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Original article

Stimulation of colony initiation and colony development in *Bombus terrestris* by adding a male pupa: the influence of age and orientation

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Abstract – Hibernated queens of *Bombus terrestris* must be stimulated to start egg laying while kept in confinement. In this study, the stimulating effect of a male pupa and the impact of the age and the fixation angle of the pupa was examined. Queens started egg laying very soon if they were provided with a young male pupa. It took queens longer to oviposit with increasing age of the supplied pupae. Moreover, queens that were provided with older pupae produced fewer eggcups and fewer workers in the first brood. Queens with a horizontally fixed pupa did better than with a vertical or oblique oriented pupa. Of the queens that were provided with a young pupa, 80% produced colonies. In contrast, only 30% of the queens that were provided with an old pupa produced a colony. In addition, the latter colonies performed worse during the entire period of colony development. In conclusion, providing one horizontally fixed young male pupa to a hibernated, single queen resulted in the production of good colonies.

***Bombus terrestris* / oviposition stimulation / male pupae / colony foundation**

1. INTRODUCTION

Bumblebees are used extensively in greenhouses for pollination of different crops. Several techniques have been described to rear colonies in captivity (Sladen, 1912; Holm, 1960; Holm, 1964; Pouvreau, 1976; Van Honk and Hogewek, 1981; Röseler, 1985; Ptacek, 1985; Van Doorn and Chrambach, 1989; Duchateau and Velthuis, 1988). In all cases, the queens had to be stimulated to start egg-laying. Sladen (1912) started colonies by putting two queens together, by putting one queen and one to a few workers together, or by putting both workers and some brood with the queen to stimulate her to initiate egg laying. Zapletal (1965) supplied the queen with a piece of honey bee comb containing several drops of honey and some pollen. Another method used to stimulate a queen to lay eggs was to provide her with young honey bee workers (Ptacek, 1985; van den Eijnde et al., 1991). Plowright and Jay (1966) reported that for *Bombus ternarius*, the presence of a piece of cardboard coated with wax was enough to stimulate a queen to initiate egg laying. In nature, after hibernation, bumblebee queens initiate egg laying in wax cups, which they build on pollen they collect. Therefore, Plowright and Jay (1966) provided pollen clumps on which the queens started to make eggcups. Frison (1927) made an attempt to

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induce broodiness in 10 queens of *B. ternarius* by confining them together in a cage with plenty of pollen and honey. Free and Butler (1959), Duchateau (1991) and van den Eijnde et al. (1991) confirmed the observations made by Sladen (1912) that oviposition occurred more often when several queens, or one to two queens and one to four workers, were confined together, compared to when a solitary queen was left alone in a cage. Gretenkord and Drescher (1997) compared all these methods using *B. terrestris* and found that all were less effective for successful colony foundation and that the combination of workers and larvae was the most successful. However, for commercial rearing the latter method is less attractive because it is more laborious. Another successful method for stimulating a *B. terrestris* queen is the use of a cocoon containing a male pupa, fixed on cardboard (Duchateau, 1995, 2000; Duchateau et al., 1994; Yeninar et al., 2000). However, Gretenkord (1997) considered this method ineffective. For mass rearing, methods need to be both effective and economical. Supplementing a queen with one male pupa seems a promising method, because it was used successfully in several studies, it is less labor intensive and, in a mass rearing system, male pupae are always available. Because the age of the pupae and the orientation of these cocoons appeared to influence stimulation of the queen to start egg laying and colony development, we studied these aspects.

2. MATERIALS AND METHODS

2.1. Age of the male pupae

After the queens of *Bombus terrestris* L. had hibernated for three months in peat at 4 °C and 80% RH, they were activated in a flight cage containing fresh pollen and sugar water, and illuminated with a Mercury bulb (100 W). After one week in the flight cage, each queen was transferred into a small box (16 × 11 × 7 cm) with fresh pollen and sugar water (1:1). These boxes were placed in a climate room, maintained at 28 ± 2 °C and 60 ± 10% humidity, and illuminated with red light of 10 lux enabling observation (bumble bees are color blind in the red spectrum). After two days, the queens were given a cocoon containing a male pupa, collected from already available colonies. The pupae were divided in 5 age groups (1–2, 3–4, 5–6, 7–8. and 9–11 day old), determined accurately by the moment the males emerged. The duration of the prepupal stage of males, from the time the cocoon is closed and oriented in a vertical position, until the emergence of an adult male, is 10.8 days (n = 32, range 9–12 days). Each pupa was fixed with paraffin on stiff drawing paper, in a near horizontal orientation, so that the pupa could not roll and the queen could sit on it for egg laying. In each age group, the pupae were changed every week for up to eight weeks until the queens started to lay eggs. This means that in the age groups of 5–6, 7–8 and 9–11 days, the queen had an empty cocoon for 1–6 days, because the males had already hatched before the replacement of the cocoons that occurred once a week. The age of the replaced cocoon was determined by color. Cocoons of 1–2 day old were whitish and soft, while the cocoons of 3–4 days were more yellow and slightly hard. The older cocoons became more and more grayish with aging. Twenty queens were used per age group. Fresh pollen was provided daily and sugar water was changed once a week. Clean conditions were maintained inside the boxes. Queens were shifted into big colony boxes (27 × 18 × 13 cm) after emergence of all workers of the first brood. Data were recorded for the date of egg laying after the first and last pupa were supplied, the number of eggcups in the first brood, the date of the emergence of the first worker, the number of workers in the first brood, the date of initiation of the second brood, the competition point (the onset of worker oviposition and egg robbery and the presence of more than one open eggcup), the total number of workers, the total number of queens, and the total number of males produced by each colony. Data were recorded for a month after the competition point.

2.2. Orientation of the fixation of the male pupae

Male pupae of different ages were fixed with paraffin on cardboard at different angles: vertically (↑) (the normal orientation in the colony), obliquely (↖) and horizontally (→). The same procedure was followed for the experiments on the effect of age of the male pupae. Data were collected on the date of egg laying after the first and last pupae were supplied, the number of eggcups in the first brood, and the number of workers in the first brood.

A one-way analysis of variance (ANOVA) was used to analyze the data from the first experiment. When the variances were not homogeneous, log transformed data were used. We expected that there

Table I. Percentage of queens that produced a colony successfully and that consumed their own eggs from the first egg cups, and the percentage of colonies that produced young queens relative to the age group of the provided male pupa (n = number of queens or colonies; X_2 and *P*-value in the last row).

	Age of male pupae (days)					
	1–2	3–4	5–6	7–8	9–11	X_2
Successful colony production (%)	80 (n = 20)	70 (n = 20)	65 (n = 20)	65 (n = 20)	30 (n = 20)	11.5174 $P = 0.0213$
Queen eaten 1st brood eggs (%)	0 (n = 20)	15 (n = 20)	30 (n = 20)	50 (n = 20)	45 (n = 20)	17.1627 $P = 0.0018$
Colonies with new queens (%)/	92.9 (n = 14)	88.9 (n = 9)	83.1 (n = 12)	81.8 (n = 11)	60.0 (n = 6)	3.2105 $P = 0.5232$

would be a linear relation between the age of the male pupa and the date of first egg laying, so a Linear Contrast was used to analyze the data further in the case the null hypothesis was not accepted, while a linear relation was expected between the age of the male pupa and the colony characteristics. Tukey post hoc test was used to determine which groups differed. For the parameter of colony initiation, the non-parametric Jonckheere's trend test was used because the data did not meet the assumption of homogeneity of variance, even after log transformation, and because the alternative hypothesis is that the data of the colony initiation are ordered per age group. The data of the second experiment were analyzed by analysis of covariance (ANCOVA) with age as covariant.

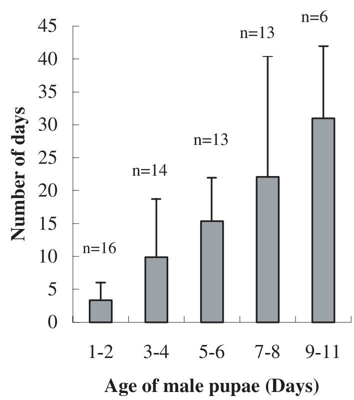

Figure 1. Number of days from the providing of 1st male pupa until the formation of first eggcup of the queen, relative to the age of the male pupae (n = number of colonies observed).

3. RESULTS

3.1. The age of the male pupae

3.1.1. Success of colony production

Table I shows that 80% of the queens that were provided a young male pupa produced a colony. In contrast, only 30% of the queens that were provided with an old male pupa (9–11 days) produced a colony. In this latter group of queens, on average 45% consumed their own eggs. The queens that were provided young male pupae did not show this behavior. Egg eating occurred in 15% of the queens provided with 3–4 day old pupae. Of the queens that produced colonies when provided 1–2 day old male pupae, 92.9% of those colonies produced daughter queens, but only an average of 60% of the colonies produced daughter queens when provided with a 9–11 day male pupa. However, there was no significant difference between the percent queens produced between groups ($X_2 = 2.9914$, df = 1, $P = 0.087$).

3.1.2. Time to colony initiation

Figure 1 shows that the age of a male pupa, provided for stimulation of egg-laying by a hibernated queen, had a significant effect on colony initiation by the queen (Jonckheere's Trend test, SD J.T. = 5.754, n = 59, $P < 0.001$). Queens began building eggcups on a young male pupa and laid eggs in the cups within a few days after the pupa was introduced (on average 3.4 ± 2.71 days (n = 16) on pupae 1–2 day old; whereas it took the queens an average 31.0 ± 10.9 days (n = 6) to start egg laying on pupae of 9–11 day old. It took the queens an average of 9.9 ± 8.9 days (n = 14)

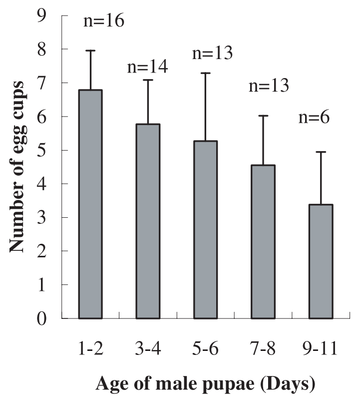

Figure 2. Number of eggcups of the first brood, relative to the age of male pupae (n = number of colonies observed).

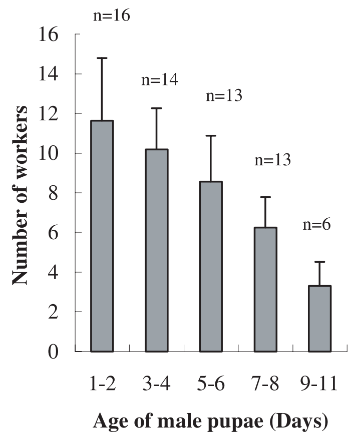

Figure 3. Number of workers of first brood relative to the age of male pupae (n = number of colonies observed).

to start egg laying on 3–4 day old male pupae, which was significantly longer than the 1–2 day group (Mann-Whiney U test, U = 49, $P = 0.013$, n = 16,14), although in both groups the cocoons always contained pupae. This indicates that indeed the age of the pupa is of importance and not only the presence of a empty cocoon for several days, as occurred in the 9–11 age group were the male hatched before the weekly replacement of the cocoons. This is confirmed by the fact that with the age of the male pupae the time till the first eggcups increases significantly as indicated by the significance of the Jonckheere's Trend test.

3.1.3. Number of eggcups and workers of the first brood

Figure 2 shows that with increasing age of the male pupa, the number of eggcups in the first brood decreased significantly (ANOVA, F = 8.947, df = 4, $P < 0.001$; Linear Contrast, F = 30.508, df = 1, $P < 0.001$). On average, 6.8 ± 1.2 eggcups of the first brood were produced on a young pupa of 1–2 day old and only 3.4 ± 1.6 eggcups were produced on a 9–11 day old pupa. Figure 3 shows that the number of workers in the first brood decreased significantly with increasing age of the male pupae (ANOVA, F = 25.184, df = 4, $P < 0.001$; Linear Contrast, F = 98.877, df = 1, $P < 0.001$). On average, 11.6 ± 3.2 workers emerged in the first brood when eggcups were constructed on a young pupa, while only 3.3 ± 1.2 workers emerged when eggcups were on an old pupa. Both numbers of eggcups and workers differed significantly between the colonies provided 1–2 day male pupae and the colonies provided 3–4 day male pupae (*t*-test, $t = 2.538$, $P = 0.018$, n = 16,14 and $t = 2.065$, $P = 0.005$, n = 14, 9, respectively).

Although the number of workers produced was affected by the number of eggcups, the number of workers that emerged per eggcup in the first brood was also significantly different and linearly related among the different age groups: 2.0 ± 0.6 workers per eggcup emerged from eggcups with pupae of 1–2 day old; 1.5 ± 0.5, 1.4 ± 0.7, 1.5 ± 0.9 and 1.1 ± 0.4 workers per eggcup from male pupae of 3–4 days, 5–6 days, 7–8 days and 9–11 days respectively (ANOVA, F = 2.660, df = 4, $P = 0.043$; Linear Contrast, F = 8.266, df = 1, $P = 0.006$).

3.1.4. Total colony productivity: workers, queens and males

The developmental time of the first workers was different for the five age groups (ANOVA, F = 3.013, df = 4, $P = 0.025$), although there was no linear relation among the age groups (Linear Contrast, F = 2.604, df = 1, $P = 0.112$). Table II shows that the workers from eggcups constructed on young pupae had the shortest development time on

Table II. Colony characteristics relative to the age of male pupa (n = number of colonies observed).

Colony characteristics	Age of male pupae (days)				
	1–2	3–4	5–6	7–8	9–11
Developmental time first workers (days)	20.5 ± 1.7 (n = 16)	21.4 ± 1.5 (n = 14)	22.6 ± 2.8 (n = 13)	20.8 ± 2.1 (n = 13)	22.8 ± 2.1 (n = 6)
Start 2nd brood (days after 1st egg)	13.1 ± 2.1 (n = 16)	13.1 ± 1.6 (n = 14)	15.3 ± 3.7 (n = 13)	15.7 ± 3.5 (n = 13)	15.5 ± 1.5 (n = 6)
Competition point (days after 1st worker)	21.5 ± 3.9 (n = 13)	22.8 ± 4.6 (n = 10)	28.3 ± 8.0 (n = 10)	33.8 ± 9.9 (n = 8)	32.3 ± 11.1 (n = 3)
Total number of workers	145.4 ± 48.4 (n = 14)	135.3 ± 42.1 (n = 11)	108.4 ± 37.8 (n = 13)	151.6 ± 42.7 (n = 9)	81.2 ± 49.7 (n = 5)
Total number of new queens	89.5 ± 59.6 (n = 14)	26.4 ± 15.1 (n = 9)	32.4 ± 21.9 (n = 12)	28.0 ± 41.6 (n = 11)	24.2 ± 37.0 (n = 5)
Total number of males	155.5 ± 62.5 (n = 13)	164.1 ± 61.5 (n = 11)	151.6 ± 62.5 (n = 12)	152.7 ± 51.6 (n = 9)	184 ± 56.3 (n = 5)

average (20.5 ± 1.8 days). The initiation of the second brood was not different among the five age groups (ANOVA, F = 2.323, df = 4, $P = 0.067$). The average number of workers produced per colony was significantly different among the five age groups (ANOVA, F = 2.725, df = 4, $P = 0.04$). However, there was no linear relationship with the age of the male pupae (Linear Contrast, F = 2.668, df = 1, $P = 0.109$). The colonies in which the queens had initiated egg-laying on older pupae produced the lowest number of workers. There was no significant difference in the numbers of males produced in the five age groups (ANOVA, F = 0.578, df = 4, $P = 0.68$). There was a large difference in the average numbers of young queens that were produced per age group. The colonies that were initiated on a 1–2 day old pupa produced 89.5 ± 59.6 queens, significantly more than from the number of queens produced in colonies on the other age groups (ANOVA, F = 2.859, df = 4, $P = 0.034$). There was no significant difference in the number of queens produced in colonies that were given 3–4 day and 9–11 day old pupae, (Tukey post hoc test), but the 1–2 day age group differed significantly from the 3–4 day group (Mann-Whitney U Test, U = 20, $P = 0.005$, n = 14 and 9). The timing of the competition point (calculated from the emergence of the first worker) was much later in the colonies provided the older male pupae than in the colonies provided with younger pupae (ANOVA, F = 5.052, df = 4, $P < 0.001$; Linear Contrast, F = 18.402, df = 1, $P < 0.001$).

Table III. Effect of fixation angles of the male cocoon on the number of eggcups in the first brood, relative to the age of male pupae (n = number of colonies observed).

Age of male pupae (days)	Number of first brood egg cups		
	Orientation of pupae		
	Vertical (↑)	Oblique (↖)	Horizontal (→)
9–11	1.56 ± 0.71 n = 3	2.5 ± 1.53 n = 3	4.25 ± 0 n = 3
7–8	4.33 ± 1.37 n = 6	4.42 ± 1.68 n = 12	4.25 ± 0.5 n = 4
5–6	4.66 ± 2.07 n = 6	5.42 ± 2.82 n = 7	5.5 ± 1.31 n = 8
3–4	5.33 ± 1.41 n = 5	6 ± 2.12 n = 3	5.72 ± 1.17 n = 10
1–2	6.25 ± 0 n = 2	6.14 ± 1.35 n = 7	6.85 ± 2.06 n = 4

3.2. Orientation of male pupae

In each age group, the horizontal fixation of the male pupal cocoon on the cardboard gave the best results (Tab. III). The age of the male pupa had no influence on the results of the fixation angle (no interaction orientation*age, ANCOVA, F = 0.010, df = 2, $P = 0.990$ for the number of eggcups of the first brood and, F = 0.114, df = 2, $P = 0.893$ for the number of workers in the first brood. The fixation angle had no significant effect on the number of eggcups of the first brood (ANCOVA, age as covariant, F = 1.494, df = 2, $P = 0.231$). However, the fixation angle of the male cocoon had a significant effect on the number of workers that emerged from the first brood (ANCOVA, age as covariant, F = 6.675, df = 2, $P = 0.003$).

4. DISCUSSION

Under laboratory conditions, a bumblebee queen must be stimulated to initiate egg laying, as was discovered by Sladen (1912). Gretenkord (1997) compared several methods for stimulating the queen to oviposit, and found that the most successful method was the combined addition of workers and larvae to the nest box. For commercial rearing, this method is less practical because it is more laborious and when the larvae is detached from a colony, the cell covering of wax over the larvae is usually damaged, which results in the death of the larvae. Gretenkord (1997) was unsuccessful in providing pupae. However in several other studies, male pupae were used successfully (Duchateau, 1995, 2000; Duchateau et al., 1994; Yeninar et al., 2000). Our study shows that providing a queen with just one young male pupa, fixed horizontally, was effective to stimulate colony initiation: 80% of the queens provided with such a male cocoon produced a colony, and most of them produced also young queens. Gretenkord (1997) used *B. terrestris* queens for his experiments, which were treated with CO_2 for additional stimulation after a short hibernation. He did not mention the age or the orientation of the male pupa. It could be that he used old pupae and/or did not replace them with new cocoons after emergence of the male. Duchateau used 1–2 day old male pupae that were replaced weekly if the queens did not produce eggcups. Yeninar et al. (2000) replaced the cocoon if the male emerged, but they did not mention the age of the cocoon. The colonies that started with a young male pupa produced on average 89 queens, 155 males and 145 workers. This result is comparable with data of Gretenkord (1997) who reported that 70% of the queens produced big colonies (more than 100 workers) with an estimated 66 queens, 450 males and 190 workers on average. However, if old pupae were used, in which the male emerged 1–2 days after being supplied, colony production was much lower (30%). This was caused partly by the behavior of the queens that ate their own eggs in 45% of the cases. Queens that could build their eggcups on young pupae never showed this behavior. Table IV gives an overview of colony initiation and colony production of other studies in comparison with this study. Although other factors such as hibernation and queen cohorts also could have an effect on colony initiation and colony production, this overview shows that the provision of one male pupa can be successful.

The age of the pupa can also affect the other colony characteristics. On young male pupae, the queens constructed more eggcups from which more workers emerged. If old pupae were provided, the males emerged soon after, and the queens probably were not stimulated by an empty cocoon. Queens might not continue laying eggs on empty cocoons, and the available eggcups were incubated and cared for less, as seen in the longer development of the first worker on an old pupa in comparison with one young pupa. On a horizontally fixed cocoon, the queen had more area to build eggcups and also to incubate, which could explain why more workers emerged from queens that were stimulated with a horizontally fixed cocoon compared to those with a vertical or oblique cocoon. In nature, however, the queens build eggcups of the first brood on a pollen clump and those of the second and third brood on the available cocoons that are vertically orientated. There is probably enough space for egg cups and incubation on a pollen clump. In a colony, there are enough cocoons so that the queen constructs only 1–2 eggcups per cocoon and has more area to hold on to these cocoons for incubation.

The effect of the age of the male pupa in itself is very strong; when the results of the age group of 1–2 day old pupa and 3–4 day old

Table IV. Overview from various studies on the origin of the queens and CO_2 treatment, the stimulation method, days until colony initiation and colony production (All *B. terrestris).*

Study	Origin of queen	Stimulation	Initiation (days)	Colony production
Present study	Reared (no CO_2 treatment)	1–2 days old pupa	3.6	80%
Present study	Reared (no CO_2 treatment)	9–11 days old pupa	31.3	30%
Gretenkord (1997)	Reared (CO_2 treatment)	Workers + Larvae	12	70%
Gretenkord (1997)	Reared (CO_2 treatment)	Honey bee workers	22	50%
Bilinski (1998)	-	6–10 young male pupae	2–3	-
Yeninar and Kaftanoglu (1997)	Field (no CO_2 treatment)	One male pupa	6.3	-
Duchateau (data of 1996)	Field (no CO_2 treatment)	One young male pupa	10	67%
Duchateau (data of 1994)	Reared (CO_2 treatment) (1month hibernation)	One young male pupa	18.9	79%
Duchateau (data of 1994)	Reared (No CO_2) (3–6 months hibernation)	One young male pupa	9.5	73%

pupa were compared, there were significant differences in colony initiation, numbers of eggcups and workers in the first brood, and in numbers of queens. In both groups, males did not hatch before replacement of the cocoons, thus these differences were an effect of the male pupa age as such, and not of partly emptied cocoons. Colony development was negatively affected by the poor start of queens provided a cocoon containing an old male pupa. On old pupae, colonies produced on average 81 workers, 24 queens and 184 males, in total about 289 individuals, which is 100 individuals less than if the colonies started with young pupae. This finding appears odd considering the competition point where the rearing of new brood comes to an end due to egg robbery by workers and queens. In the colonies which were initiated on old pupae, the competition point occurred much later than in the colonies that started on young pupae (32 and 21.5 days respectively). It would therefore be expected that the colonies on the old pupae would have produced more individuals, because the queen could lay eggs during a longer time. As this was not the case it shows that the colonies performed worse during whole colony development. For commercial rearing, rapid colony initiation by the queen, with enough workers in the first brood is a prerequisite to obtain good colonies. This quick start can be achieved by providing a hibernated queen with one cocoon containing a young male pupa, horizontal fixed.

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Résumé – Stimulation de la fondation et du développement de la colonie chez *Bombus terrestris* par l'ajout d'une nymphe de mâle : influence de l'âge et de l'orientation. Les reines de *Bombus terrestris* qui ont hiberné doivent être stimulées pour se mettre à pondre en conditions de confinement. Il est nécessaire de disposer d'une méthode efficace et économique pour élever en masse les bourdons. L'utilisation d'un cocon renfermant une nymphe mâle est une solution prometteuse car elle a été utilisée avec succès dans plusieurs études ; elle requiert moins de travail et les mâles sont toujours disponibles dans un élevage en masse. Des études antérieures ont montré que l'âge de la nymphe et l'angle de fixation du cocon semblent influencer le comportement de la reine. Des reines, ayant hiberné durant trois mois puis ayant été stimulées en cage de vol durant une semaine, ont reçu chacune un cocon contenant une nymphe mâle.

Dans la première expérience, les nymphes ont été réparties en cinq groupes (de 1–2 j à 9–11 j) selon leur âge au moment de l'introduction. Elles étaient remplacées chaque semaine par d'autres nymphes du même âge si les reines n'avaient pas commencé à pondre. Vingt reines ont été utilisées pour chaque groupe d'âge. Les cocons étaient fixés à l'horizontale avec de la paraffine sur du papier à dessin épais. Les résultats montrent que plus l'âge de la nymphe était élevé, plus il fallait de temps à la reine pour pondre (Fig. 1). Pour le premier couvain, le nombre de cellules à couvain construites par les reines, le nombre d'ouvrières et le nombre d'ouvrières par cellule à couvain décroissaient quand l'âge des nymphes introduites augmentait (Figs. 2 et 3). Quand des nymphes âgées étaient introduites, les mâles émergeaient peu après et la reine avait un cocon vide pendant un à six jours. Les reines ne sont probablement pas stimulées par des cocons vides puisqu'elles ne construisaient aucune nouvelle cellule à couvain et qu'elles incubaient moins et prenaient moins soin des cellules à couvain présentes. En outre, le développement des premières ouvrières sur une nymphe âgée était plus long que sur une nymphe jeune (Tab. I). Seulement 30 % des reines ayant reçu une nymphe âgée ont produit une colonie. L'une des raisons est que la reine avait mangé ses œufs dans 45 % des cas. Les reines qui pouvaient construire leurs cellules à couvain sur de jeunes nymphes n'ont jamais présenté ce comportement. Les colonies produites par les reines ayant reçu une nymphe âgée ont eu une plus mauvaise performance durant toute la période de développement de la colonie (Tab. II). Au contraire, 80 % des reines ayant reçu une nymphe jeune ont produit une colonie comportant en moyenne 145 ouvrières, 89 reines et 155 mâles.

Dans la seconde expérience, nous avons comparé divers angles de fixation du cocon : à l'horizontal, à la verticale ou en oblique. Les cocons fixés horizontalement ont donné de bien meilleurs résultats (Tab. III). Cela peut s'expliquer par le fait que la reine a plus de place pour construire les cellules sur un cocon fixé horizontalement et peut mieux incuber les œufs et les larves.

***Bombus terrestris* / stimulation de la ponte / nymphe mâle / fondation de colonie**

Zusammenfassung – Stimulation der Volksgründung und Entwicklung bei *Bombus terrestris* durch Zusatz von Drohnenpuppen: Der Einfluss von Alter und Orientierung. Königinnen von *Bombus terrestris* müssen nach der Überwinterung bei Käfighaltung zur Eilage stimuliert werden. Für die Massenaufzucht von Hummeln wird daher eine effektive und wirtschaftliche Methode benötigt. Die Nutzung von Kokons mit Drohnenpuppen ist eine vielversprechende Methode, weil sie sich bereits in mehreren Versuchen als erfolgreich erwiesen hat. Auch ist sie weniger arbeitsintensiv und Drohnen sind jederzeit in einem System der Massenzucht verfügbar. Frühere Untersuchungen weisen darauf hin, dass das Alter der zugefügten Puppen und der Winkel bei der Befestigung des Kokons einen Einfluss auf das Verhalten der Königin zu haben schien. Isolierten Königinnen (nach 3 monatiger Überwinterung und anschließender einwöchiger Aktivierung im Flugkäfig) wurde ein Kokon mit einer Drohnenpuppe zugegeben. Im ersten Versuch bestanden die Puppen beim Zusetzen aus 5 Altersgruppen (1–2 bis 9–11 Tage); die Puppen wurden wöchentlich gegen die gleiche Altersgruppe ausgetauscht, wenn die Königinnen noch nicht mit der Eilage begannen. Für jede Altersgruppe wurden 20 Königinnen eingesetzt. Die Kokons wurden fast horizontal mit Paraffin an einer Pappe befestigt. Die ersten Versuche ergaben, dass die Königinnen mit zunehmenden Alter der Puppen später mit der Eilage begannen (Abb. 1). Bei der ersten Brut nahm die Zahl der von der Königin gebauten Eizellen, die Anzahl der Arbeiterinnen und die Anzahl der Arbeiterinnen per Eizelle signifikant mit zunehmenden Alter der eingebrachten Puppen ab (Abb. 2 und 3). Wurden alte Puppen zugefügt, schlüpften die Drohnen kurze Zeit später und die Königin hatte für 1–6 Tage einen leeren Kokon. Königinnen werden wahrscheinlich nicht durch leere Kokons zur Eilage stimuliert, weil keine neuen Eizellen gebaut werden. Die schon vorhandenen wurden weniger gewärmt und versorgt. Außerdem ist die Entwicklungszeit der ersten Arbeiterinnen bei Zugabe von alten Puppen länger als bei jungen Puppen (Tab. I). Nur 30 % der Königinnen, die alte Puppen bekamen, erzeugten ein Volk. Die Ursache war in 45 % der Versuche das Fressen der Eier durch die Königin. Königinnen, die ihre Eizellen auf jungen Puppen bauen konnten, fraßen die Eier nie. Insgesamt entwickelten sich die Völker von Königinnen, die alte Drohnenpuppen erhalten hatten, während der ganzen Zeit schlechter (Tab. II). Das steht im Gegensatz zu Königinnen, die junge Puppen bekommen hatten und die zu

80 % Völker mit im Durchschnitt 145 Arbeiterinnen, 89 Königinnen und 155 Drohnen aufbauten. Im 2. Versuch wurden die Winkel verglichen, mit denen die Kokons angebracht wurden: horizontal, vertikal und schräg. Dieser Versuch zeigt deutlich, dass Königinnen mit horizontal befestigten Kokons mit jungen Puppen größere Völker erzeugten als solche mit vertikal oder schräg angebrachten Kokons (Tab. III). Das Ergebnis wird mit dem größeren Platz erklärt, der für Eizellen auf einem horizontal befestigten Kokon zur Verfügung steht. Außerdem können Eier und Larven besser gewärmt werden.

***Bombus terrestris* / Stimulation der Eilage / Drohnenpuppen / Volksgründung**

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