ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS
SBORNÍK MENDELOVY ZEMĚDĚLSKÉ A LESNICKÉ UNIVERZITY V BRNĚ

Ročník XLVI 7 Číslo 1, 1998

ACTIVATION OF LABORATORY-REARED BUMBLEBEE QUEENS (*HYMENOPTERA: APIDAE, BOMBUS SPP.*)

A. Přidal, J. Hofbauer

Received: September 23, 1997

Abstract

PŘIDAL, A., HOFBAUER, J.: *Activation of laboratory-reared bumblebee queens (Hymenoptera: Apidae, Bombus spp.) (in English).* Acta univ. agric. et silvic. Mendel. Brun. (Brno), 1998, XLVI, No. 1, pp.: 79–84

In the Forage Crop Research Institute, the activation of queens of laboratory-reared bumblebees (*B. terrestris* and *B. lucorum*) was tested using male cocoons, photoperiod and social contact between queens.

Neither the food enriched with vitamin D, dried egg yolk or honey nor photoperiod affected oviposition of the queens. The queens established their nests only rarely, but after the insert (after 2 months) of male cocoons they started their oviposition. On average 20 % of the queens did not lay an egg even after repeated insert of the cocoon, the queens ignored the cocoon only sporadically. The placing of queens in pairs was not a sufficient stimulus to their activation. Nonetheless, an accidental experiment in combination with these two stimuli encouraged the immediate location of the queens on the cocoons provided. The number of accepted cocoons was higher in queens with longer "precocoons" period than in queens with shorter this period. With one exception the colonies did not raise a higher number of young queens. In a total of 68 reared colonies it was never observed that only males appeared in the first brood. The colonies of *B. lucorum* come soon into switch point, develop only weak colonies and are consequently not suitable for laboratory rearing and for pollination purposes. Without social stimuli, the queens of *B. pascuorum* and *B. hypnorum* established nests in the laboratory within one week, but the brood never completed its development. They did not accept the cocoons of *B. terrestris* and *B. lucorum*. The method of cocoon activation cocoons is also associated with larval blackening in the first brood. Other potential physiological aspects of queen activation are discussed.

Bombus spp., laboratory rearing, activation of queens, egg-laying

In connection with the pollination of greenhouse tomatoes the development of laboratory rearing of bumblebees was studied. The main aim of studies are the possibility of closed rearing cycles and predominantly the activation of queens. Year-round rearing was first studied by RÖSELER (1985). Utilizing anaesthesia with CO_2 inhibits diapause and induces the development of ovaries. The CO_2 treatment also terminates diapause at both newly described stages (LARRERE et al.), 1993 — developed diapause and postdiapause inactivity). Nevertheless, the queens require another "stimulus" to start establishing the first brood (GRETENKORD et al. 1996). The authors found that the addition of bumblebee workers and larvae was most effective. Simultaneously, they introduced two stimuli — narcotic treatment and a stimulus provoking the establishment of the first brood. The addition of bumblebee workers is also a social stimulus to colony establishment.

PTÁČEK (1989) compared three different methods: queen activation without narcotic treatment, the use of blooming dead nettle plants and the use of honeybee workers in our conditions. Another factor studied is photoperiod (TASEI et

al., 1994). The queens untreated with a narcotic were subjected to different photoperiod regimes. Queen activation and subsequent production of young queens was best under a photoperiod regime of 8 h light and 16 h dark. The experiments showed that higher temperature (TASEI, 1994) did not have a significant effect. However, in honeybees (*Apis melifera* L.) higher temperature affected pH of titre in foragers (HUANG et al., 1995). Another potential social stimulus is the addition of male cocoons (DUCHATEAU et al., 1994).

The aim of the investigation of queen activation, especially *Bombus terrestris* and *Bombus lucorum*, using male cocoons and special application of CO_2. The experimental conditions also involved photoperiod and activation using two queens.

MATERIAL AND METHODS

The queens of *B. terrestris* in a winter experiment originate from the summer rearing of the year 1996. The process of rearing is described in the study of PŘIDAL et al., 1996. Spring experiments were conducted with the queens caught in field shortly after leaving winter hideouts. The spring experiments involved queens of *B. terrestris* and *B. lucorum*.

All experimental groups were reared in plastic boxes 8.5 x 12.5 x 12.5 cm regularly lined with clean paper. The temperature was 28°C and humidity was ca. 60 %. Sucrose solution was provided in vertical feeders and pollen in Petri dishes 2 cm in diameter. The pollen collected on pollen traps by honeybees was not dried but frozen immediately after removal from the beehive entrance to at least — 20°C, as proposed by RIBEIRO et al., 1996. The sucrose solutions were prepared in the following weight proportions:

1. 1:2:1 (distilled water: sugarbeet sugar: honey. Fumagilin 2 g/l, 600 mg vitamin E
2. 1:1 Fumagilin 2 g/l, 200 mg vitamin E, 0.6 g sorbic acid (the solution with Fumagilin was administered with an interval of 14 days (TASEI et al., 1994). The queens were fed on pollen every other day with a two-day interval once a week.

In winter rearing there were two narcotic treatments for 10 min. at 3–4°C 24 hours apart (LARRERE et al., 1993). In the active narcotic treatment of 5 queens in group N was made in such a manner that the queens were put into an airtight vessel for 21 days at 4°C and subsequently kept in pairs. The effect of photoperiod (13 h light and 11 h dark) was also tested. A list of experimental groups is given in Table I. The queens which did not establish the first brood until 19 December 1997 were given a male cocoon stuck with beewax to a small square of paper and fed pollen from *Papaver somniferum* L. In group 5q one cocoon was added and was left there until one of the queens established a brood on it. Then it was removed with the establishing queen. The entire process was repeated.

The spring rearing of queens was performed without narcotic treatment. After approximately 10 days the queens were provided with a male cocoon. A list of experimental groups is shown in Table II. Group SP was composed of *B. pascuorum, B. hortorum, B. lapidarius* and *B.hypnorum*.

RESULTS

The queens without male cocoons established nests only sporadically. Neither food enriched with vitamin D, dried yolk and honey nor photoperiod triggered oviposition in the queens. Only one of the queens, which established brood after 58 days, developed into a very strong colony (group 2q). However, this colony raised only one young queen. In group N only one queen formed a nest without a male cocoon (after 21 days). The colony developed into a medium-sized one. The same also applies to the other 4 queens.

The queens started to establish their first brood after the insert of male cocoons (Table II and III). On average 20 % of them accepted the cocoon but did not lay eggs (only warmed up the cocoons). Only rarely did the cocoon remain unaccepted. The value followed by a question mark in Table IIIa gives the number of queens which did not clearly demonstrate their behaviour. In group S (activated as the last) all the queens warmed up the cocoon for the first three days and only then started to abandon it. The queens in group S (Table IIIc) and also the queens, which established brood and later "cancelled" it, accepted the cocoons provided for the second and the third time more readily than the first cocoon. In group 5q all five queens gradually established nests on the cocoons provided.

I: *List and characteristics of winter (a) and spring (b) experimental groups*

groups	narcosis	starting date	photoperiodic regime	food	queens n
a) winter experimental groups					
R	yes	17.X.	13D/11L do 23.X.	sucrose solution, rape pollen	7
KTR	without	17.X.	13D/11L do 23.X.	sucrose solution, rape pollen	8
CH	yes	22.X.	13D/11L do 23.X.	sucrose solution, rape pollen with 15%yellow powder	8
T	yes	12.XI.	24h dark	sucrose solution, rape pollen	9
D	yes	12.XI.	24h dark	sucrose solution with vitamin D, rape pollen	10
2q	yes	12.XI.	24h dark	sucrose solution, rape pollen	10 pairs
N	active	2.XII.	24h dark	sucrose solution, rape pollen	5 pairs
2q-r	2x after 2h interval	26.XI.	24h dark	sucrose solution, rape pollen	11 pairs
5q	yes	17.XII.	24h dark	sucrose solution, poppy pollen	5
S	yes at 22C	17.XII.	24h dark	sucrose solution, poppy pollen	10
b) spring experimental groups					
HM	without	28.IV.	24h dark	sugar solution with honey, poppy pollen	9
HC	without	28.IV.	24h dark	sucrose solution, poppy pollen	13
Menv	without	3.IV.	24h dark	sugar solution with honey, poppy pollen	7
SP	without	3.IV., 28.IV.	24h dark	sugar solution with honey, poppy pollen	6
env	without	10.III.-3.IV.	24h dark	sucrose solution, poppy pollen	7

II: *Egg laying of queens after insert of male cocoons (winter experiments)*

groups	number of				
	queens	egg-laying queens (%)	cocoons only heated by queen	cocoons emerged untimely	cocoons non-accepted
a) date of cocoons insert 19.XII.1996 date of experiment evaluation: 6.1.1997					
KTR	4	2 (50)	1	1	–
R	1	1 (100)	–	–	–
D	1	–	1	–	–
T	3	3 (100)	–	–	–
N	2	1 (50)	1	–	–
CH	1	–	1	–	–
2q + 2q-r	3	1 (33)	2	–	–
S	10	1 (10)	7?	7?	2
b) date of cocoons insert 10.I.1997 date of experiment evaluation: 20.1.1997					
KTR	5	4 (80)	–	–	1
R	2	2 (100)	–	–	–
D	3	2 (66)	–	1	–
T	4	2 (50)	–	–	2
N	2	1 (50)	–	1	–
CH	5	3 (60)	1	1	–
2q + 2q-r	14	8 (86)	2	–	–
S	10	5 (50)	4	1	–
c) date of cocoons insert 12.III.1997 date of experiment evaluation: 26.3.1997					
S	8	7 (88)	1	–	–

In group SP (spring experiments) the species *(B.pascuorum and B. hypnorum)* refused to accept the cocoons of *B. terrestris* and *B. lucorum*. Was confirmed that *B. pascuorum* and *B. hypnorum* established nests in the laboratory within 1 week of capture without any social stimulus, but their brood only developed into the second instar. The queens of *B. hortorum* and *B. lapidarius* accepted honeybee (*Apis mellifera*) workers but did not make nests. The results concerning the activation of queens of *B. terrestris* and *B. lucorum* are summarized in Table II and III. The reason why the number of cocoons until the first emergence of the first imago is reported is that the first brood usually dies of the so-called larval blackening. The queens of *B. terrestris* formed 4 times as large colonies as *B. lucorum*, but in all cases they came into switch point very soon and did not raise young queens (the only exception was one colony with 3 young

III: *Egg laying of queens after insert of male cocoons (spring experiments)*

groups	number of					
	queens	egg-laying queens (%)	cocoons only heated by queen	cocoons emerged of 1st eggs*	applied cocoons till 1st brood	applied cocoons till 1st imago
Menv	7	6 (86)	1	17,33 ± 13,65	2,00	3,00
HC	13	10 (77)	3	15,08 ± 6,14	1,30	2,50
HM	9	8 (89)	1	20,25 ± 16,54	2,00	2,00
env	7	5 (71)	2	18,20 ± 17,92	1,60	2,20

queens). Only 4 colonies of *B. terrestris* out of 42 colonies of winter rearing and one colony out of 26 colonies of spring rearing managed to raise queens. In winter rearing 5 young queens and in spring rearing 73 young queens were obtained from one colony. In all 68 reared colonies it happened only once that the first brood was composed of males and workers.

In two out of five cases the queen died and a new fertilized queen was added to the colony. In both cases the queens exhibited pheromone dominance and the switch point by laying diploid eggs was prevented.

DISCUSSION

The addition of male cocoons confirmed that the queens needed the so-called social stimulus to laying their first eggs (GRETENKORD et al., 1996). Without this stimulus the experimental groups from winter rearing established nests only rarely. The rearing of queens in pairs (2q and 2q-r) cannot be considered a stimulus similar to that of male cocoons. However, in queen pairs the activation occurred without cocoons. A combination of the above-mentioned stimuli seems satisfactory but it is necessary to conduct the experiment again under strict scientific conditions.

The non-accepting of the cocoon by the queen, which happened predominantly in spring rearing, was most probably due to the unsatisfactory interruption of diapause. The queens (6) were subsequently subjected to narcotic treatment for 10 minutes at 22°C but only 3 established a colony. For this reason the result cannot be considered significant. In winter rearing this phenomenon appeared more frequently in group S, which was activated as the last. Our assumption is that the queens need more time after diapause termination to establish a colony. This fact may be related to the nutrition of the queens (WHEELER, 1996). In spring rearing the queens established a nest as early as two days after their capture in nature.

Furthermore, this fact is also suggested by a high success rate of accepting cocoons subsequently provided.

A great problem of this and other methods of nest establishing is the so-called larval blackening (PŘIDAL et al., 1997). After disinfection when a new cocoon was provided the queens established a nest again. However, a recurrence occurred. Very rapid establishment of the nest by the queens of *B. hypnorum* and *B. pascuorum* is satisfactory. It is not clear, however, how to ensure the complete development of the brood. We assume that the the so-called larval blackening is not involved.

We have not found that the addition of male cocoons promoted the occurrence of males in the first brood or premature switch point (DUCHATEAU *et al.*, 1994). However, only a few colonies raised young queens. Queen with cocoons-inserted must be in quite well condition for success of colony establish (DUCHATEAU, 1991).

SUMMARY

Neither the food enriched with vitamin D, dried egg yolk or honey nor photoperiod affected the oviposition of the queens. The queens established their nests only rarely, but after the insert after 2 months of the male cocoons they started their oviposition. On average 20 % of the queens did not lay an egg even after the repeated insert of the cocoon, the queens ignored the cocoon only sporadically. The placing of queens in pairs was not a sufficient stimulus to their activation. The number of accepted cocoons was higher in early-reared groups than in late-reared groups. With one exception the colonies did not raise a higher number of young queens. In a total of 68 reared colonies it never happened that only males appeared in the first brood. The colonies of *B. lucorum* come soon into switch point, develop into only weak colonies and are not therefore suitable for laboratory rearing and for pollination purposes. Without social stimuli, the queens of *B. pascuorum* and *B. hypnorum* established nests in the laboratory within one week, but the brood never completed its development. They did

not accept the cocoons of *B. terrestris* and *B. lucorum*. The method of activation using cocoons is also associated with larval blackening in the first brood.

ACKNOWLEDGMENTS

We wish to express our special thanks to Ing. Pavlína GOTTWALDOVÁ and Alice PROCHÁZKOVÁ for their technical assistance and participation in the continuous rearing of bumblebees. This study is part of the project supported by the National Agency for Agricultural Research at the Ministry of Agriculture of the Czech Republic. Project No. EP 0960986289.

SOUHRN

Aktivace matek čmeláků v laboratorních podmínkách (*Hymenoptera: Apidae, Bombus spp.*)

Obohacení potravy vitamínem D, sušeným žloutkem či medem, ale ani světelný režim neovlivnil ovipozici matek. Matky zakládaly hnízda pouze ojediněle, avšak po pozdějším poskytnutí samčích kokonů (2 měsíce po začátku aktivace) začaly tytéž matky ihned s ovipozicí. V průměru 20 % matek nepoložilo vajíčko ani po opakovaném podání kokonu, ale jen ojediněle matky o kokon nejeví zájem. Umístění matek ve dvojicích nebylo dostatečným stimulem pro jejich aktivaci. Nicméně, náhodný pokus s kombinací těchto dvou stimulů vyvolal okamžité kladení matek na poskytnuté kokony. Počet přijatých kokonů byl vyšší ve skupinách s dřívějším datem startu chovu než ve skupinách, kde datum startu bylo pozdější. Až na jednu vyjímku kolonie neodchovaly větší množství mladých matek. Nikdy se však v 68 celkem odchovaných koloniích neobjevili v prvním plodu pouze samci. Kolonie B.lucorum brzo přichází do zvratu, dospívají jen do slabých kolonií a zdá se tedy, že nejsou vhodné pro laboratorní chovy a k účelům opylování. Matky *B. pascuorum* a *B.hypnorum* založily hnízda v laboratoři do jednoho týdne bez sociálních stimulů, ale plod nikdy nedokončil vývoj a kokony druhu *B. terrestris* a *B. lucorum* nepřijmuly. Při této metodě aktivace pomocí kokonů se rovněž vyskytuje tzv. černání larev prvního plodu.

Bombus spp., laboratorní chov, aktivace matek, kladení vajíček

REFERENCES

Duchateau, M.J. (1991): Regulation of colony development in bumblebees. Proc.6th Int.Symp.Poll., Acta Horticulturae, 288:139–143.

Duchateau, M.J., Hoshiba, H., Velthuis, H.H.W. (1994): Diploid males in the bumble bee Bombus terrestris — sex determination, sex alleles and viability. Ent.Exp.Appl. 71: 263–269.

Gretenkord, C., Drescher, W. (1996): Successful colony foundation and development of experimentally hibernated Bombus terrestris queens depending on different starting methods. Proc.VII.Int. Pollination Symp. June 1996 — Alberta Canada.

Huang, Z.Y., Robinson, G.E. (1995): Seasonal changes in juvenile hormone titers and rates of biosynthesis in honey bees. J.Comp.Physiol B 165: 18–28.

Larrere, M., Lavenseau, L., Tasei, J.N., Couillaud, F. (1993): Juvenile hormone biosynthesis and diapause termination in Bombus terrestris. Invertebrate Reproduction Development 23: 7–14.

Ptáček, V. (1985): Testing three methods of bumble bee rearing (Hymenoptera, Bombidae). [In Czech]. Sborník věd. prací v Troubsku 9: 59–67.

Přidal, A., Hofbauer, J. (1996): Laboratory rearing and nutrition of young queens of bumblebee (Bombus terrestris L.) from emergence to diapause. Scientific Studies in Troubsko 14: 125–131.

Přidal, A., Sedláček, I., Marvanová, L. (1997): Microbiology of bumble bee larvae (Bombus

terrestris L.) from laboratory rearing. Acta Univ. Agric. et Silvic. Mendel. Brun (Brno) 45 (314): 51–58

Ribeiro, M.F., Duchateau, M.J., Velthuis, H.H.W. (1996): Comparison of the effects of two kinds of commercially available pollen on colony development and queen production in the bumble bee Bombus terrestris L. (Hymenoptera, Apidae). Apidologie 27: 133–144.

Röseler, P.F. (1985): A technique for year-round rearing of Bombus terrestris Apidae, Bombini) colonies in captivity. Apidologie 16: 165–170.

Tasei, J.N., Aupinel, P. (1994): Effect of photoperiodic regimes on the oviposition of artificially overwintered Bombus terrestris L. queens and the production of sexuals. J. Apic. Res. 33: 27–33.

Tasei, J.N. (1994): Effect of different narcosis procedures on initiating oviposition of prediapauzing Bombus terrestris queens. Ent.Exp.Appl. 72: 273–279.

Wheeler, D. (1996): The role of nourishment in oogenesis. Annu.Rev.Entomol. 41:407–431.

Adresa

Ing. Antonín Přidal, Ústav zoologie a včelařství, Mendelova zemědělská a lesnická univerzita, Zemědělská 1, 613 00 Brno,
RNDr. Jan Hofbauer, CSc., Výzkumný ústav pícninářský, spol. s r.o., Zahradní 1a, 644 41 Troubsko u Brna, Česká republika