autogamy was investigated on 20-30 cm long branches of different orientation (N, E, S, W) covered still at bud stage with parchment paper bags. 5-10 isolated units comprising 100-400 flowers represented each variety. Paper bags were eliminated 3-4 days after the blooming period ended. The effect of artificial self pollination or geitonogamy was checked on isolated branches prepared as in the case of autogamy, except that the fully open flowers were hand pollinated once with previously collected and stored (at +4°C) pollen of the same variety, subsequently, the bags were restored until the end of blooming period as in the case of autogamy.

Open pollinated flowers were observed, equally, on 5-10 branches 1.5-2 meter above ground level of four different orientations and comprising 100-500 flowers per variety.

Fruit set was registered and compared in all treatments at 1-2 weeks before fruit ripening.

RESULTS AND DISCUSSION

Blooming time

Sour cherry belongs to the group of medium early-blooming fruit trees but it is the latest among the stone fruits. At the start of blooming cultivated varieties cover a period of 4-7 days, during some seasons exceptionally also 10 days. Most authors distinguish three blooming time groups in sour cherry varieties. Present observations derived from three growing sites, 101 varieties, and a long period of years (1972-2002), which allowed the establishment of five blooming time groups. In Table 1 the propagated varieties are shown separately from the most important Hungarian and foreign varieties.

Neighbouring blooming time groups differ by 1-2 days only, depending on the season. In order to achieve considerable (more than 20%) fruit set a self-incompatible variety should be pollinated by a compatible polliniser, when their blooming period is overlapping each other by 70%, at least. In a system of 3 blooming time groups varieties belonging to the same group should be combined, whereas with 5 groups the overlap also of the neighbouring groups may prove to be sufficient for safe fruit set. Synchronous blooming or the extent of overlapping, however, may change yearly.

Variants of the variety *Pándy* differ in blooming time, thus Pándy 48 is medium

Table 1

Blooming time groups of sour cherry varieties

Early	medium early	intermediate	medium late	late
Érdi bõtermõ	Meteor korai	Favorit	Érdi jubileum	Pándy 279
	Csengõdi	Érdi nagygyümölcsû	Cigánymeggy 7	Debreceni bõtermõ
	Cigánymeggy C. 404	Korai pipacsmeggy	Cigánymeggy 59	Kántorjánosi 3
	Maliga emléke		Pándy Bb. 119	Újfehértói fürtös
	Pándy 48			Oblacsinszka
Török meggy		Montmorency (Nagy Gobet)	Hartai meggy	Paraszt meggy
Egri fürtös 102		Cigánymeggy 3	Fanal	Schattenmorelle (Latos meggy)
Törpe meggy			Cigánymeggy 215	

Table 2

Minimal and maximal overlap (%) of blooming periods in the *Pándy meggy* group of sour cherries with the potential polliniser varieties (Érd, 1972-1974)

♀	♂				
	Cigánymeggy 7	Cigánymeggy 59	Parasztmeggy	Schattenmorelle	Germersdorfi 57
Pándy 48	57-82	57-64	57-73	57-57	71-100
Pándy 279	75-100	75-90	80-90	75-80	60-90

early blooming, *Pándy 279* is late blooming. Blooming of *Pándy 48* is variable from year to year, thus the 70% overlap was hardly achieved with the medium late blooming *Cigánymeggy 7*, *Cigánymeggy 59* and late blooming *Parasztmeggy* and *Schattenmorelle* (Table 2). After having studied five potential pollinisers, only *Germersdorfi 57*, a sweet cherry variety proved to be a safe polliniser. *Pándy 279* seemed to overlap (by more than 70%) the bloom of *Cigánymeggy 7*, *Cigánymeggy 59*, *Parasztmeggy* and *Schattenmorelle*, whereas *Germersdorfi 57* was not sufficient every year.

Self-fertility

Self fertility of sour cherry varieties is a genetically determined property. In the extent of self-fertility there are substantial differences. In the growing practice, the self-incompatible varieties (e.g. types of the *Pándy meggy* group), as unsafe yielders are loosing space, whereas the popularity of self-fertile varieties is increasing.

Nyéki (1989) rated stone fruit varieties according to their extent of self-fertility into five groups. Sour cherry varieties observed on those three growing sites are assigned to those five groups as shown in Tables 3 and 4. In the assortment of sour cherries all variants between complete self-incompatibility and highly self-fertility are represented.

A high yield requires a 20-30% fruit set. Most of the sour cherry varieties are unable to set as much fruit at natural self pollination. Our data corroborate the statement of Rjabov & Rjabova (1970), which means that self-fertile sour cherry varieties are yielding safely every year because they are less subject to adversities due to weather and to growing site. Partially self-fertile varieties may produce 0-1% as well as more

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Table 3

Comparison of fruit set obtained by open pollination and by self-pollination in sour cherry varieties

Variety	Self-fertility groups	Self--pollination (%)	Open pollination (%)	Difference between self-and open pollination (%)
Cigánymeggy 1317[1]	completely self--incompatible (0%)	0	19.5	19.5
Törpe meggy[1]		0	4.7	4.7
Mean		**0**	**12.1**	**12.1**
Cigánymeggy 60[1]	self-incompatible (0.1-1 %)	0.2	6.1	5.9
Cigánymeggy 215[1]		0.1	15.5	15.5
Török meggy[1]		0.3	6.4	6.4
Mean		**0.2**	**9.3**	**9.1**
Cigánymeggy C.404[2]	partially self-fertile (1.1-10 %)	6.3	28.8	22.5
Debreceni bőtermő[2.3]		5.5	22.8	17.3
Diemitzer[1]		7.1	20.2	13.1
Kántorjánosi[2,3]		5.0	20.1	15.1
Montmorency 1[1]		5.3	32.5	27.2
Montmorency 2[1]		2.0	17.5	15.5
Nagy Gobet[1]		1.2	9.5	9.5
Újfehértói fürtös[2,3]		6.0	23.1	17.1
Mean		**4.8**	**21.8**	**17.0**
Cigánymeggy 7[1]	self-fertile (10.1-20%)	14.4	23.3	8.9
Cigánymeggy 59[1,2]		13.6	23.9	10.3
Hartai meggy[1,2]		11.8	28.2	16.4
Latos meggy[1]		10.4	27.3	16.9
Montmorency 3[1]		11.6	41.9	30.3
Parasztmeggy[1,2]		19.5	30.3	10.8
Schattenmorelle 2[1]		18.5	38.9	20.4
Schattenmorelle 3[1]		16.7	32.8	16.1
Schattenmorelle 4[1]		16.9	28.3	11.4
Mean		**14.5**	**29.0**	**14.5**
Schattenmorelle 1[1]	highly self-fertile (more than 20%)	25.3	29.4	4.1

Site and date of the observations: [1] Érd-Elvira 1972-1974,
[2] Helvécia 1987-1988,
[3] Újfehértó 1983-2002.

Table 4

Extreme values of fruit set obtained by self- and open pollination in sour cherry varieties and types

Variety (type)	Self-incompatibility group	Self-pollination (%)			Open pollination (%)		
		minimum	maximum	difference	minimum	maximum	difference
Cigánymeggy 1317[1]	completly self--incompatible (0.0 %)	0.0	0.0	0.0	12.6	26.0	13.4
Törpe meggy[1]		0.0	0.0	0.0	2.6	6.7	4.1
Mean		**0.0**	**0.0**	**0.0**	**7.6**	**16.4**	**8.8**
Cigánymeggy 60[1]	self--incompatible (0.1-1%)	0.0	0.6	0.6	0.0	8.9	8.9
Cigánymeggy 215[1]		0.0	0.2	0.2	8.5	27.4	18.9
Török meggy[1]		0.0	0.9	0.9	1.4	12.7	11.3
Mean		**0.0**	**0.6**	**0.6**	**3.3**	**16.3**	**13.0**
Cigánymeggy C.404[2]	partially self--fertile (1.1-10%)	4.4	8.2	3.8	24.5	33.1	8.6
Debreceni bőtermő[2,3]		0.5	12.7	12.2	6.6	38.3	31.7
Diemitzer[1]		6.4	8.8	2.4	14.2	26.2	12.0
Kántorjánosi[2,3]		0.0	10.9	10.9	3.0	37.1	34.1
Montmorency 1[1]		2.9	7.6	4.7	2.8	36.0	33.2
Montmorency 2[1]		0.7	3.0	2.3	7.7	27.5	19.8
Nagy Gobet[1]		0.2	2.2	2.0	5.0	14.0	9.0
Újfehértói fürtös[2,3]		0.7	18.7	18.0	3.0	42.5	39.5
Mean		**2.0**	**9.0**	**7.0**	**8.4**	**31.8**	**23.4**
Cigánymeggy 3[1]	self-fertile (10.1-20%)	8.4	13.1	4.7	4.7	24.4	19.7
Cigánymeggy 59[1,2]		8.5	26.7	18.2	144	225	8.1
Hartai meggy[1,2]		6.9	17.1	10.2	18.2	39.7	21.5
Latos meggy[1]		8.6	12.1	3.5	17.9	36.7	18.8
Montmorency 3[1]		3.7	19.5	15.8	32.3	51.5	19.2
Paraszt meggy[1,2]		10.8	30.0	19.2	15.9	42.7	26.8
Schattenmorelle 2[1]		12.8	29.0	16.2	27.9	46.5	18.6
Schattenmorelle 3[1]		16.1	17.3	1.2	25.6	40.0	14.4
Schattenmorelle 4[1]		9.6	24.2	14.6	27.5	29.1	1.6
Mean		**8.8**	**21.6**	**12.8**	**19.3**	**35.2**	**16.9**
Schattenmorelle 1[1]	highly self--fertile (more than 20%)	15.7	33.5	17.8	19.0	39.7	20.7

Site and date of the observations: [1]Érd-Elvira 1972-1974,
[2]Helvécia 1987-1988,
[3]Újfehértó 1983-2002.

Table 5

Self- and open fertilisation of sour cherry varieties (%) (Újfehértó, 1983-2002)

Variety	Type of fertilisation	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	mean of years
Újfehértói fürtös	self	18.7	11.8	2.7	10.0	3.2	4.2	2.4	9.2	6.3	5.7	2.6	3.2	1.3	6.9	-	1.7	0.7	1.4	5.0	13.0	5.8
	open	14.3	21.7	22.9	41.0	22.1	31.5	12.2	16.0	40.7	25.6	28.7	-	3.0	18.2	13.2	24.3	22.8	19.9	19.3	36.5	22.8
Kántor-jánosi	self	9.1	7.9	1.8	4.2	3.9	3.9	6.3	6.0	40.4	8.2	4.1	5.1	0.0	4.4	-	1.7	5.5	1.8	4.1	10.9	4.9
	open	9.2	16.9	11.9	28.6	16.5	20.7	30.5	14.2	29.4	27.6	27.0	-	3.0	26.7	16.2	26.7	22.0	16.1	8.0	16.3	19.4
Debreceni bőtermõ	self	8.5	2.2	2.2	5.4	5.4	6.3	12.7	5.5	10.6	6.1	3.6	4.8	8.3	2.9	-	4.0	5.0	0.5	3.9	11.2	5.7
	open	16.7	-	16.5	24.7	26.8	35.6	25.6	18.5	29.5	36.8	38.3	-	6.6	13.8	13.9	22.8	25.2	8.1	11.7	42.5	23.0

Table 6

Comparison of fruit set (%) in sour cherry varieties obtained by natural self-pollination (autogamy), artificial self pollination (geitonogamy) and free pollination (Helvécia, 1988-1989)

Variety	1988			1989		
	Natural self-pollination (autogamy)	Artificial self-pollination (geitonogamy)	Open pollination	Natural self-pollination (autogamy)	Artificial self-pollination (geitonogamy)	Open pollination
Pándy 7	0	0	7.0	0	0	27.0
Debreceni bőtermő	1.4	17.2	9.4	6.8	21.8	31.8
Kántorjánosi	5.2	10.2	17.4	6.7	10.8	37.1
Cigánymeggy C 404	4.4	17.0	24.5	8.2	21.8	33.1
Újfehértói fürtös	3.2	1.5	15.7	12.2	21.6	35.7
Cigánymeggy 59	8.6	28.7	31.0	11.7	32.6	36.6
Cigánymeggy 7	6.8	16.7	21.4	17.4	34.5	37.4
Parasztmeggy	14.7	23.1	33.6	17.1	39.7	42.7
mean	**5.5**	**14.3**	**20.0**	**10.0**	**22.9**	**35.2**

than 10% fruit set; variability of their yield is much higher than that of the self-fertile varieties (Table 4). Maximum fruit set of partially self-fertile varieties may amount 4.5 times their minimum set, whereas only 2.5 times in self-fertile varieties.

The high yearly variation of fruit sets is shown in Table 5. In the *Pándy meggy* type of varieties being partially self-fertile the yields show multiple differences depending on the season. Selfed flowers yield nearly 0% fruit, and the maximum yields did not reach 20% in any of them. As a mean of 19 years there were three varieties with similar rates of fruit set. Earlier observations (Nyéki, 1974) proved the beneficial effect of geitonogamy on the rate of fruit set in relation to natural self pollination or autogamy even in self-fertile varieties. In partially self-fertile varieties the advantage of geitonogamy was much less expressed than in the self-fertile group. However, natural autogamy allowed a higher variation in fruit set than did geitonogamy within the clone. Table 6 proves that with geitonogamy (hand pollination with pollen of the same clone) fruit set increased by a factor of 2-3 in relation to natural self-pollination or autogamy. Self-incompatible varieties (e.g. *Pándy 7*) did not set fruit even after geitonogamous hand pollination.

Open pollination

Fruit set on open pollinated flowers varied substantially according to growing site as well as to the season, which proves the high susceptibility of sour cherry to ecological adversities. Most decisive is the influence of weather on fruit set during the blooming period. Nyéki (1989) stated that fruit set of open pollinated *Pándy meggy* types is low and seasonally highly variable.

Tables 3 and 4 shows fruit sets of the varieties assigned to five self-fertility groups. Within the group of *Cigánymeggy* there are varieties, which are very poorly (e.g. *Cigánymeggy 60*), or well fertilised (e.g. *Hartai meggy*, *Paraszt meggy*). Some favourable seasons may produce high (26-27%) fruit sets even in self- -incompatible varieties (e.g. *Cigánymeggy 215*, *Cigánymeggy 1317*). At present, only the best fertilised *Cigánymeggy* types are admitted to be propagated (*Cigánymeggy 7*, *Cigánymeggy 59*). Some varieties (e.g. *Montmorency 3*) may set fruit at rates more than 50%.

As was mentioned, self-incompatible and partially self-incompatible varieties yield more variably not only after self pollination but also with free pollination in relation to the self-fertile varieties.

Nyéki (1989) stated that the higher the self-fertility of a variety the higher yields are expected also with open pollination. A linear correlation exists between the rate of self-fertility and the productivity at open pollination at P=0.01 level. It is also proved by data appearing on Table 3. Completely and highly self-incompatible varieties set fruit with open pollination at rates of 12.1% and 9.3%, whereas self-fertile and highly self-fertile sour cherry varieties produced the highest fruit set: 29.0% and 29.4%.

CONCLUSIONS

1. Self-incompatible and partially self- -incompatible sour cherry varieties must be planted with associated polliniser varieties. Appropriate polliniser varieties are to be chosen from groups of synchronous blooming period. If three blooming time groups are considered, the partners should belong to the same group, whereas from the five groups, they may be taken from the neighbouring blooming time groups too.
2. The rate of self- and open fertilisation is highly variable depending on the growing site as well as on the season. Variation of fruit set (and yield) is a good deal lower in self-fertile varieties than in self-incompatible and partially self-fer-

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tile varieties. Consequently, the safest yields are expected in self-fertile varieties.

3. Artificial self-pollination (geitonogamy) resulted in 2-3 times higher rates of fruit set in relation to natural self-pollination (autogamy), except in completely self-incompatible varieties. Highest fruit set is expected on open blooming flowers visited by bees. The advantage of open pollination is clearly evident in self-fertile and completely self-fertile varieties too. Consequently, it should be stated that bee pollination increased the yield not only in self-incompatible varieties but also in self-fertile sour cherry plantations.

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BIOLOGIA KWITNIENIA I ZAPYLANIE WIŚNI (*Prunus ceraus* L.) ODMIAN WYSELEKCJONOWANYCH NA WĘGRZECH

Nyéki J., Szabó T., Szabó Z.

Streszczenie

Doświadczenia prowadzono w latach 1972 - 2002 w trzech miejscowościach: Erd, Helvecia i Ujfeherto, w których badano odmiany wiśni uprawiane w różnych zestawach odmianowych. Obserwacje obejmowały: biologię kwitnienia (czas rozpoczęcia, okres trwania, największe nasilenie), dynamikę kwitnienia (liczba otwieranych codziennie kwiatów) oraz zawiązywanie owoców i wpływ na to sąsiedztwa odmian znajdujących się w sadzie.

Opierając się na uzyskanych wynikach oceniono stopień nakładania się okresów kwitnienia różnych odmian, a na podstawie okresu największego nasilenia kwitnienia wszystkie odmiany podzielono na 5 grup. Badano samopłodność i zdolność do naturalnego samozapylania się oraz wybrano dobre zapylacze dla odmian samosterylnych i częściowo samosterylnych.

Potrzebę zapylenia przez pszczoły oceniono stosując różne sposoby zapylania; naturalne samozapylanie, sztuczne samozapylanie i wolne zapylanie.

Słowa kluczowe: zapłodnienie, kwitnienie, zapylanie, wiśnia.

SITUATION AND PERSPECTIVE OF THE HUNGARIAN BEE-KEEPING

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S u m m a r y

Apiculture constitutes a sector of Hungarian agriculture, which has not been big yet perspective in respect of its development possibilities. The ratio of apiculture shared 0.5 percent of the gross production value of agriculture, and 1.03 percent of that of animal husbandry in 2000. Due to its natural conditions the whole territory of Hungary is suitable for apiculture. The latter is a complex activity: along honey production, the pollinating activity of bees must not be neglected (positive externality). The participants of the sector are mainly small-scale producers. Concentration due to economic reasons will be increased in the future. Concerning the quantity Hungary cannot compete with large honey exporting countries therefore Hungary has to penetrate the EU honey markets with highly processed and branded products.

Keywords: honey production, cost-income situation, production structure, honey export, EU accession, service organisations.

INTRODUCTION

According to FAO statistics, the world honey production was 1 262 thousand metric tons in 2001. Global output increased by 30 percent from 1979 to 1989, which subsequently stabilised in the following decade, with an increase of 3 percent from 1989 to 1999. The world's major producing region is Asia, which is followed by Europe, Northern and Central America. In the context of the world trade, China is the major exporter and the European Union is the major import market.

The honey production of the world becomes more concentrated from step to step. Either the price competition or the production and the trade of the special honey products make influence on market. Traditions, natural and economic conditions reflect wide variations of honey in the large honey producing countries, namely China, the CIS countries, USA, EU, Argentina and Mexico.

METHODS

Increasing competitiveness gives much more emphasis to the cost reduction, standardisation and intensive production. The characteristics of concentration can also be determined in this sector by the increasing number of bee colonies and decreasing number of honeybee-keeping farms. In certain countries, like USA, Canada and Mexico, the demand for pollination with honeybees increases. The participants of the sector also have to meet the customers' demand; this concerns mainly those honey products that originate from GMO crop production. The honeybee species and their treatments determine mainly the honey production.

In Hungary, the annual honey production oscillates about 16 thousand metric tons. According to the Hungarian Central Statistical Office (2001a), 68 per cent of the produced honey is black locust, 19 per cent is blossom, 6 per cent is sunflower, 5 per cent is rape and 2 per cent is other type of honey.

Table 1

Honey production in Hungary

Year	Production, Tons	Value of gross production, Million HUF	Share of honey production in value of gross production of agricultural [%]	Share of honey production in value gross production of animal husbandry [%]
1995	16 050	3 679	0.51	1.14
1996	15 811	3 948	0.43	1.06
1997	15 652	4 431	0.42	1.01
1998	16 739	8 035	0.77	1.55
1999	15 431	6 149	0.58	1.27
2000	15 165	5 536	0.50	1.03

Source: Statistical Yearbooks of Hungary 1995-2000, Hungarian Central Statistical Office, Budapest, 1996-2001

Table 2

Honey production costs and returns in 2001 (horizontal hives with frames)

Colonies				
Denomination	50	100	150	200
Average yield per colonies, kilogram's	**10**	**25**	**25**	**25**
Total production, tons	**0.5**	**2.5**	**3.75**	**5**
Honey sales, tons	**0.5**	**2.5**	**3.75**	**5**
Black locust honey, tons	0.5	1.25	1.875	2.5
Blossom honey, tons	0	1.25	1.875	2.5
Revenues	**1 609**	**3 653**	**5 481**	**7 304**
Black locust honey, euro	1 609	2 193	3 288	4 382
Blossom honey, euro	0	1 460	2 193	2 922
Subsidies, euro	390	861	1 293	1 725
Total revenues, euro	**1 999**	**4 514**	**6 774**	**9 029**
Total production costs, euro	**2 123**	**5 212**	**8 099**	**11 379**
Profit, euro	-124	-698	-1 325	-2 350
Gross income euro/kg	-0.2	-0.3	0.3	0.5
Gross income per colonies, euro	-2.5	-6.98	-8.8	-11.8
Profit as proportion of sales revenue, %	**-6**	**-15**	**-19**	**-26**
Profit as proportion of costs, %	**-6**	**-13**	**-16**	**-21**

Source: Nyárs L. (2001): Situation of the Hungarian honey bee-keeping sector and the opportunities for development, Research and Information Institute for Agriculture Economics, Agriculture Studies. Budapest, Number 9., Comment: at 50 colonies only direct sales, 1 Euro = 256.68 HUF

Table 3

Honey production costs and returns in 2001 (super boxes)

Colonies					
Denomination	200	400	600	800	1000
Average yield per colonies, kilogram's	**50**	**50**	**75**	**75**	**75**
Total production, tons	10	20	45	60	75
Honey sales, tons	**10**	**20**	**45**	**60**	**75**
Black locust honey, tons	5	10	22.5	30	37.5
Blossom honey, tons	5	10	22.5	30	37.5
Revenues	**14 610**	**29 219**	**65 743**	**87 354**	**109 572**
Black locust honey, euro	8 766	17 531	39 446	52 591	65 743
Blossom honey, euro	5 844	11 688	26 297	35 063	43 829
Subsidies, euro	1 724	3 448	5 172	6 896	8 619
Total revenues, euro	**16 334**	**32 667**	**70 915**	**94 250**	**118 191**
Total production costs, euro	**13 253**	**25 050**	**39 227**	**54 149**	**68 100**
Profit, euro	**3 081**	**7 617**	**31 688**	**40 101**	**50 091**
Profit, euro/kg	0.30	0.40	0.70	0.66	0.66
Profit per colonies, euro	15.4	19.0	52.8	50.1	50.0
Profit as proportion of sales revenue, %	**19**	**23**	**45**	**43**	**42**
Profit as proportion of costs, %	**23**	**30**	**81**	**75**	**74**

Source: Nyárs, L. (2001): Situation of the Hungarian honey bee-keeping sector and the opportunities for development, Research and Information Institute for Agriculture Economics, Agriculture Studies. Budapest, Number 9

Honey produced in Hungary accounts for about 1-1.2 per cent of the total world production and 4-4.5 per cent of total world honey trade. According to Halmágyi L. and Keresztesi B. (1991), honey producers could collect 40-46 thousand metric tons of honey and this quantity could also be processed by the present processing capacity. In Hungary, 12 honey processors have more than 1 000 tons/year honey processing capacity. Of these plants, 4 work with 4-6 thousand tons/year capacity. Another 6 processors capacity's are between 1-2.5 thousand tons/year. There exist 4 plants, which deal with 100-1 000 tons/year capacity (Nyárs 2001).

The costs-income analyses do not justify the exploitation of the acacia forests. The use of the production potential is limited by the dominance of horizontal hives with frames, which are labour intensive and cannot be mechanised. Up to the EU accession the most important task of the sector is the modernisation of the honeybee-keeping equipment. Subsidies for such technological improvements are available only to a limited extent in the support programs of the EU.

In the cost-income calculations the difficulty is caused by the low representative samples of the data available and this is the reason why conclusions referring to the

whole sector cannot be drawn. Consequently, these data are only for information. For the analyses static models are applied. Calculations were made in two different categories. In one category of horizontal hives with frames the calculations refer to honeybee-keeping farms with 50, 100, 150, 200 bee colonies. In the other category of hives with supers (boxes) honeybee-keeping with 200, 400, 600, 800, 1000 colonies was referred to. Table 2 and table 3 provides estimates of the revenues, costs and returns from operating horizontal hives with frames and super boxes. Yields were determined by categories. Pricing is an important issue in achieving sales and profitability. The price depends on what kind of marketing channels the honey is sold to. The minimum acceptable price should cover all production, marketing, transportation and labour costs. Competitor prices are an important consideration in pricing honey for sale to consumers. Advantages of farm gate sales include: cash payments from consumers without incurring transportation cost, opportunity to develop products for niche markets. The potential to increase sales is limited and producers may not be able to market all of the product through their outlet. As a result, producers need other marketing channels for honey that cannot be sold at the farm gate. In our model, the honeybee-keeping farm with 50 colonies sold honey to the consumers directly. Based on estimates other beekeeping farmers sold their honey to packers and honey agents. Operating with horizontal boxes, revenues cannot hold the wage costs and the depreciation of equipment and vehicles. Due to the low yields, the production is showing loss. In the Hungarian honeybee-keeping sector, the producers do not account for their wages and their contributions.

Real incomes were generated at honeybee-keeping farms provided with hives with super boxes.

According to profitability calculations, the optimal production scale is about 600 bee colonies per apiary in the Hungarian honey bee-keeping sector. Larger stocks with higher yield resulted in higher profitability indicators; however, the high risks involved in large yields (risks of animal health) cannot be ignored (Nyárs L., 2001).

RESULTS

The production structure and specific indicators of the honeybee keeping by stock size do not show the real situation. The participants of the sector are mainly small--scale producers and due to the special tax allowances the ownership of bee colonies is distributed among the family members. The total number of beekeepers in Hungary was approximately 19 thousand in 1991 (Table 4).

Table 4

Number of beekeepers and bee colonies in Hungary

Denomination	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000
Number of beekeepers	19 923	19 013	17 598	16 970	16 887	15 372	15 677	16 672	17 087	16 579
Number of bee colonies (thousand)	716	725	674	646	669	604	642	690	806	840
Bee colonies/ beekeeper	36	38	38	38	39	39	41	41	47	51

Source: Honey Product Council

Table 5

Hungarian exports of honey

Year	Export, tons	Export prices, USD/kg	Export prices, €/kg	Import, tons	Import prices, USD/kg
1995	13 028	1.85	1.47	760	1.33
1996	13 137	1.90	1.55	710	1.20
1997	7 675	1.80	1.66	410	1.20
1998	9 262	2.10	1.92	548	1.20
1999	9 889	1.61	1.53	441	1.03
2000	12 806	1.29	1.40	857	0.94

Source: Statistical Yearbook of External Trade 1995-2000, Hungarian Central Statistical Office, Budapest, 1996-2001

By 2000 the total number of beekeepers had decreased to 16 thousand, 3.7 per cent of whom were declared as professional beekeepers (in order to be regarded as a professional, a beekeeper must operate at least 150 bee colonies in the European Union).

Over the same period, 1991-2000, the number of colonies increased by 17 percent. The 17.2 per cent of the hives are operated by professionals. Economic reasons for the concentration will increase in the future, which also has to be encouraged by the regulations and supports. Honey market following EU, China is the second largest honey producing country of the world, however, its self-supply is particularly low, altogether 47 per cent. The most important export market of Hungary is the European Union; therefore, producers should be informed about market developments and positions. Export of honey decreased by 41,6 per cent from 1996 to 1997 because of the low priced Romanian honey export to European Union (Table 5). In 2000, the main markets for Hungarian honey were Germany, Italy, France, Austria and Finland.

The EU can only satisfy the domestic demand by a high import volume, that is, by 130-150 thousand tons annually. During the period of 1994-2000 the competition strengthened between three main honey suppliers of the EU, namely China, Argentina, Mexico. There is also a hard competition for markets between the small-scale suppliers in Hungary, Romania and Bulgaria. Romania's market share in Germany increased from 1.2 percent in 1994 to 5.8 percent in 1999 while during the same period the market share of Hungary decreased from 4.8 percent to 2.7 per cent. In the dynamic increase of Romanian honey export the foreign companies with large working capitals played a significant role by supporting producers with current assets and operating on site quality assurance systems.

The situation has become much more difficult since 1st January 2001 as Romania, Bulgaria and Czech Republic may export honey to EU markets with zero in-quota tariffs. Within the trade negotiations with the EU Hungary could "only" achieve a (10.38 per cent) tariff cut, which was based on the data of the twelve months -which had an unfavourable impact on the Hungarian honey export. Hungary has exported honey to EU market with zero in-quota tariffs since July 1, 2002. In addition to the competitors mentioned above Mexico was allocated a quota of 30 thousand metric tons with a 50 per cent MFN (Most Favoured Nation) bound rates.

Concerning the quantity Hungary cannot

compete with the large honey exporting countries. Therefore, Hungary has to penetrate the EU honey markets with highly processed and branded products. In the EU member states there are purchasing, packaging and marketing co-operatives in the honey sector. It is important for Hungary as well, because an export price increase is not possible with a bulk product. At present, the interest of the Hungarian honey processors and commercial companies lies in packaging and exporting the honey in barrels. The low producer prices do not favour the producers and the retailers transfer the price reductions to the producers.

In Hungary 90 percent of the honey production is for human consumption and based on the estimations the remaining 10 percent of the honey production is used by the industry (baking industry, sweets industry, pharmaceutical industry and cosmetics) and social programmes. The honey industry also produces beeswax, which is used in making candles and cosmetics. As for the sales of honey there are several marketing channels. The wholesalers purchase 10-13 thousand metric tons of honey from the producers each year depending on the fluctuation of the honey production; that is, the most significant part of the total production annually. The nominal capacity of the Hungarian honey processing plants is about 40 thousand metric tons, which is double of the highest production level of the last ten years. Most of the commercial companies (all the large ones) operate their own honey processing plants. This is the reason why at present the capacities of honey processing exceed the level of production in Hungary.

The regulation system of the Hungarian honeybee keeping is harmonized with the EU regulations; concerning in particular the animal health and the food hygiene legislation. After the EU accession the present Hungarian market order will be replaced by the market orders of the EU Member States. The harmonisation process requires national programmes with accurate data register system. The national programmes have to include both the current and the fixed costs (European Commission (2001 b)). If the sector is not prepared for the EU accession the Hungarian beekeepers will not be eligible for the EU subsidies. The interest groups and professional bodies together with the participants involved in honey production will have to prepare jointly their annual work plan based on the requirements defined by the regulation.

The national development plans and the measures have had to contain also plans for monitoring the implementation and for the evaluation since the use of the financial resources from the EU budget is regulated. The needed information of the sector has not been provided for the preparation of EU accession since the data of the Honey Produce Council and that of the Central Statistical Office did not correspond to each other. At present there are only estimates concerning the marketing channels used by the producers for sales (direct sales to consumers, wholesaler, and retailers, sales to the industry).

CONCLUSIONS

Honey beekeeping is an integrated agricultural activity. In addition to the honey production the pollination by bees is also a significant positive impact externality. In Hungary pollination is considered as a significant part of the technology and for that reason crop production and horticulture are supposed to pay for this service. In the USA and Canada these activities are done by contracts.

Within the Hungarian bee-keeping the most important task is the mechanisation of honey production; and in this way its present labour demand can be lowered. It is only for stand sizes of 200-300 bee colonies that developments will pay off and tolerate interest rates, as well as it is these stand

sizes that permit the financing of production for the following year. The major obstacle to development is the lack of capital and credit.

The development of the sector could be encouraged by establishing service organisations and penetrating both the export and the domestic markets, and branded products with quality assurance certifications in consumer-friendly packages produced by producer's organisations since bee keeping farms with less than 100 bee colonies cannot be competitive on the market. Besides the activities listed above, the service organisation could also get involved in joint material purchase (medicines, sugar, apicultural implements), as in the case of big quantities the service organisation is able to achieve considerable price concessions. Service organisations will be operable only if backed by capital-intensive producers' communities or marketing companies.

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STAN OBECNY I PERSPEKTYWY WĘGIERSKIEGO PSZCZELARSTWA

Nyárs L.

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

Pszczelarstwo jest częścią rolnictwa na Węgrzech, które obecnie nie ma możliwości rozwojowych. Udział pszczelarstwa w całkowitej wartości produkcji rolniczej w roku 2000 wynosił 0,3%, a w produkcji zwierzęcej 1,03%. Warunki naturalne sprawiają, że całe terytorium Węgier jest przydatne do produkcji pszczelarskiej. Na aktualne znaczenie pszczelarstwa patrzeć należy nie tylko ze względu na produkcję miodu, doceniana winna być działalność pszczół jak zapylaczy roślin. Udziałowcami w produkcji pszczelniczej są głównie mali producenci. Ze względów ekonomicznych w przyszłości będzie następowała koncentracja produkcji. Węgry ze względu na wielkość nie są w stanie konkurować z wielkimi eksporterami miodu jednak dostarczają na rynki UE wysoko przetworzone markowe produkty.

Słowa kluczowe: produkcja miodu, koszty produkcji, struktura produkcji, eksport miodu, Unia Europejska, organizacja obsługi.

DELIBERATE PRODUCTION OF YOUNG QUEENS IN *Bombus terrestris* L. (*Hymenoptera*, *Apoidea*) COLONIES REARED IN LABORATORY

V l a d i m i r P t a c e k

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S u m m a r y

Two possible ways of obtaining young queens in laboratory were tested: to rear them in groups of workers, and to make the regular colonies queenless in comparatively early stage of their development.

In 1997 four groups of four workers in each did not accept young diploid larvae either from their maternal colony or from another one. Workers, which already have established dominance in the groups, destroyed the inserted brood and laid their own eggs. The method still should be tested under different conditions, because, exceptionally, larger groups of workers did accept alien brood and raised queens from it.

From 7 colonies deliberately made queenless at the stage of 20 - 30 workers 6 did raise young queens (20-40 per colony) from remaining brood. One colony produced males only. Young queens reared on this way behaved according to the queen-model and were able to start normal colonies.

Keywords: bees, *Bombus terrestris*, bumble bees, queen rearing.

INTRODUCTION

According to the scheme presented first by Duchateau (1991), in laboratory only some colonies produce queens. They are reared from a portion of diploid brood available in a colony when it reaches a physiological stage called as the "switch point". No other young queens emerge then in such colony as its old queen turns to laying haploid eggs only. However a large proportion of colonies do not produce queens at all. After having reared several generations of workers, they turn to mail producers for the rest of their life. However, the rearing methods of bumble bees will not be complete without having a possibility to rear young queens intentionally from any colony or even any queen.

Since the first laboratory colonies of *B. terrestris* were reared from queens accompanied by honeybee workers (Ptacek, 1983, 1985), and the queens showed a behaviour partially similar to that of honey bee queens, it seems to be logical to use the model of a queenless honey bee colony for raising emergency queens also in the case of the mentioned bumble bee species.

MATERIALS AND METHODS

Two possible ways of obtaining young queens in laboratory were tested:

1. To rear them in groups of workers taken from regular colonies,
2. To make the colonies queenless in comparatively early stage of their development.

In the pilot trial in1997 four groups of four workers in each (taken from a queen right colony) were given young larvae originating either from their own queen (two groups) or from the alien ones (the other two groups).

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In the second case the old queens were removed from colonies at the stage of 20-30 workers with the expectation that at least a part of the remaining diploid brood will be reared into young queens. In the years 1999 - 2001 the influence of queen-absence was tested on the total of 7 colonies.

RESULTS AND DISCUSSION

The worker groups with 4 individuals in each did not accept young larvae for further feeding. The larvae were left do die.

The second method brought better results. From the 7 colonies made queenless 6 did raise young queens. In one colony solely males were produced, probably because it had reached the switch point at the stage of removing its queen. We were not able to register the accurate numbers of developed queens but guessingly they were from 20 to 40 per a hive.

To be suitable for affected queen production a colony should have much brood in the stage of eggs and young larvae (Fig. 1). After the old queen is removed, a part of the diploid brood is fed as queens. Such larvae enlarge in size noticeably (Fig. 2). Also their cocoons are much larger then those of workers and males (Fig. 3). Cocoons can then be removed from a colony and treated in the already known way (Ptacek 2000). According to our experience the queens raised from them behave normally and are able to start and develop standard colonies.

Evaluating the gained experience we should consider the second method - removing the queen - to be more successful. It gives queens from nearly any colony. On the other hand we do not want to put aside the first method - workers with added brood, either. In our trials, which were only very simple, maybe the age of workers and/or their number in groups might play their role, as well as the age of the brood given to them. Workers in groups used by us already had established dominance before they were given the alien brood which also might contribute to its rejection. For the possibility of further improvement of this method speaks also the fact, that later on, when we by chance gave young brood to queenless but more numerous colonies,

Fig. 1 The colony suitable for affected queen production has brood in all stages and round 20 workers. Queen (right in the picture) can be removed

Fig. 2 Some of the former worker larvae of another queen less colony have been raised into young queens (the large ones in the middle and lower part of the picture)

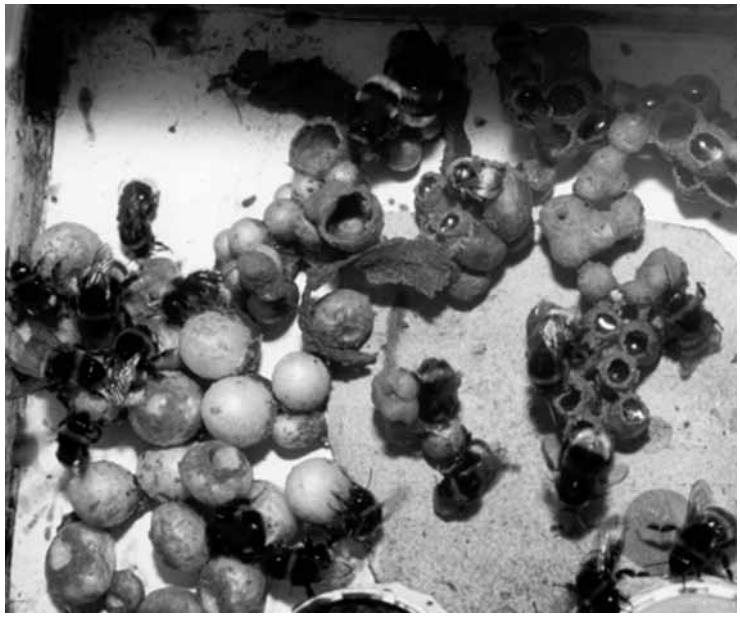

Fig. 3 The young queen larvae (left in the lower corner) of the third experimental colony at the end of heir larval development. Some of them have just finished their cocoons

some of them exceptionally did rear young larvae from it (Fig. 4). Further experiments in the field surely will bring more explanatory results.

The brood from a certain "breeder" queen can be obtained easily by keeping it in a small colony with several workers of hers. When such a colony is given a cluster of male cocoons from any colony, the queen covers it by egg cells within several days. This brood can than be taken off for raising young queens.

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Fig. 4 One of the group of queen-less workers (beyond the experiments), which **did accept** alien brood and raised queens (now in cocoons) from it

CONCLUSIONS

According to the gained experience it seems to be possible to rear new queens in nearly any colony of *B. terrestris* L. by removing the old queen at the stage when the colony has 20 -30 workers.

Further research is necessary in the field of producing queens by deliberately made groups of workers.

Similar responses of workers to the absence of queen as they were observed in B. terrestris can be expected also in other bumblebee species.

ACKNOWLEDGEMENTS

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ROZWAŻANIA NAD MOŻLIWOŚCIĄ WYCHOWU MŁODYCH MATEK TRZMIELA ZIEMNEGO - *Bombus terrestris* L. (*Hymenoptera*, *Apoidea*) W RODZINACH HODOWANYCH W LABORATORIUM

P t a c e k V .

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

Oceniano dwie metody otrzymywania młodych matek w warunkach laboratoryjnych: wyprowadzanie ich w grupach robotnic i w rodzinach osieroconych we względnie wczesnym stadium ich rozwoju.

W 1997 roku robotnice trzmieli (po cztery w każdej grupie) nie przyjmowały młodych diploidalnych larw pochodzących zarówno z ich matecznej rodziny, jak i z obcych. Robotnice przejmując dominację w grupach, zniszczyły wstawiany czerw i składały własne jaja. Badania powinny być jeszcze kontynuowane, ponieważ okazało się, że większe grupy robotnic przyjmowały obcy czerw i wyprowadzały z niego młode matki.

Z 7 umyślnie osieroconych rodzin w stadium 20-30 robotnic 6 wyprowadziło młode matki (20-40 na rodzinę), za wyjątkiem jednej, która produkowała tylko samce.

Słowa kluczowe: pszczoły, *Bombus terrestris*, trzmiele, wychów matek.

DISTRIBUTION AND RELATIVE ABUNDANCE OF BUMBLE BEES (*Bombus* and *Psithyrus*) IN HUNGARY

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Summary

The distribution data of the *Bombus* and *Psithyrus* species occurring in Hungary were collated into a database. Over 6,500 occurrence data represented 25 *Bombus* and 6 *Psithyrus* species. Our summarised data covered the 42% of the UTM squares in Hungary. Three of the bumble bee species (*B. distinguendus*, *B. elegans* and *B. serrisquama*) had only occurrence data from before 1953 so these species are probably extinct in Hungary. Regarding the relative occurrence frequency of the species, we found that the Hungarian bumble bee fauna has a great percentage of rare species. We found that most of the bumble bee species had shown a declining relative distribution in the last 50 years.

Keywords: bumblebee, Bombus, cucoobumblebee, Psithyrus, distribution, Hungary.

INTRODUCTION

In addition to the well known honeybee (*Apis mellifera*) there are about 600 wild bee species in Hungary. They have a very important role both in agricultural and natural habitats with pollinating several plant species. The best known and most commonly observed group of wild bees is the bumble bee group (*Bombus* Latr. and *Psithyrus* Lep.). These species are widespread in the Palearctic region (Prys-Jones & Corbet 1987). The bumble bee fauna of the Carpathian basin is rich as compared with other parts of Europe (Móczár 1953). Several plant species need bumble bee pollination, because these species have unique pollination quality (large body size, buzz pollination etc). In the last few decades the number and distribution of wild bees showed a decreasing tendency all over the Europe and North America (Kwak 1996, Kwak et al. 1996, Westrich 1996, Williams 1996, Kearns & Thomson 2001). So it is very important from a nature conservation point of view as well to collect all of the available information about species distribution and frequency of bumble bee species (Sárospataki et al. 2002).

MATERIALS AND METHODS

We gathered together the distribution data of bumble bees from different insect collections (Hungarian Natural History Museum, Natural History Museum of Bakony, private collections of Zsolt Józan and Pál Benedek) and also from the available Hungarian scientific literature (for the list of publications see Sárospataki et al. 2002). We collected approximately 13-14 thousands data, and after the erasing of the duplicates the database consists of about 5200 records.

The method of UTM- (Universal Transverse Mercator) mapping was used for visualise our data. This method covers the whole surface of the Earth with a uniform net, consists of 100x100 km, 10x10 km and even smaller units (Bácsatyai 1997). In

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Table 1

The relative distribution frequency of the Hungarian bumble bee species

Species names	Number of UTM squares	Relative frequency (%)	Frequency categories
Bombus consobrinus	1	0.23	-
Bombus distinguendus	1	0.23	-
Bombus serrisquama	2	0.45	-
Psithyrus sylvestris	5	1.13	I.
Bombus soroeensis	6	1.36	I.
Bombus elegans	7	1.58	I.
Bombus fragrans	16	3.62	I.
Bombus haematurus	17	3.85	I.
Bombus paradoxus	17	3.85	I.
Psithyrus bohemicus	19	4.30	I.
Psithyrus campestris	21	4.75	I.
Bombus hypnorum	28	6.33	I.
Bombus argillaceus	31	7.01	I.
Bombus laesus	38	8.60	I.
Bombus subterraneus	40	9.05	I.
Bombus pomorum	46	10.41	II.
Psithyrus vestalis	54	12.22	II.
Bombus lucorum	55	12.44	II.
Psithyrus barbutellus	55	12.44	II.
Bombus confusus	58	13.12	II.
Bombus pratorum	62	14.03	II.
Bombus ruderatus	78	17.65	II.
Psithyrus rupestris	78	17.65	II.
Bombus muscorum	87	19.68	II.
Bombus humilis	162	36.65	III.
Bombus ruderarius	164	37.10	III.
Bombus hortorum	165	37.33	III.
Bombus sylvarum	179	40.50	III.
Bombus pascuorum	208	47.06	IV.
Bombus lapidarius	254	57.47	IV.
Bombus terrestris	300	67.87	IV.

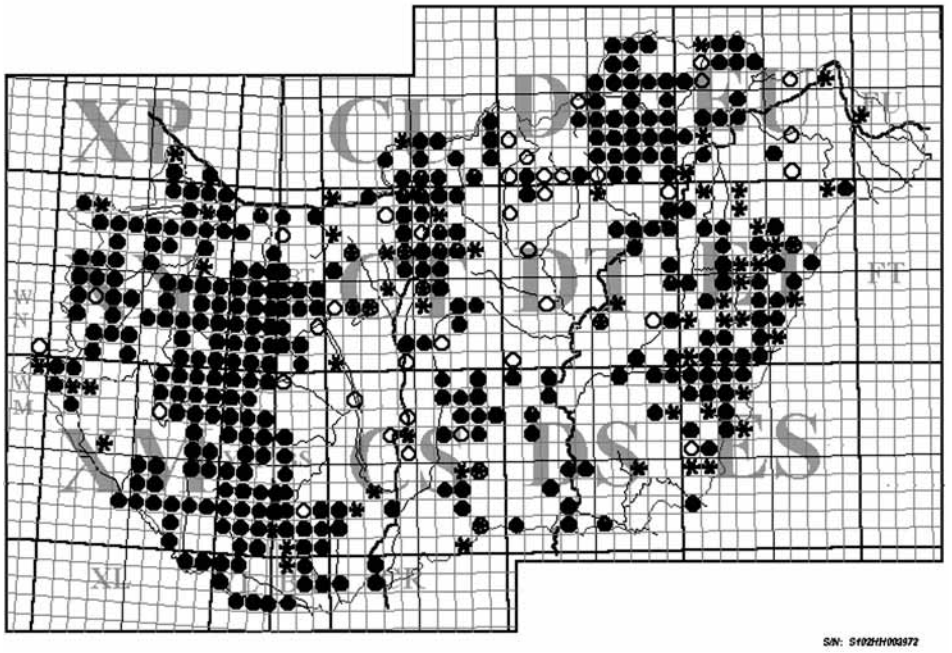

Fig. 1 Summarised distribution data of *Bombus* and *Psithyrus* species in Hungary.
○: records before 1953., ✶: records between 1954 and 1970., ●: records after 1971

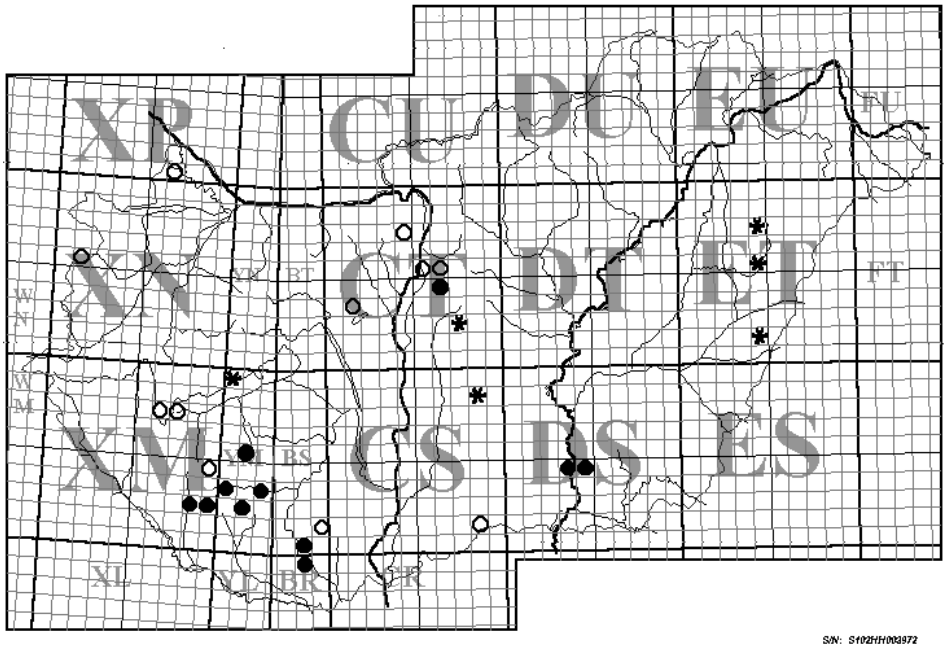

Fig. 2 Distribution data of *B. argillaceus* in Hungary. See abbreviation in fig 1

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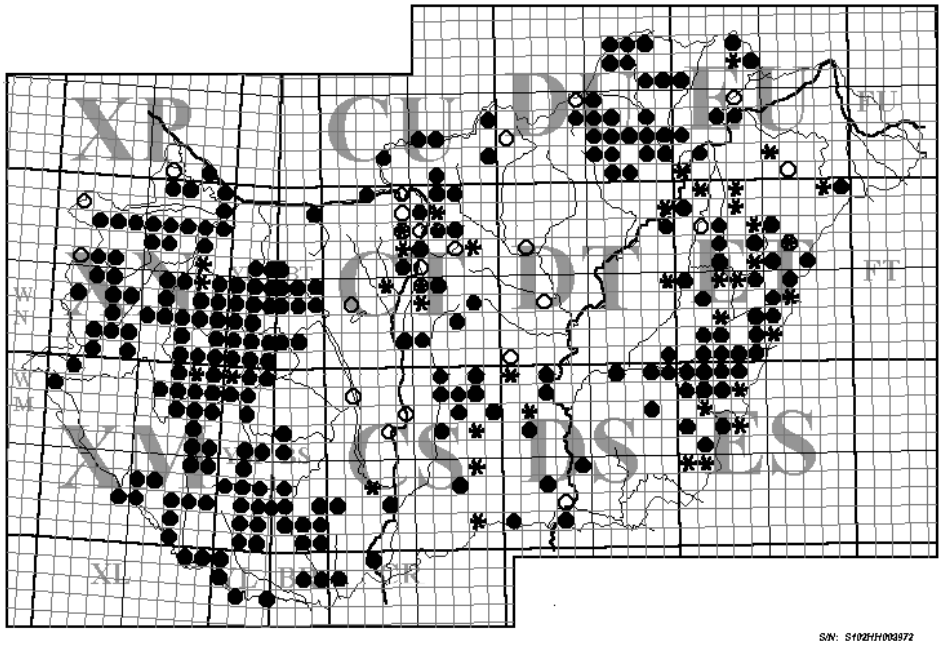

Fig. 3 Distribution data of *B. terrestris* in Hungary. See abbreviation in fig 1

our work we used the 10x10 km units for mapping the distribution data of the species. We used the "BioTér" software package for creating the maps (Dévai et al. 2000). On the maps we separated the data into three categories on the basis of collection date: before 1953, between 1953 and 1970, after 1970.

The relative distribution frequency of species was measured by the percentage of all the UTM squares covered by the database.

RESULTS AND DISCUSSION

Our database contains the distribution data of 25 *Bombus* and 6 *Psithyrus* species (table 1). From the 1052 UTM squares of Hungary our data cover 442 squares (fig. 1). This is 42%, so the coverage is higher than in other similar publications on other animal groups in Hungary (Dévai & Miskolczi 1987, Bakó & Korsós 1999). Two examples of species maps are published here (fig. 2 and 3): one rare species (*B. argillaceus*, the only protected species) and one common species (*B. terrestris*, one of the most well-known and common species).

Three species (*B. distinguendus B. elegans* and *B. serrisquama*) can be seen as extinct from Hungary, because we found occurrence data there only before 1953. We have only one data on *B. consobrinus*. The more detailed observation of this only one collection site is needed to find out the consistency of this population in Hungary.

The relative frequencies (table 1) show about the half of the Hungarian species to be rare. However, only one species (*B. argillaceus*) is protected in Hungary. The high percentage of rare species in the Hungarian bumble bee fauna shows that much more species need official protection.

It is very important to consider some difficulties of the UTM mapping methods:

1. We have a lot of UTM squares (58%) without any data. The absence of data can mean not only the absence of the

species, but frequently the absence of the research in the given area as well.

2. The distribution of data sometimes shows the site preference of the collectors.
3. The common species are probably underrepresented in the database, because collectors usually have a preference for rare, "more interesting" species (Móczár 1953). That is shown by the rather low relative frequencies of the most common species, which probably can be found almost all UTM squares of Hungary (Józan, personal communication).
4. From similar consideration the rare species are overrepresented in the database, in other words they are more scarce than the data shows. This underlines the need of protection of these species.

ACKNOWLEDGEMENT

We are grateful for everybody who helped as to collect the occurrence data of bumble bees, namely: Zsolt Józan and Pál Benedek (making available their private collections) as well as Lajos Zombory (Hungarian Natural History Museum) and Csaba Kutasi (Natural History Museum of Bakony). The work was partly financed by the grants of the Oak Association for Nature Conservation. During the study Miklós Sárspataki had a Bolyai János Research Grant.

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WYSTĘPOWANIE TRZMIELI (*Bombus*) I TRZMIELCÓW (*Psithyrus*) NA WĘGRZECH

Sárospataki M., Novák J., Molnár V.

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

Opracowano ponad 6 500 danych dotyczących występowania 25 gatunków trzmieli (*Bombus*) i 6 gatunków trzmielców (*Psithyrus*) na Węgrzech, które pokrywają 42% kwadratów mapy UTM. Trzy gatunki trzmieli (*B. distinguendus*, *B. elegans* i *B. serrisquama*) uznano za wymarłe, ponieważ dane o ich występowaniu pochodzą sprzed 1953 roku. Pozostała fauna trzmieli węgierskich należy w znacznym procencie do gatunków rzadkich. Stwierdzono względne obniżenie występowania trzmieli na przestrzeni ostatnich 50 lat.

Słowa kluczowe: trzmiel, *Bombus*, trzmielec, *Psithyrus*, rozmieszczenie, Węgry.

REVIEW ARTICLES

HOW OLD ARE BEES? - A LOOK AT THE FOSSIL RECORD

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Summary

How old are bees? Which came first, bees or flowers? When did bees first develop social behaviour? Fossil bees are found in amber and copal but are quite rare as inclusions except for *Proplebeia* in amber from the Dominican Republic. Amber occurs throughout the world and in Europe the main deposits are in the Baltic region, Germany, Romania and Sicily. Baltic amber is approximately 40 million years old (Myr). Amber belonging to the Cretaceous period is found in several parts of the world and is about 120 Myr. Lebanese amber is the oldest and dates back to the early Cretaceous period some 135 Myr. At the time there were tropical rain forests where the resins from these trees preserved the insect fossils, which we have today. Copal by comparison is "modern", less than 2 Myr and is mainly from Africa, Colombia and the Dominican Republic.

"The oldest fossil bee" was described by Michener and Grimaldi in 1988 and is now referred to as *Cretotrigona prisca*. It is from the Cretaceous amber of New Jersey and believed to be 96 to 74 Myr before the present. Rasnitsyn and Michener (1991) questioned the date of this amber and Rasnitsyn believes that late Eocene Baltic amber is where the oldest fossil bees are to be found. He is still strongly opposed to the Cretaceous origin of bees. Prior to the discovery of *Cretotrigona*, the oldest fossil bees known were all from late Eocene Baltic amber, about 40 Myr. Engel (2001) has written a comprehensive monograph on Baltic amber bees and firmly believes bees originated in the Cretaceous and that the oldest possible age for them is 125 Myr. This is after the origin of flowering plants. No evidence exists (Engel 2001) to suggest bees arose prior to the origin and early diversification of flowering plants. Nests are also known from the fossil record and provide some additional information for dating the origin of bees.

Photographs of bees in Baltic and Dominican amber are displayed and included bees from the Museum of Amber Inclusions, University of Gdańsk; the Natural History Museum, Kraków; the Museum of Earth, Warsaw and the Natural History Museum, London.

The paper is review of the present state of knowledge and attempt to answer the questions raised at the beginning of this presentation. Relevant references are given.

Keywords: amber, *Apis*, bees, fossils, museum collections.

INTRODUCTION

Fossil bees are rare. They are found mainly in Baltic amber which is about 30 to 40 million years old (myBP = million years before present). Amber occurs throughout the world and in Europe the main deposits are in the Baltic region, Germany, Romania and Sicily. Copal by comparison is less than 2 million years old and is mainly from Africa, Columbia and the Dominican

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Republic. Bees in amber are "probably biased toward bees that used resin in nest construction" (Michener 2000).

Predatory sphecid wasps (*Sphecoidea*) are generally believed to be the ancestors of present day bees (*Apoidea*). The oldest of these wasps date from the early Cretaceous. Why they changed from being predatory to collecting pollen and nectar is not known.

The genus *Apis* Linnaeus 1758 also has a fossil record. *Apis mellifera* L. fossils have been found as far back as 1.5 to 2 myBP. Specimens of honey bees from the copal of East Africa in the Natural History Museum in London are described by Zeuner and Manning (1976).

RESULTS AND DISCUSSION

The Oldest Bee

The oldest fossil bee, that is the earliest bee in the fossil record, is a worker described by Michener and Grimaldi (1988) from the late Cretaceous (80 myBP) amber of New Jersey, USA. It is referred to as *Trigona prisca* (Michener et Grimaldi) and is a stingless bee (*Apidae*, *Meliponinae*). Engel (2000) has redescribed the fossil and transferred it to a new genus *Cretotrigona*. However a "cloud hangs over the dating for the piece of amber" (Michener 2000).

Rasnitsyn (see: Rasnitsyn and Michener 1991) has challenged the dating of the amber in which *Trigona prisca* has been found and believes (personal communication) the oldest body fossils of bees are those from late Eocene Baltic amber and opposes the idea of the Cretaceous age of *Trigona*. Prior to this report, the oldest bees known were found in late Eocene Baltic amber (40 myBP). Several species from the extinct genus *Electrapis* have been described from Baltic amber. These bees are considered to be social because: 1) many specimens have been found together in amber and 2) the presence of pollen collecting apparatus (Poinar 1992).

Specimens of amber with bee inclusions can be found in museums throughout Europe including Gdańsk, Hamburg, Kaliningrad, Kraków, London, Paris and Warsaw (see captions to photographs (Table 1; Figs 1-6)).

Fossil Honey Bees

No honey bees are known from before the Oligocene. T.D.A. Cockerell (1866--1948) was one of the earliest workers on fossil honey bees and the first to describe a

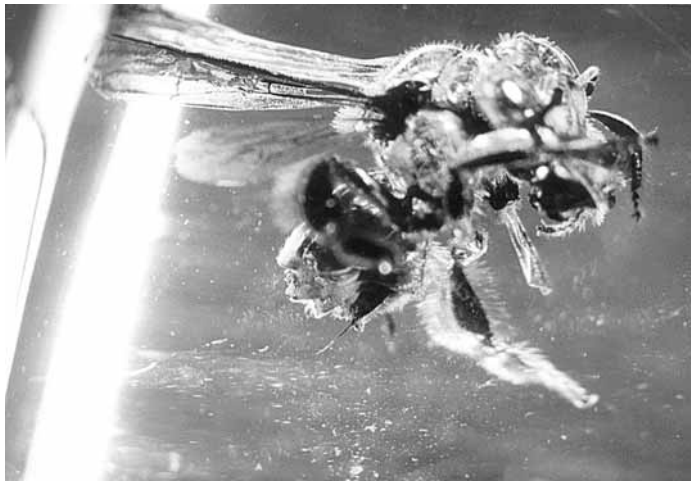

Fig. 1 *Apoidea* (*Hymenoptera*) in Baltic amber. Museum of Amber Inclusions, University of Gdańsk (photo. E. Sontag).

Table 1

Geochronological history of the Earth

Era	Period	Epoch	Duration (My)	Mya
Cenozoic	Quaternary	Holocene	10000 yrs	
		Pleistocene	1.64	1.7
	Neogene (Tertiary)	Pliocene	3.5	
		Miocene	18.3	23.5
	Paleogene (Tertiary)	Oligocene	12	
		Eocene	21	
		Palaeocene	8.5	65
Mesozoic	Cretaceous	Late Cretaceous	32	
		Early Cretaceous	49	146
	Jurassic	Late Jurassic	c. 10	
		Middle Jurassic	c. 20	
		Early Jurassic	c. 29	205
	Triassic	Late Triassic	25	
		Middle Triassic	12	
		Early Triassic	9	251
Palaeozoic	Permian	Late Permian	c. 10	
		Early Permian	c. 29	290
	Carboniferous	Stephanian	12	
		Westphalian	11	
		Namurian	10	
		Visean	18	
		Tournaisian	12	353
	Devonian	Late Devonian	c. 18	
		Middle Devonian	c. 10	
		Early Devonian	c.28	409
	Silurian	Pridoli	c. 2	
		Ludlow	13	
		Wenlock	6	
		Llandovery	9	439

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Fig. 2 *Apidae (Hymenoptera*) (492) in Baltic amber. Natural History Museum, Kraków (photo. W. Krzemiński).

Fig. 3 *Electrapis krishnorum* Engel (*Apidae*, *Hymenoptera*) (TG 5589). Museum of the Earth, Warsaw (photo. J. Kupryjanowicz).

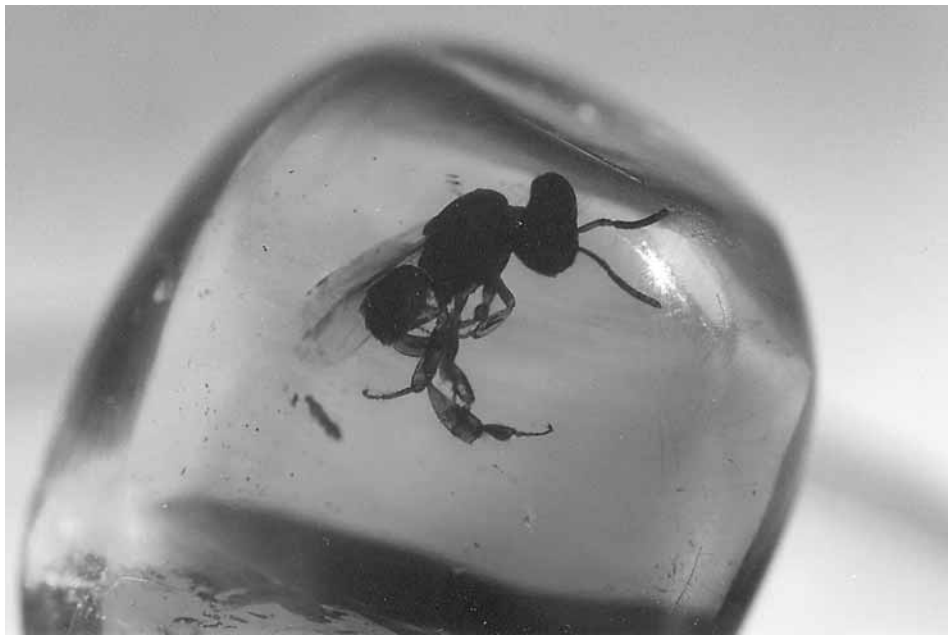

Fig. 4 *Proplebeia dominicana* (Wille et Chandler) (*Apidae*, *Hymenoptera*) in Dominican amber (photo. J. Kupryjanowicz).

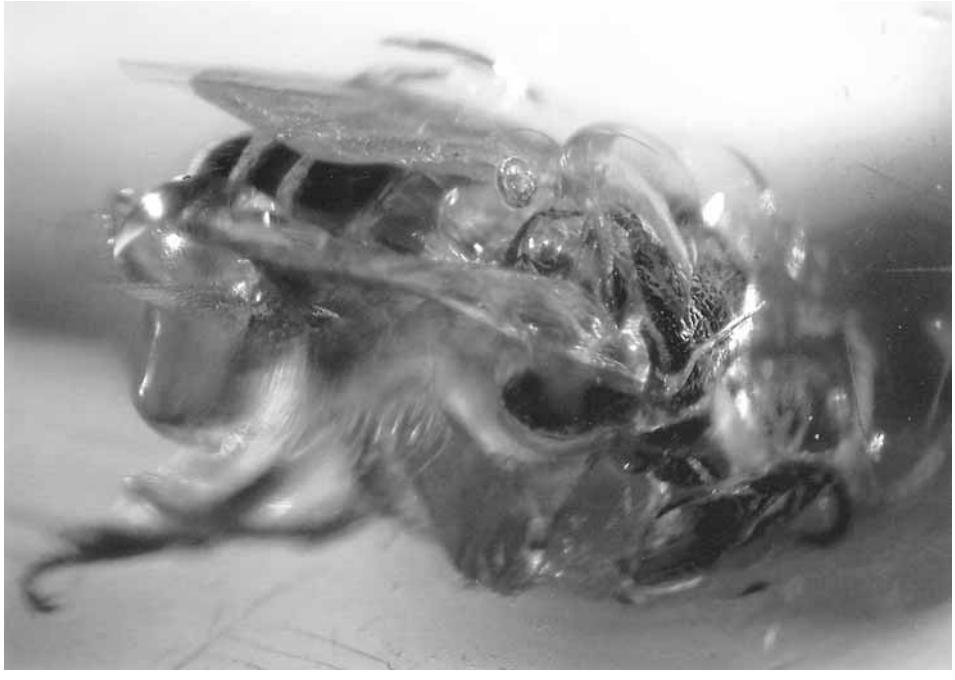

Fig. 5 *Apidae* (*Hymenoptera*) (In. 64560) in Dominican amber. Natural History Museum, London (photo. P. J. Evennett).

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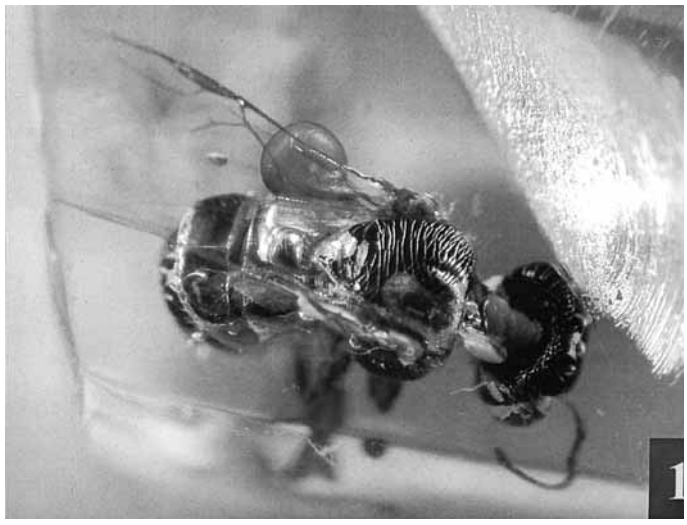

Fig. 6 *Proplebeia* sp. (*Apidae*, *Hymenoptera*) in Dominican amber. Natural History Museum, London (Baker, Chmielewski and Evennett - 2002 (in press) - photo. P. J. Evennett).

fossil species of the genus *Apis*. In 1907 he described *Apis henshawi* Cockerell. The holotype of this, "the most famous of the fossil honey bees" (Engel 1998), is to be found in the Museum of Comparative Zoology at Harvard University.

Zeuner and Manning (1976) referred to 17 fossil honey bees but now only 8 or 9 fossil species are recognized. Of the to-day living species of honey bee, only one has been found in the fossil record (Engel 1998). This is *Apis mellifera* L. and is known from the Pleistocene-Holocene, less than 2 myBP. Zeuner and Manning (1976) refer to specimens in the Natural History Museum in London from the Pleistocene in East African copal.

Cockerell (1909) examined a piece of "amber" said to be from the coast off Yarmouth, England containing two *A. mellifera* L. and discusses their antiquity, suspecting the bees to be in a piece of copal (not amber) and not of English origin.

Engel (1998), lists the fossil taxa, provides a revision and proposes a phylogeny for *Apis*.

Which Came First - Bees or Flower Plants?

Recent studies have shown that angiosperm (*Angiospermae*) developed early in the Cretaceous and by the end of this period there was a great deal of diversity in flowering plants. Bees probably arose before the middle Cretaceous at a time when angiosperms were becoming more abundant.

However there is a possibility that bees arose earlier and made use of pollen collected from gymnosperm plants (*Gymnospermae*) and may therefore have arisen before the angiosperms became common.

It is probably true to say that bees arose at the same time as or after the flowering plants.

CONCLUSIONS

This brief review of fossil bees highlights recent work and illustrates the need for further on their biology and geology, including taxonomy and dating.

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JAK STARE SĄ PSZCZOŁY? - SPOJRZENIE NA REJESTR SKAMIENIAŁOŚCI

Baker R. A., Chmielewski W.

Streszczenie

Przedstawiono przegląd obecnego stanu wiedzy na temat skamieniałości owadów pszczołowatych z pszczołą miodną włącznie, na tle geochronoligcznego układu dziejów Ziemi. Omówiono nazewnictwo najstarszej kopalnej pszczoły stwierdzonej w zarejestrowanych dotychczas skamieniałościach i przedyskutowano kontrowersje w odniesieniu do wieku tego znaleziska w świetle literatury. Zestawiono wykaz ważniejszego piśmiennictwa na temat znalezisk tego typu i stwierdzenia skamieniałości (fosylia) *Apis mellifera*. Podano wykaz kilku europejskich muzeów, które posiadają zbiory bursztynów zawierających inkluzje pszczele.

Słowa kluczowe: *Apis*, bursztyn, pszczoły, skamieniałości, zbiory muzealne.

BEE POLLINATION OF FRUIT TREES: RECENT ADVANCES AND RESEARCH PERSPECTIVES I.

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Summary

Intense research was carried out on the bee pollination of temperate zone deciduous fruit trees during the past decade. Much progress was achieved to explore the flower characters of a great number of cultivars affecting honeybee activity at flowers and their pollinating efficiency. Flower characters were found to be consequently different among apple and pear cultivars in consecutive years but in case of some stone fruits differences between consecutive years were greater than between cultivars in given years. The necessity of bee pollination was clearly demonstrated both at self-sterile and at self-fertile fruit cultivars. The rate of flower constancy of honeybee foragers was different at different fruit tree species but the role of competing plants was found to be much less deleterious than stated in the literature earlier. However, a number of questions arose partly from the results of latest pollination research and partly from practical experiences in commercial plant production. These indicate several research tasks to understand and to solve the problems possibly in the near future. The questions concentrate on the effectiveness of bee visits in the pollination of individual fruit crops and their different cultivars and on the reliable estimate of the overall amount of bees as well as on the control of bee density during the flowering period of fruit orchards for optimal, controlled honeybee pollination. Much less effort was made to manage native wild bees for fruit tree pollination, however, some mason bees seem to be promising for this purpose in Europe, The Far East and North America.

Keywords: insect pollination, honeybees, temperate zone deciduous fruit crops.

INTRODUCTION

Almost all temperate zone fruit tree species need insect pollination, the only exception is the horse chestnut, which is wind pollinated, but partly benefits insect pollination, too (Pesson and Louveaux 1984). The role of insects in fruit crop pollination has been recognized long ago. The information accumulated on this field has been reviewed by Free (1993) in the comprehensive handbook "Insect pollination of crops (second edition)" some ten years ago. After the appearance of this book fruit pollination research has remained intense. Important new information accumulated in this field, however, several new problems arose that needs further research. The aim of this paper is to give an outlook onto the state of knowledge in this field and to point out the need for further research.

RESULTS

Improving the efficiency of honeybees in crop pollination

A number of techniques have been known to increase the pollinating efficiency of honeybees by increasing the bee visitation to target crops, increasing the ratio of pollen collecting foragers, improving their pollen dispersal, and so on (Free 1993).

However, to increase the bee visitation of the target crop is rather a hard task, hence

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it depends on several characteristics of the crop and of cultivated or wild plants grown in the surroundings, on the food demand of the honeybee colonies, and of course, on the weather. This may be the explanation why a number of different methods, neither the application of bee attractants, nor sequential introduction of colonies to the flowering crop, nor feeding bees, nor diluting the nectar with overhead irrigation, nor interplant alfalfa increased the number of honeybees and increased the yield significantly at seed onion fields (Mayer and Lunden 2001).

On the other hand, Winston and Slessor (1993) reviewing their results on the recognition and the use of the queen mandibular pheromone (QMP), predicted that its most significant commercial application would be in crop pollination. This statement was based on good results obtained in field experiments. Spraying flowering crops increased the number of honeybees foraging on apple, pear, cranberry, and blueberry (Currie et al. 1992a, 1992b, 1994, Hugo et al. 1995, MacKenzie and Averill 1992). The effect of QMP, however, is greatly dependent on dosage and partly on crop. Namely, 0.1 queen equivalents/ha was ineffective, but 1000 was effective on apple and pear (Currie et al. 1992b), the same dosage failed to increase bee visits on sweet cherry (Naumann et al. 1994), while 100 was the most effective on cranberry and 100 was as effective as 1000 on blueberry (Currie et al. 1992a). In other instances, with pear and apple, QMP application increased only fruit sizes without actual yield increase, and this still resulted in higher profits (Currie et al. 1992b, Naumann et al. 1994). On he contrary, on sweet cherry a negative correlation arose between bee visits and fruit size. These experiences, however, are not consistent enough, and therefore further research is needed on this topic, not only in the US but also in other countries with different weather conditions. Also, the profitability of the method should be analysed, as its acceptance in the practice depends on its profitability. There is one other, cheaper kind of application of QMP in crop pollination, because the pollination value of queenless "disposable" pollination units can be improved when QMP is added, which may increase the foraging activity; moreover should brood be present, even pollen collecting activity is increased (Currie et al. 1994). Other kinds of bee attractants (e.g. Beeline, Be-Q, Beehere, lavender oil, citral, geraniol, etc.), however, did not increase the bee visitation at a number of different crops (Ambrose et al. 1995, Neira et al. 1994, Ortiz-Sanchez 1993, Sing and Sinha 1996, Zvedenok 1996).

As in-hive pollen transfer is recognized to be an important factor in pollen dispersal among compatible but self-sterile or self-fertile but readily cross-fertilizing cultivars of some crops (e.g. Dag and Kammar 2001, DeGrandi-Hoffman et al. 1984, DeGrandi-Hoffman and Martin 1993, 1995, McLaren et al. 1996), the methods increasing pollen exchange between foragers of the honeybee colonies can be regarded to be very important. Following the pioneer work of Free et al. (1991), several kinds of hive entrance devices were used lately to brush pollen from incoming and deposit on departing bees (Hatjina 1996, Eijnde and Velde 1995, Szalai 2000). This type of research should be continued in order to develop the most effective devices without harming foragers and without decreasing germination capacity of the pollen.

However, the mechanism and the effectiveness of in-hive pollen exchange are not adequately studied and its role in crop pollination is probably underestimated. Latest research on the fruit set of large single-cultivar blocks of apple indicates that in-hive pollen exchange (or possibly carrying pollen on the body hairs of bees from one foraging trip to the next) could be much

more important in the effective pollination of orchards than believed so far, because even large and very large single-cultivar blocks in apple plantations can effectively be pollinated in lack of pollinizer cultivars inside the block (Blažek 1996, Benedek unpublished). Therefore, the in-hive pollen transfer among bees needs to be studied thoroughly with fruit trees and other crop plants.

Recently, the role of electrostatic charges for non-contact pollen detachment between pollinating honeybees and flowers is confirmed, and this can be utilized for supplementary pollination in commercial production (Vaknin et al. 2000), this new approach, however, needs further research to base commercial implementation.

A need of controlled honeybee pollination

As successful pollination may be a limiting factor of a good yield, growers would like to know how many honeybee (or bumblebee) colonies are needed and how to organize them for optimal pollination at the field. We can give general instructions based on experiments and/or measurements about timing the placement and the arrangement of the location of the bee colonies inside or round the target crop, but the number of bee colonies needed is much more difficult to define on a reliable basis.

On the other hand, the recommendations on the stocking rates of bee colonies needed to pollinate the crop plants are based on assumptions rather than on experimental results. There is very little information in the literature how to estimate the necessary number of bee visits/flower or the necessary bee density/per unit area of optimal pollination or about the optimal set of various crops (the examples are sunflower, cotton, hairy vetch as well as fruit plantations to some extent: see in Free 1993). This kind of information would, however, be very important in the practice, because not only the inadequate activity of pollinating insects, but their excessive activity can also be disadvantageous to the yield.

The unpleasant consequences of oversetting, numerous but too small fruits, poor quality, the need of laborious and expensive thinning, are well know for some fruit trees in the practice, for peach and nectarine for example. Lately, both insufficient and excessive visiting of bees (honeybees and bumblebees) for pollination of strawberry have been found to be undesirable, both causing faulty pollination under cover. A density of 10 - 15 thousands of worker bees/1000 sq. metres in plastic tunnels or glasshouses is recommended, but neither the lower nor the higher densities are desirable (Lieten 1993). Similar results were obtained with cucumber; fruit set and the number of filled seeds were the higher when there were some 0.5 honeybee colonies per some 2000 plants, reduced number of bees was resulted in lower yields, on the contrary overpopulation caused so strong competition for flowers (for pollen) which reduced the yield (Cervancia and Forbes 1993). In case of sunflower crops some 10 thousand bees/hectare was found to be optimal for pollination in Hungary, however, increased bee visitation did not affect the yield (Benedek 1972). Similarly, additional extra hives at flowering pear increased bee numbers in the orchard, but not the fruit set in New Zealand (McLaren et al. 1996).

For this reason, it is important to explore the relationship between the number of bee visit/flower and the set. In case of pumpkin, for example, latest results show that some 10 insect visits are needed o that enough pollen grains are deposited onto the stigma needed for a good set (Masierowska and Wien 2000). In case of squash each plant requires at least one honeybee visit during the optimum pollination time (that is between 06.00 and 09.00 in this case) (Al-Fattah 1991), and some 5-6 bee-visits/flower are needed for maximum

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productivity of raspberry (Oliveira et al. 1991). Based on experimental results 6-12 bee visits were necessary in Hungary during the life of a single apple flower to set a fruit (Benedek et al. 1989). Both fruit set and quality were related to the number of bee visits/flower in experiments with apple in Canada, but an other experiment gave inconclusive result due to overall poor fruit set caused by bad weather (Brault et al. 1995).

In fact, the present practice to move certain number of bee colonies to the target crop just prior to, or at the start of flowering, and to leave them on site until petal fall, is an inadequate solution to crop pollination problems. This can be exemplified by a sequential introduction of honeybee colonies to pear pollination; namely, placing additional bee colonies in pear orchards at 50 % flowering resulted in more bees visiting the flowers for at least one day which significantly increased fruit set in 10 out of the 14 experimental orchards (Mayer 1994). However, growers need some kind of method, as simply as possible, to decide if they should do something with bees during the flowering period of crops. A simple solution was suggested in Hungary. Taking some factors, the number of bee visits per flower necessary to set a fruit, the length of the receptive period of the flowers, the required ratio of fruit set and the patters of the daily bee activity into account, 3-6 bee visits per 50 opening flowers during a ten minutes period was recommended as the optimal intensity of bee visitation in apple orchards to get a good crop (Benedek 1996). The growers were recommended to control the intensity of bee pollination during the flowering period and to move additional colonies to the orchard immediately, when the bee visitation was much lower than the proposed optimal level thus avoiding insufficient, low sets. At other instances, however, excessive bee visitation was decreased during the flowering period to avoid unwanted oversetting at some crops. This kind of bee pollination management should be implemented during the flowering period of crops, which would lead to controlled bee pollination in the agriculture.

Consequently, intense research is needed to base controlled bee pollination experimentally for those crop plants, of different fruits first of all, where lower and higher than optimal sets are equally undesirable for profitability reasons. Growers should be recommended to carry out simple observations or should be given sophisticated, but user-friendly computer simulations, as the well-known PC-REDAPOL for example, which can reliably predict the apple yield (DeGrandi-Hoffman et al. 1995). This enables growers to decide what to do during the flowering period for the optimal pollination of their insect pollinated crops. The problem is especially crucial in the new type, high density orchards with semi-dwarf or dwarf fruit trees being the productive life much shorter, 10 to 15 years at most, than of traditional, large crown fruit plantations, which can stand and produce fruit for decades. During the short productive period of high density orchards no single year with bad yield or with bad fruit quality can be suffered, because the investment cost of this type of orchards is very high and also their cultivation is much more expensive than of a traditional plantation, consequently the production must be profitable each year of their short productive life. Accordingly, pollination must not be an unstable element of their management system.

There is a host of factors affecting the general behaviour of bees towards the rewards that different crops and competing other plants, flowering simultaneously offer to them. Other environmental factors affect also the foraging behaviour, and consequently the pollinating efficiency of bees visiting the flowers of crop plants (more of social than of solitary bees). Weather and

competitor plants seem to have the most decisive influence on bee activity. The effect of weather conditions is fairly well known, but the effect of competing plant species is contradictory and confusing. Lately, even massive appearance of flowering dandelion, that is known to have very strong competitive effect on honeybees in fruit orchards, failed to affect bee visitation in pear orchards, neither sour cherry attracted bees from pear (Benedek et. al. 1998a). There are some other indications that the effect of competitor plants is not sufficiently explored, accordingly this item needs further studies to understand why certain plant species are strong competitors in some cases and no similar effect can be recognized at other instances.

CONCLUSIONS

Based on the above discussion there seem to be a number of topics that greatly needed intense research in the near future to improve the knowledge on as well as the technology of the insect pollination of crop plants cultivated in temperate zone regions. These are partly related to the pollination requirements of selected crops and to managing some solitary wild bee species for crop pollination. The problems, however, concentrate on the effectiveness of bee visits in the pollination of individual fruit crops (and their different cultivars) and on the reliable estimate of the stocking rates of bees for crop pollination as well as on the management of actual bee density during the flowering period of fruit orchards for optimal controlled bee pollination. The following topics are proposed as the subject of further research in the coming years:

1. Improving the efficiency of honeybees in crop pollination

— The effect of the queen mandibular pheromone sprays is to be further studied to increase the pollination efficiency of honeybees at target crops in flower.

— Further research is needed on the mechanism and the effectiveness of in-hive pollen exchange among honeybees during crop plant pollination.

— Further research is required to develop the most effective devices increasing pollen exchange between foragers of honeybee colonies for pollination.

2. A need of controlled honeybee pollination

— Intense research is needed to base controlled bee pollination experimentally for those crop plants, of different fruits first of all, where lower and higher than optimal sets are equally undesirable for profitability reasons. Growers should be recommended to carry out simple observations or should be given sophisticated, but user- friendly computer simulations to manage flower visiting bee populations at an optimal level during the flowering period of insect--pollinated crop plants.

— Further studies are needed on the effect of competing plant species to understand why certain plant species are strong competitors in some cases and no similar effect can be recognized at other instances.

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ZAPYLANIE DRZEW OWOCOWYCH PRZEZ PSZCZOŁY: OSTATNIE POSTĘPY I PERSPEKTYWY BADAŃ I.

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

Podczas minionej dekady prowadzono wiele szczegółowych badań dotyczących zapylania przez pszczoły drzew owocowych strefy umiarkowanej. Wielki postęp został osiągnięty kiedy odkryto, że kwiaty wielu odmian posiadają cechy oddziałujące na aktywność pszczół miodnych na kwiatach i ich wydajność jako zapylaczy. Odnalezione cechy kwiatów były zdecydowanie różne w przypadku odmian jabłoni i gruszy w poszczególnych latach ale w przypadku niektórych odmian gruszy różnice między kolejnymi latami były większe niż między odmianami w danych latach. Konieczność zapylania przez pszczoły została wyraźnie wykazana zarówno w przypadku obcopylnych jaki i samopylnych odmian owoców. Stopień wierności kwiatowej zbieraczek pszczoły miodnej był różny na poszczególnych gatunkach drzew owocowych ale rola pożytków konkurencyjnych okazała się być mniej szkodliwa niż stwierdzano to wcześniej w literaturze. Jednakże, pewna liczba pytań powstała częściowo w wyniku najnowszych badań nad zapylaniem i częściowo z praktycznych doświadczeń w polowej produkcji roślin. Pytania te wskazują kilka zagadnień do badania, by zrozumieć i rozwiązać problemy prawdopodobnie w najbliższej przyszłości. Koncentrują się one na skuteczności wizyt pszczół w zapylaniu poszczególnych upraw owoców i różnych ich odmian oraz na wiarygodnej ocenie całkowitej liczby pszczół jak również na kontroli zagęszczenia pszczół podczas okresu kwitnienia sadów owocowych dla optymalnego, kontrolowanego zapylania ich przez pszczoły. Dużo mniej wysiłku zrobiono, by wykorzystać rodzime dzikie pszczoły samotnice dla zapylania drzew owocowych, jednakże, niektóre murarki wydają się być odpowiednie do tego w Europie, na Dalekim Wschodzie i Ameryce Północnej.

Słowa kluczowe : zapylanie przez owady, pszczoły miodne, plonowanie owoców w strefie umiarkowanej.

BEE POLLINATION OF FRUIT TREES: RECENT ADVANCES AND RESEARCH PERSPECTIVES II.

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Summary

Intense research was carried out on the bee pollination of temperate zone deciduous fruit trees during the past decade. Much progress was achieved to explore the flower characters of a great number of cultivars affecting honeybee activity at flowers and their pollinating efficiency. Flower characters were found to be consequently different among apple and pear cultivars in consecutive years but in case of some stone fruits differences between consecutive years were greater than between cultivars in given years. The necessity of bee pollination was clearly demonstrated both at self-sterile and at self-fertile fruit cultivars. The rate of flower constancy of honeybee foragers was different at different fruit tree species but the role of competing plants was found to be much less deleterious than stated in the literature earlier. However, a number of questions arose partly from the results of latest pollination research and partly from practical experiences in commercial plant production. These indicate several research tasks to understand and to solve the problems possibly in the near future. The questions concentrate on the effectiveness of bee visits in the pollination of individual fruit crops and their different cultivars and on the reliable estimate of the overall amount of bees as well as on the control of bee density during the flowering period of fruit orchards for optimal, controlled honeybee pollination. Much less effort was made to manage native wild bees for fruit tree pollination, however, some mason bees seem to be promising for this purpose in Europe, The Far East and North America.

Keyworlds: insect pollination, honeybees, temperate zone deciduous fruit crops.

INTRODUCTION

Almost all temperate zone fruit tree species need insect pollination, the only exception is the horse chestnut, which is wind pollinated, but partly benefits insect pollination, too. The role of insects in fruit crop pollination has been recognized long ago. The information accumulated on this field has been reviewed by Free (1993) in the comprehensive handbook "Insect pollination of crops (second edition)" some ten years ago. After the appearance of this book fruit pollination research has remained intense. Important new information accumulated in this field, however, several new problems arose that needs further research. The aim of this paper is to give an outlook onto the state of knowledge in this field and to point out the need for further research.

RESULTS

Flowers characters and other conditions affecting bee activity

Pollination requirements of temperate zone fruit crops, their pollinating insects, the rewards to attract bees and the foraging behaviour of bees on their flowers as well as the effect of bee pollination on the yield is fairly well known (Free 1993). Lately, intensive studies were carried out on variety features affecting bee activity,

There were some few indications in the

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literature that some differences between cultivars, for example the relative position of petals and stamens, or the nectar and pollen production of flowers had a definite influence on bee behaviour and on the pollinating efficiency of bees at some instances (see in Free 1993), this problem, however, has not been not adequately studied and no more than sporadic information was available on very few cultivars of some selected fruit species, first if all of apple and pear. For this reason lately large series of cultivars were inspected for various fruit species, first of apple (Benedek et al. 1989b, Benedek and Nyéki 1996a, Devary-Nejad et al. 1993), thereafter of apricot (Benedek et al. 1991b, 1995), peach and nectarine (Benedek et al. 1991a, Benedek and Nyéki 1996b), sweet and sour cherry (Benedek et al. 1990, 1996) and plum (Szabó et al. 1989, Benedek et al. 1994). Differences were detected among cultivars in flower sizes, in the number of stamens/flower, in the relative position of petals to stamens and to pistils, in the nectar content of flowers, in the sugar concentration in nectars, in the anther size and in the pollen production, and finally in the intensity of bee visitation at the flowers. In case of apricot, flower characteristics varied considerably depending on the year and site, greater differences occurred at the same cultivar in consecutive years than between cultivars in given years. Similarly, no consistent differences were found in the bee visitation at a number of almond cultivars in India; floral preferences varied from year to year (Thakur et al. 1995). Contrarily, at the rest of the mentioned fruit species, the differences in the flower characteristics of cvs were consistent in different years, and consequently, also their bee visitation differed. In the case of pear, the opening order of flowers within inflorescence was different; cvs fell into three types of opening order which also differed in the average number of bee visits per flower; this was explained by the different number of flowers and differences in the longevity of flowers within the inflorescence of the tree types (Dibuz el al. 1997, 1998).

In other studies the nectar content and the bee visitation of several almond (Abrol 1995), peach (Nyéki et al. 2000), pear (Benedek et al. 2000a) and quince cultivars were compared (Benedek et al. 2000d, 2000e); cultivars with higher sugar concentration, i.e. with higher caloric rewards usually attracted more foraging insects compared to other cultivars of the same fruit species, except pear (Abrol 1995, Benedek et al. 1997, 1998b, 2000e, Benedek 2000). Nectar production of pear is generally regarded to be very poor in the literature (Free 1993); in fact, it has been shown lately that pear flowers could produce a plenty of nectar, but the amount of nectar was extremely dependent on the weather (Benedek et al. 2000a). However, pear nectar always contains very little amount of sugar (Benedek et al. 2000a) which is not attractive to bees at all (Free 1993). That is why honeybees collect almost exclusively pollen on pear, even with high nectar production (Benedek et al. 1997, 1998b, Benedek and Nyéki 1997a, Benedek 2000).

Comparing the nectar production and the bee visitation of fruit tree flowers at the species level, the amount of nectar/flower was the highest at sour cherry, followed by apricot, apple, plum, peach and nectarine; pear always produce very small but sometimes can produce very high amount of nectar (Benedek and Nyéki 1997a). The mean sugar concentration of pear nectar was always very low, regardless the amount, and the sugar concentration of nectar was the highest at sour cherry, followed by apple, plum, peach and nectarine (Benedek and Nyéki 1997a). Mean intensity of bee visitation was also different according to the fruit species, and again, it was clearly

related to the sugar concentration of the nectar (except pear), but the amount of nectar had no effect on the bee visitation at the species level (Benedek and Nyéki 1997a).

Different factors, weather conditions first of all, can restrict pollinating insects to work the flowers of fruit trees during their blooming period. In spite of this, very little information has been available in the literature on the rate of limitation that affected the fruit set and yield. For this, reason experiments were made on this issue lately with apple (Benedek et al. 1989a, Benedek and Nyéki 1996c, 1997b), with pear (Benedek et al. 2000b), with quince (Benedek et al. 2000f), with sour cherry (Benedek et al. 1990), with plum (Szabó et al. 1989, Benedek et al. 1994) and with apricots (Benedek et al. 2000b). Summarizing the results of relevant experiments, the main conclusion was, that in the case of self-sterile fruit species and cultivars (apple, pear, quince, some plums, some sour cherries) even partial limitation of the effective duration of the bee pollination period significantly reduced the fruit set and the yield; in the case of self-fertile fruits (some plums, some sour cherries, some apricots), on the other hand, the effect of partial limitation was usually small, and the complete (or incomplete but strong) limitation resulted in a strong reduction of yields; it means that not only self-sterile, but also self-fertile fruit cultivars definitely need insect pollination (Benedek and Nyéki 1995, 1996d, Benedek et al. 2000b).

Lately flower constancy of honeybees at fruit trees was investigated by analysing the pollen loads of pollen gatherer honeybees captured at fruit tree flowers (Benedek and Nagy 1995, Benedek et al. 2000c); the fidelity of bees was fairly high for pear, but it was smaller for apricot, much smaller for apple and even smaller for sour cherry. Competing plants influenced the flower constancy of honeybees and the influence was greatly different for fruit species; it was very little at pear (Benedek et al. 1998a, Benedek 2000). High fidelity of pollen gatherers was also observed towards cultivars, since bees returning to the hives had 80-90% of their loads from only one cultivar out of the 5 or 6 in an almond orchards (Vezvaei and Jackson 1997, DeGrandi-Hoffman et al. 1992). However, bees with high amount of pollen from a single cultivar usually had some pollen from other cultivars, too (DeGrandi--Hoffman et al. 1992).

The gene flow, as investigated with isozyme markers, was quite restricted in almond orchards in Australia, being the strongest between neighbouring halves of cross--compatible pairs of trees (Jackson and Clarke 1991, Vezvaei and Jackson 1997). However, the nut set on branches adjacent to compatible pollen sources did not differ significantly from the set on branches adjacent to the trees of the same cultivar in one other experiment in the US (DeGrandi-Hoffman et al. 1992).

Managing wild bees and other insects for fruit tree pollination

Managing and utilizing wild bees as pollinators attract more and more interest, because some wild bees are relatively easy to manage and they posses several advantages as pollinators compared to honeybees. Wild bees, some solitary bees and bumblebees, however, have been exploited for field crop pollination and for insect pollination under cover because (Richards 1993, Cane 1997, Ruijter 1997) because the managed species are not specialised visitors of the Rosaceae plant family.

Managing mason bees for fruit tree pollination: It is a fairly new development to try managing different mason bees for pollination. In temperate regions, Osmia cornuta seems to be the most promising mason bee to this purpose. Its normal seasonal activity starts very early spring, and coincides well with the flowering period of fruit

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trees (Bosch et al. 1993). There are some serious parasites that can restrict its population size (Bosch 1992, Bosch et al. 1993). Pollen collected for provisions show that the species prefers to collect Prunus pollen, and switches to Malus pollen when it is more readily available (Márquez et al. 1994). It is calculated that during almond flowering, each female potentially visit as much as 9.5 to 23 thousand flowers, and therefore 3 females/tree are sufficient for almond pollination (Bosch 1994a). Cocoons were exposed to different overwintering and incubating temperatures trying to manipulate the time of emergence but with little success so far (Bosch and Blas 1994). Suitable nesting material was searched for (Bosch 1994b, 1995), but further research is needed. Osmia cornuta has been tried to be used for blackberry pollination in plastic tunnel with success (Pinzauti et al. 1997). Very probably it will be suitable to pollinate other fruit crops too, hence this issue also needs further studies. Some other mason bees, Osmia lignaria propinqua, O. californica, O. Montana, were also successfully managed for mobile orchard pollination in the US (Torchio 1991), and O. cornifrons for Japanese pear and apple pollination in the Far East (Maeta et al. 1993, DooHyun et al. 1996, Sekita 2001). There are some additional proposals to manage some other solitary bees for crop pollination; Andrena flavipes - which also occurs in Europe - was proposed to manage in India (Abrol 1993) and Anthophora pilipes villosula in Japan (Batra 1994).

An other European early spring mason bee species, Osmia rufa, closely related to O. cornuta, is regarded as unsuccessful for orchard pollination because this species exploits much wider range of pollen sources and no pollen preference exists towards fruit trees (Ricciardelli d'Albore et al. 1994).

CONCLUSIONS

Based on the above discussion there seem to be a number of topics that greatly needed intense research in the near future to improve the knowledge on as well as the technology of the insect pollination of crop plants cultivated in temperate zone regions. These are partly related to the pollination requirements of selected crops and to managing some solitary wild bee species for crop pollination. The problems, however, concentrate on the effectiveness of bee visits in the pollination of individual fruit crops (and their different cultivars) and on the reliable estimate of the stocking rates of bees for crop pollination as well as on the management of actual bee density during the flowering period of fruit orchards for optimal controlled bee pollination. The following topics are proposed as the subject of further research in the coming years:

1. Flowers characters and other conditions affecting bee activity
   - Further studies are needed on the flower constancy of honeybees on fruit trees as related to their pollinating efficiency.
   - The pollinating efficiency of honeybees on self-incompatible fruit tree species should be re-evaluated by investigating the gene flow with isozyme markers.
2. Managing wild bees for orchard pollination
   - Further research is needed to develop rearing techniques for commercial usage of the European orchard mason bee, Osmia cornuta, for fruit tree pollination. One other closely related species, Osmia rufa, might be successful for a number of crops under cover.

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ZAPYLANIE DRZEW OWOCOWYCH PRZEZ PSZCZOŁY: OSTATNIE POSTĘPY I PERSPEKTYWY BADAŃ II.

Benedek P.

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

Podczas minionej dekady prowadzono wiele szczegółowych badań dotyczących zapylania przez pszczoły drzew owocowych strefy umiarkowanej. Wielki postęp został osiągnięty kiedy odkryto, że kwiaty wielu odmian posiadają cechy oddziałujące na aktywność pszczół miodnych na kwiatach i ich wydajność jako zapylaczy. Odnalezione cechy kwiatów były zdecydowanie różne w przypadku odmian jabłoni i gruszy w poszczególnych latach ale w przypadku niektórych odmian gruszy różnice między kolejnymi latami były większe niż między odmianami w danych latach. Konieczność zapylania przez pszczoły została wyraźnie wykazana zarówno w przypadku obcopylnych jaki i samopylnych odmian owoców. Stopień wierności kwiatowej zbieraczek pszczoły miodnej był różny na poszczególnych gatunkach drzew owocowych ale rola pożytków konkurencyjnych okazała się być mniej szkodliwa niż stwierdzano to wcześniej w literaturze. Jednakże, pewna liczba pytań powstała częściowo w wyniku najnowszych badań nad zapylaniem i częściowo z praktycznych doświadczeń w polowej produkcji roślin. Pytania te wskazują kilka zagadnień do badania, by zrozumieć i rozwiązać problemy prawdopodobnie w najbliższej przyszłości. Koncentrują się one na skuteczności wizyt pszczół w zapylaniu poszczególnych upraw owoców i różnych ich odmian oraz na wiarygodnej ocenie całkowitej liczby pszczół jak również na kontroli zagęszczenia pszczół podczas okresu kwitnienia sadów owocowych dla optymalnego, kontrolowanego zapylania ich przez pszczoły. Dużo mniej wysiłku zrobiono, by wykorzystać rodzime dzikie pszczoły samotnice dla zapylania drzew owocowych, jednakże, niektóre murarki wydają się być odpowiednie do tego w Europie, na Dalekim Wschodzie i Ameryce Północnej.

Słowa kluczowe : zapylanie przez owady, pszczoły miodne, plonowanie owoców w strefie umiarkowanej.

METHODS OF CLASSIFICATION OF HONEYBEE RACES USING WING CHARACTERS - A REVIEW

Dorothea Kauhausen, Roland Keller

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Morphometric analysis for classification of races and breeding lines of the honey bee, *Apis mellifera*, is still in use today, as has been for the past 40 years. Even with the advent of genetic analysis on a molecular level, an inexpensive and easy-to-use method is needed to supply wide spread information for the bee breeders. Most importantly, it must be a method that a beekeeper can use without special training or expensive equipment.

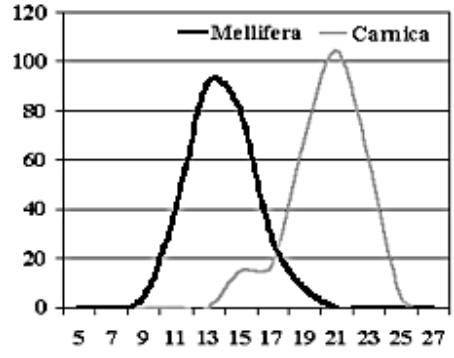

Fig. 1 Frequency distribution of cubital index for *A. m. carnica* and *A. m. mellifera*

Over 40 years ago, the imported carnica race needed to be differentiated from the predominant mellifera race in Germany. For this purpose the cubital index (Fig. 1) was good for discrimination, and is still in use today. For other races like ligustica, caucasica and the breeding line Buckfast this method failed.

Kruber (1994) suggested to expand this method of the cubital index, to allow a more precise selection of pure breed carnica from hybrids. Further wing characters, formerly established by Goetze (1964), were added - the "Hantelindex" and the "Discoidal-

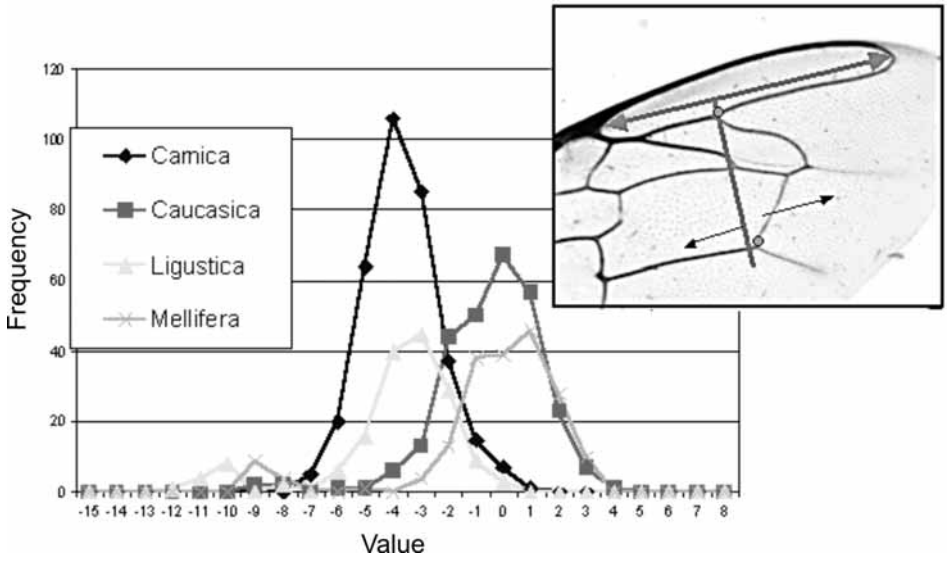

Fig. 2 Distribution of Discoidal displacement for different races

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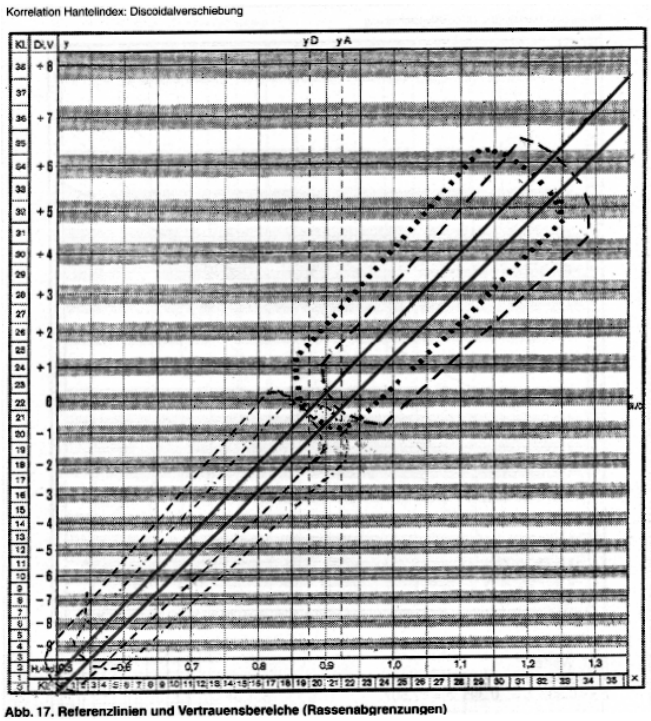

Fig. 3 Reference lines and confidence intervals for discrimination of races (Kruber 1994)

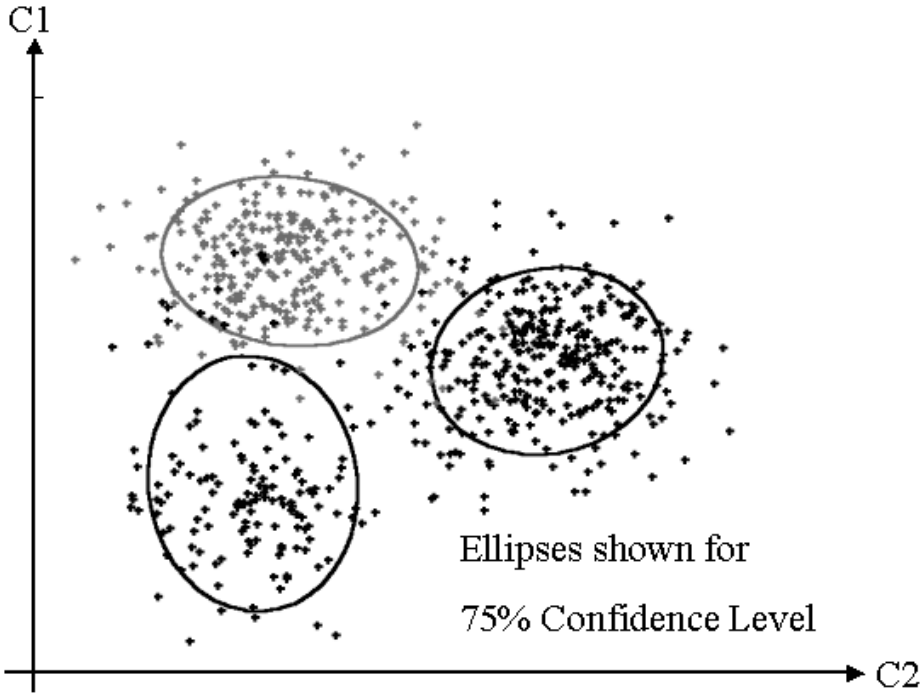

Fig. 4 Discriminant analysis showing C1 and C2 as canonical coordinates and 3 sample groups

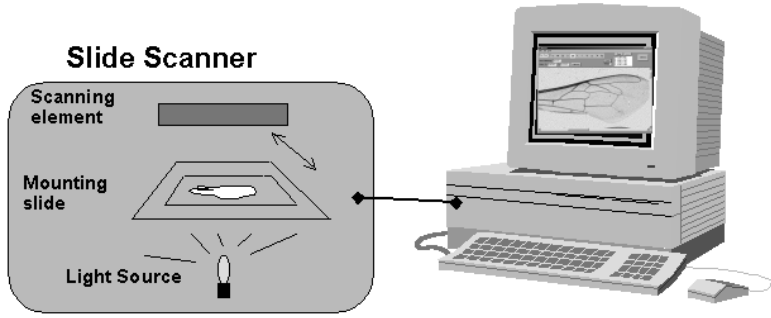

Fig. 5 Slide scanner with PC

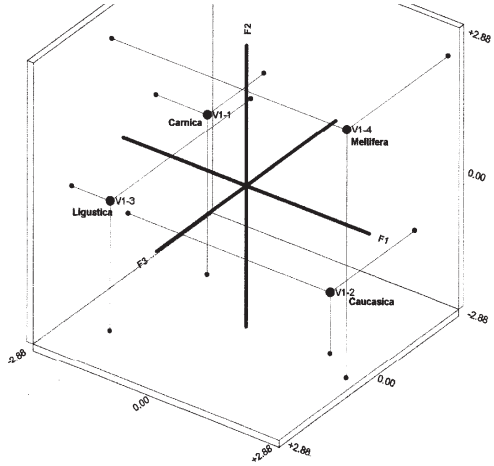

Fig. 6 Position of group centroids using only wing characters (introduced by DuPraw 1964)

verschiebung" (Fig. 2). The results of these two characters represented in a diagram, would allow better differentiation between carnica and mellifera, and their hybrids (Fig. 3). With other races, like *A. m. ligustica* or *A. m. caucasica*, however, a differentiation is still not better than with the cubital index.

A major step forward was undertaken by Prof. F. Ruttner (1988), who used 15 wing characters, 13 size characters, length of cover hair and 2 characters of the tomentum as well as 5 characters of pigmentation. These 36 different characters were used in a statistical method called "discriminant analysis" which reduces the many characters to a few "best canonical factors" from which two or three dimensional distribution plots could be made. A priori predictions were made by choosing certain confidence levels expressed in distance from the group centroid (Fig. 4). Using this method, it was possible for Ruttner to classify among twenty four different subspecies of *Apis mellifera*. The preparation and measurement of samples was quite tedious - nearly a whole week was required for one single sample of twenty working bees from a colony.

In the following years, various development in using digitizers linked with a computer for faster data taking went under way. Video cameras soon took the place of digitizing tablets, and finally slide scanners (Fig. 5), which did not need optical lenses. Since measurements of wing characters were the most precise, with a high repeatability, focus was set on these, and the other body characters were neglected.

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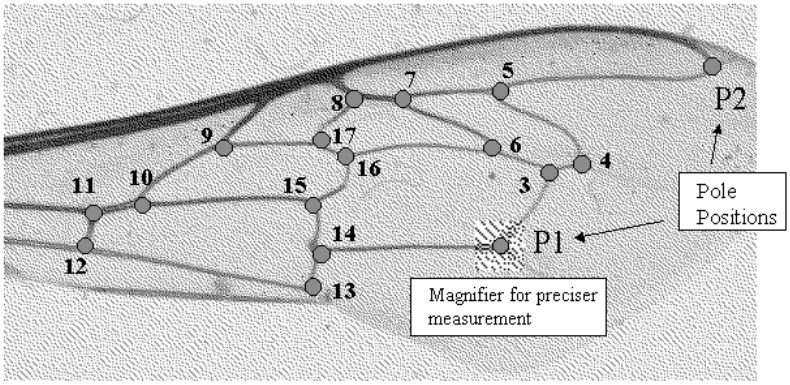

Fig. 7 Location of geographic coordinates on the forewing

Using only the wing characters, introduced by DuPraw (1964), gives a fairly good discrimination between mellifera, carnica, ligustica and caucasica, to take a few of the common Central European subspecies (Fig. 6). DuPraw took the position of 18 vein intersections on the forewing, drew a standard wing diagram and measured 13 angles and two size characters.

An attempt was made by Kauhausen--Keller and Keller (1994) to improve this discrimination by adding characters of the associated hind wing - with success. However, the hind wing is quite difficult to handle, and to mount on a slide.

A latest and simplest method of using wing characters was to discard the measuring and calculating of wing characters like DuPraw, and using pure geographic coordinates of the centres of the vein intersections on the forewing (Fig. 6). Also the scanning and digitizing techniques were improved to such a degree that easy handling and precise measurements were possible. The small hind wing can bee neglected. Sufficient discrimination can be achieved among the mentioned subspecies (Fig. 7). Using this method, the determination of racial purity is as simple to handle as when determining the cubital index.

CONCLUSIONS

Wing characters have proven to be the most reliable in measurement, and easiest to handle. During mounting, the wing does not change in size or structure, as it is with other body parts. Also, the geographic coordinates seem to exhibit a wide variability, correlated with different races. Hence, for the determination of race or racial purity (or degree of hybridization), this method proves to be a quick and reliable technique which can also be used by bee breeders.

Keywords: morphological characters, wing, discriminant analysis, honeybee races.

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METODY OCENY PRZYNALEŻNOŚCI RASOWEJ PSZCZÓŁ ZA POMOCĄ CECH MORFOLOGICZNYCH SKRZYDEŁ - PRZEGLĄD

Kauhausen D., Keller R.

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

Pomiar cech morfologicznych pszczół do oceny ich przynależności rasowej prowadzony jest od ponad 40 lat. Rasą dominującą w Niemczech jest pszczoła kraińska. Od pszczoły środkowoeuropejskiej łatwo ją odróżnić wartością indeksu kubitalnego (rys. 1), trudniej od innych ras (włoska, kaukaska). Do przeprowadzenia pełnej analizy morfologicznej wykonywano dotychczas pomiar 36 różnych cech, jest to jednak bardzo pracochłonne. Okazało się, że do dobrego odróżnienia wystarczy pomiar 18 cech na skrzydle pszczoły. Wykonanie tych pomiarów na ekranie komputera i poddanie ich analizie dyskryminacji przy użyciu specjalnego programu komputerowego pozwala na stosunkowo szybkie i łatwe odróżnienie ras i linii pszczół.

Słowa kluczowe: cechy morfologiczne, skrzydło, analiza dyskryminacji, rasy pszczół.

RENOVATION OF J. G. MENDEL'S APIARY

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Fig. 1 J. G. Mendel (July 20,1822 - Jan. 9, 1884)

As is well known, the discoverer of genetic laws - J. G. Mendel - was also an active scientist in apiculture. He tried to find out the best way of wintering productive colonies, conducted experiments with mating queens and selected drones in a cage, and compared productivity and characters of different honey bee races. Mendel even observed and wintered a colony of Trigona sting-less bees. He presented his results mainly on meetings of the committee of the Moravian Beekeeper's Union, of which he was a member. Mediated information about his results occurred in the beekeeping magazine Včela Brněnská (Die Honigbiene von Brünn).

Mendel (1822-1884) lived in the city of Brno in the southeastern part of what is now the Czech Republic. As an abbot of the Old Brno Augustinian Monastery he had a lot of duties but nevertheless he built a nice apiary within the area of the abbey. It was constructed according to the latest knowledge of Mendel's time and has two manipulation rooms and the space for placing 15 hives. Behind the house there is a cellar for indoor wintering of colonies. Three original hives and two copies made in early 1960s remain in the bee-house till now. Unfortunately, the originals are mostly rotten. The building itself, the front wall of the cellar as well as the fence surrounding the area needs fundamental renovation, urgently.

On the 180th anniversary of Mendel's birth an excellent exhibition called “The genius of genetics” was opened in the Old Brno Abbey on May 15, 2002, and an international workshop of top scientists in the field took place on this occasion.

Because Mendel was an active member of the beekeeper's union, the beekeepers of Brno have taken part in the renovation of the apiary, which naturally belongs to Mendel's heritage. The plan is for it to become an educational site showing the basic knowledge about the life of the honey bee, the kinds of hives from Mendel's time till now, as well as the various ways of beekeeping. As a beginning, colonies in modern magazine hives were placed in the area surrounding the bee house, which itself has to be reconstructed first.

The beekeepers of Brno will be grateful for any material support in their activities. For any (urgently needed) sponsorship contact please the author of the article.

Keywords: Mendel, apiary, apiculture.

ACKNOWLEDGEMENTS

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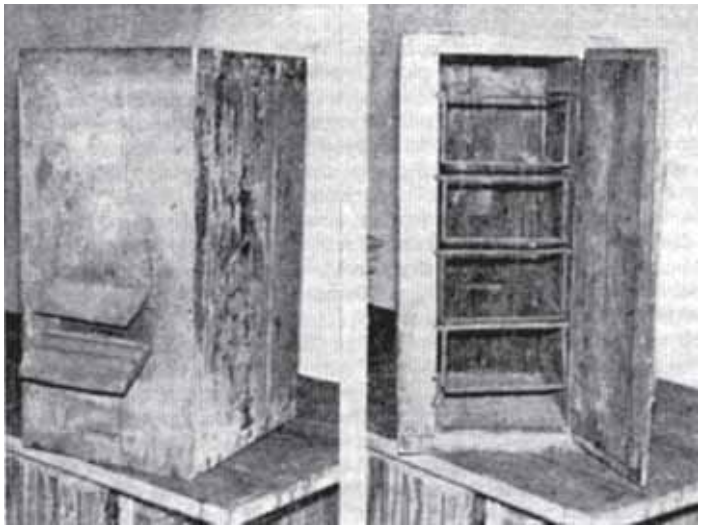

Fig. 2 Moravian hive from Mendel's time

Fig. 3 The bee room inside with the old hives (right), all is waiting for conservation and renovation

Fig. 4 The modern hives making the apiary living again

Fig. 5 Mendel's experimental garden. Right is situated the entrance to the exhibition

ODTWORZENIE PASIEK J. G. MENDLA

P t a c e k V.

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

Odkrywca praw genetycznych J. G. Mendel był także aktywnym badaczem pszczelarskim. Wyniki dwóch obserwacji przedstawiał głównie na spotkaniach Morawskiego Związku Pszczelarzy. Swoją pasiekę, w postaci pawilonu mieszczącego 15 uli posiadał w Brnie na terenie klasztoru Augustynów. Podczas obchodzonej w roku 2002, 180 rocznicy urodzin Mendla, pszczelarze z Brna podjęli decyzję o odtworzenie jego pasieki w kształcie w jakim istniała za jego czasów.

Słowa kluczowe: Mendel, pasieka, pszczelarstwo.

PROGRAMM OF THE FIGHT AGAINST RESISTANT *VARROA* MITES IN CZECH REPUBLIC

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Summary

Prevention and control of resistant populations of *Varroa* mites is based on the monitoring of the occurrence and on the measures in foci and their protection zones. Monitoring is carried out on the basis of field experiments, where natural fall and efficacy of acrinathrin, fluvalinate and amitraz are compared. Control measures are legalized. They include limitation of movement of bee colonies, rotation of preparations and increased care in winter time, especially using aerosol form of application. The objective of the measures is interruption of the generation sequence of resistant mites.

Keywords: resistance, *Varroa* mite, cross field experiments, pyrethroids, acrinathrin, fluvalinate, amitraz, aerosol administration, generation sequence.

The territory of Czech Republic is like other European countries threatened with resistant populations of *Varroa* mites (Milani 2001). But hitherto only individual foci of resistance to pyrethroids were recorded and this phenomenon was found in southern and southeastern border regions. Regardless of this fact we have introduced certain organization measures (Veselý 2002).

The Czech programm of prevention and control of resistant populations consists of two parts: First, monitoring of the occurrence and second, measures in occurrence foci and their protection zones. Monitoring is carried out on the basis of field cross experiments where the efficacy of acrinathrin and fluvalinate at late summer treatment (tab.1) and the efficacy of fluvalinate and amitraz at autumnal treatment (tab. 2) of bee colonies are compared. Besides that, there is compared the efficacy of carriers with long time effectiveness in the first days of exposition with the previous natural fall of mites. The monitoring action is completed by the declaration of the foci and 5 km protection zones.

Cross experiments are established on following principles: The experimental group is to include at least 8 bee colonies and hives are to be equipped by double nett pads to prevent the bringing out of mites by bees. This equipment enables daily monitoring of the fall of mites. Bee colonies of this experimental group are divided into odd and even colonies. Into odd colonies at late summer experiments we put long time acting strips with the content of acrinathrin (Gabon PA 92). At autumnal experiments odd bee colonies are treated by fumigation by amitraz (Varidol FUM). Into even bee colonies at late summer experiments we put long time acting strips with the content of fluvalinate (Gabon PF 90). At autumnal experiments we treat these colonies by fluvalinate fumigation (MP-10 FUM). At late summer experiments after 15 days we change the strips mutually between odd and even colonies. At autumnal experiments we carry out the second treatment by fumigation after 4 - 7 days. This treatment is reciprocal, that means odd bee colonies are treated by fluvalinate and even bee colonies by amitraz. After the finish of late summer

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Table 1

Scheme of late summer cross experiments (acrinathrin - fluvalinate)

Odd bee colonies										
Hive No.	Natural Fall date/number of females	Gabon PA 92 input Date	Fall of Mites			Gabon PA 90 input Date	Fall of Mites	Check fumigation		SUM of Fall of Mites (whole experiment)
			1st day	2 nd day	3rd-15th day		16th-30th day	Date	Fall of Mites	
Even bee colonies										
Hive No.	Natural Fall date/number of females	Gabon PA 90 input Date	Fall of Mites			Gabon PA 92 input Date	Fall of Mites	Check fumigation		SUM of Fall of Mites (whole experiment)
			1st day	2 nd day	3rd-15th day		16th-30th day	Date	Fall of Mites	

Table 2

Scheme of autumnal cross experiments (amitraz - fluvalinate)

Odd bee colonies									
Hive No.	1. fumigation Varidol FUM Date	Fall of Mites			2. fumigation MP-10 FUM Date	Fall of Mites			SUM of Fall of Mites (whole experiment)
		1. day	2. day	In total		1. day	2. day	In total	
Even bee colonies									
Hive No.	1. fumigation MP-10 FUM Date	Fall of Mites			2. fumigation Varidol FUM Date	Fall of Mites			SUM of Fall of Mites (whole experiment)
		1. day	2. day	In total		1. day	2. day	In total	

experiments, after 30 days, we treat bee colonies by amitraz fumigation and find the fall of mites after 12 hours. The efficacy is stated as percentage of fall of mites during 30 days after application of both types of carriers and of the total fall inclusive checks fumigation. Detailed estimtion evaluates the difference of the effect of acrinathrin and fluvalinate and the share of mites on adult bees, that is fall after the first two days of experiment and the share of mites on brood, that is fall after the third to thirties days of experiment. At autumnal experiments we determine the efficacy of percentage expression of mites fall after the first fumigation to the total fall after both fumigations.

The resistance of mites to pyrethroids is proved if in late summer experiments both types of carriers show no effect or lower effect below the value of 80% and when at autumnal experiments the fumigation by fluvalinate shows significantly lower efficacy than amitraz

The monitoring network is made by volunteers from beekeepers rank. The number of the monitoring sites is in individual years about two hundred and sites are distributed on the whole territory of the Czech republic. We do our best to influence the location of monitoring sites so that the network may cover regions with higher risk of the occurrence of resistance to pyrethroids. There are Czech - Austrian border regions and localities where beekeepers express suspicion that preparations based on pyrethroids show lower efficacy. All deliveries of medicaments for areal treatments are provided with leaflets as to pay the attention to possible resistance and with challenge for monitoring of the efficacy by comparison of the natural fall with the fall of mites of the first

two days with the exposition to medicaments.

Measures in resistance foci and in protection zones are as follows: ban on transport of bee colonies into not resistant regions, ban on use of pyrethroids in all application forms, substitution of pyrethroids by formic acid in season time, increased number of winter treatments by amitraz in the aerosol form, winter debris investigation and early spring treatment of sites where mites in winter debris were found and this in quantity more that one mite per bee colony. Such colonies are treated repeatedly by amitraz in the interval of 10 days and as soon as possible that means if daily temperature achieves without larger deviations 20°C, by evaporating sheets with formic acid. Methodical Instruction of the State Veterinary Administration legalized all these measures. The target is to reach an interruption of the generation sequence of resistant mites by full destruction or by the decisive reduction of their population.

All efforts connected with the implementation of the programm are justified. Combination of acrinathrin in late summer and amitraz in autumnal and winter treatment proved to be highly effective and hygienically flawless without negative side effects.

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PROGRAM WALKI Z ODPORNYMI POPULACJAMI ROZTOCZY *Varroa* W CZECHACH

Veselý V.

Streszczenie

Zapobieganie i zwalczanie odpornych populacji roztoczy *Varroa* oparte jest o monitoring występowania i o pomiary ognisk i ich stref ochronnych. Monitoring prowadzony jest w oparciu o doświadczenia terenowe, gdzie porównuje się osyp naturalny i skuteczność akrynatryny, fluvalinatu i amitrazu. Zabiegi zwalczania są zalegalizowane. Obejmują one przemieszczanie rodzin pszczelich, rotację (przemienne stosowanie) preparatów i zwiększoną opiekę w sezonie zimowym, w szczególności stosowanie preparatów w formie aerosolu. Celem zabiegów jest przerwanie następstwa pokoleń odpornych roztoczy.

Słowa kluczowe: odporność, roztocze *Varroa*, doświadczenia terenowe, pyretroidy, akrynatryna, fluvalinat, amitraz, forma aerozolowa aplikacji, następstwo pokoleń, Czechy.

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 30,000 characters.

The manuscripts should be sent to: Research Institute of Pomology and Floriculture, Apiculture Division, 24-100 Puławy, ul. Kazimierska 2, 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 2,000 characters. 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 2000 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 2000 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.