Effect of different narcosis procedures on initiating oviposition of pre-diapausing Bombus terrestris queens

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Abstract

Four experiments aimed at the stimulation of starting oviposition were carried out with bumblebee queens (Bombus terrestris L.) from colonies belonging to the ecotype of Central Western France and reared in a glasshouse. After mating, queens were narcotized with carbon dioxide, confined singly in small boxes (11 x 5 x 4.5 cm) and kept in a dark room at 28–29 °C and 60 %–65 % r.h. They were fed on a sugar solution and a pollen-syrup mixture. No effects were discernible if the narcosis was applied 20 to 30 days after mating instead of 5 days, nor if the queens were submitted to a 4 to 5 day period at 34 °C following narcosis. Survival rates ranged from 65 % to 68 %. If the queens were reared under fluorescent tubes (L8:D16) after narcosis the mean delays to egg-laying were significantly reduced compared to a dark treatment (21 days instead of 39), as was their variability (s.e. = 1.6 day instead of 3.1 days). The survival rates were respectively 73 % and 67 %. Under the same photoperiod (L8:D16) the CO₂ narcosis repeated at a 24 h interval had the same efficacy whether its duration was 10 min or 5 min. The delays to egg-laying were respectively 20 days (s.e. = 1.5) and 25 days (s.e. = 4.8) with survival rates close to 73 %. Egg-laying could also be induced in non-narcotized queens with a survival rate of 54 % and delays to oviposition close to those of queens narcotized 2 x 10 min.

Introduction

Since mass-rearing of Bombus terrestris L. began in 1987 to supply glasshouse tomato growers in Europe, bumblebee producers have used several methods to rear colonies year-round, despite the long ovarian diapause of this species (Alford, 1975; Pouvreau, 1976). Rösele & Rösele (1984) demonstrated that narcotizing pre-diapausing queens with carbon dioxide (a 30 min narcosis repeated twice) inhibited the formation of fat reserves, increased the size of the corpora allata, which produced high rates of juvenile hormone in vitro, and induced oogenesis. In 1985, Rösele, giving the first description of a year round rearing system, stated that after mating, B. terrestris queens would start laying eggs within a week following the double narcosis.

Part of the great commercial development of bumblebee rearing is due to Rösele’s narcosis method which was also recommended by van den Eijnde et al. (1991) who described how to produce colonies of B. terrestris artificially. Carbon dioxide narcosis of 10 min repeated at a 24 h interval was already used by Mackensen (1947) to stimulate egg-laying in newly inseminated honey bee queens. Enhancement of ovarian maturation after CO₂-narcosis was also reported by Nicolas (1979) in Locusa migratoria. From the information released by commercial producers of bumblebees it is obvious that the results of carbon dioxide narcosis procedures on B. terrestris queens are variable and that the treatment does not always reach an optimum efficiency, sometimes causing detrimental side effects.

Several parameters are expected to be involved in the physiological action of carbon dioxide. In this article four of them were studied in order to assess their effect on egg-laying delays:
- the duration of narcosis,
- the age of the mated queens submitted to narcosis,
- the temperature following narcosis,
- the photoperiodic regime to which narcotized queens were submitted.

Materials and methods

Origin of queens. All colonies that produced experimental queens were created by queens captured from the wild in February 1991. Bumblebees were reared either in glasshouse compartments \((3 \times 3 \times 2 \text{ m})\) or in a dark climate room. Glasshouse colonies were raised in cubic wooden boxes \((25 \times 25 \times 25 \text{ cm})\) and fed on a solution containing 73% sugar, pollen collected by honeybees and flowers of Salix and Brassica napus oleifera. In these colonies the production of queens occurred from the 20th of June until the 4th of November. In the climate room the bumblebees were kept in wooden boxes \((25 \times 25 \times 25 \text{ cm})\) each fitted with two feeders. They were supplied with sugar solution and a mixture of honeybee collected pollen and syrup. The temperature was \(28 \pm 29 ^\circ \text{C}\) and the relative humidity 60–65%. In both rearing conditions experimental queens emerging from the colonies were mated in the glasshouse with males from other colonies flying in screened cages of 0.7 m\(^3\). After mating, queens were maintained in groups in other cages of the same size in glasshouse conditions. They were fed with sugar solution and pollen collected by honeybees.

Current narcosis technique. At least five days after mating queens were cooled to \(3 ^\circ -4 ^\circ \text{C}\) for one to two hours, then narcotized for 10 min at the same temperature in plastic boxes. The carbon dioxide used in all the trials was 99.9% pure. After flowing one min into a narcosis box, its concentration in weight varied from 75% to 85% at the beginning of anaesthesia, to 51% to 77% after 10 min. \(\text{CO}_2\) narcosis was repeated after 24 h. In the interval queens remained in the dark at 23 °–24 °C.

Standard rearing conditions after narcosis. After the second narcosis queens were put into single wooden boxes \((11.3 \times 4.5 \times 4.3 \text{ cm})\) with a transparent cover. They were equipped with two feeders, one was filled with a syrup containing 73% sugar, the other received small daily deposits of a pollen-syrup mixture. To prevent Nosema multiplication, Fumidil powder at 2 g/l was added to the sugar solution during periods of 14 days alternating with two week intervals without the antibiotic. A three cm round plastic lid with a beeswax bottom was also at the disposal of the queen for egg-laying. The rearing boxes were kept in a dark climate room at 28 °–29 °C and 60%–65% R.H. A worker B. terrestris was introduced in each box to stimulate the queen. Daily observations were performed under red light to note the date of the first egg-cell building or the queen's death. We defined the survival rate as the percentage of experimental queens which laid eggs, whatever might be the duration of their life after the first oviposition. Oviposition delays are given as: \(\bar{x}\pm se\).

Variations of the procedure.

a) Trial 1. Some unpublished results suggested that queens would lay eggs if they were submitted to a temperature higher than the nest temperature for a short period. Thus we applied 34 °C for four to six days in a dark room to 44 queens just after narcosis and before introduction into the dark climate room at 28 °–29 °C. A control sample of 47 queens was reared in the dark climate room just after narcosis.

b) Trial 2. The standard \(\text{CO}_2\)-narcosis was applied to 62 queens five days after mating and to 29 queens 20–30 days after mating. Following the \(\text{CO}_2\) treatment all these bumble bees were reared in the climate room.

c) Trial 3. The standard \(\text{CO}_2\)-narcosis was applied to 136 queens, then 91 of them were kept in the dark climate room at 28 °–29 °C, and 45 were reared at the same temperature under fluorescent tubes supplying 800 lux with the photoperiodic regime: L8:D16. The 50 HZ electric equipment fed three tubes on three phases, thus suppressing light flickering. Eight hours is the lowest value of the day length in January, the month when early B. terrestris queens emerge from their hibernation lodge.

d) Trial 4. Three groups of queens were reared in the climate room under the photoperiod L8:D16 after three different treatments applied to 13, 9 and 36 queens respectively: no narcosis, five min narcosis repeated at a 24h interval, 10 min narcosis repeated at 24h interval.

Results

Influence of high temperature treatment after \(\text{CO}_2\)-narcosis and of the age of mated queens on oviposition delay. The four treatments applied:
Table 1. Influence of a high temperature period following narcosis on the mean days to egg-laying

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Egg-laying delay (days)</th>
<th>n</th>
<th>s.e.</th>
<th>Value of t</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂ narcosis</td>
<td></td>
<td>31</td>
<td>39.5*</td>
<td>0.22</td>
</tr>
<tr>
<td>CO₂ narcosis +</td>
<td></td>
<td>30</td>
<td>38.2*</td>
<td></td>
</tr>
<tr>
<td>4-6 days at 34 °C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values bearing the same letter are not different according to Student t test (P<0.05).

Table 2. Influence of the delay between mating and narcosis on the mean days to egg-laying

<table>
<thead>
<tr>
<th>Delay between mating and narcosis (days)</th>
<th>Egg-laying delay (days)</th>
<th>n</th>
<th>s.e.</th>
<th>Value of t</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td></td>
<td>42</td>
<td>40.0*</td>
<td>3.9</td>
</tr>
<tr>
<td>20-30</td>
<td></td>
<td>19</td>
<td>36.3*</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Values bearing the same letter are not different according to Student t test (P<0.05).

- standard narcosis,
- period of 4 to 5 days at 34 °C after narcosis,
- narcosis 5 days after mating,
- narcosis 20-30 days after mating

resulted in identical survival rates: 66% - 68% - 68% and 65% respectively. The delays to egg-laying were also identical according to the Student ‘t’ test: 39 - 38 - 40 - 36 days with standard errors close to each other ranging from 3.9 to 5.0 (Table 1 and 2). As shown in Fig. 1 and 2, the dispersion of data presents common characteristics: a large range from 0-10 days to 90-100 days, and modes between either 10 and 20 days or 20 and 30 days.

Influence of a photoperiod applied after narcosis on oviposition delays. The survival rate of control queens (dark treatment) was 67% compared to 73% of queens laying eggs under artificial light. The photoperiod L8:D16 reduced the mean egg-laying delays compared to the dark, respectively: 21.1 ± 1.6 days (33) and 38.9 ± 3.1 days (61). Fig. 3 shows that the distribution of data in the light-dark treatment was quite different from that of the data concerning queens reared in the dark. The range of the delays in the dark was twice as large as that in the light. The modes were identical, between 10 and 20 days. Within 40 days, 97% of the queens in the light-dark regime laid their first eggs, compared to 59% of the queens in the dark. These proportions are significantly different (Table 3: χ² = 13.56; P = 0.0003). Similarly, queens in L:D regime showed a higher frequency of oviposition within 20 days than queens in the dark; respectively: 60.6 % and 29.5 % (Table 3: χ² = 7.36; P = 0.006).

Influence of narcosis duration. If no CO₂ treatment was applied only 7 out of the 13 queens survived whereas 33 of the 45 narcotized queens did, all reared in the L8:D16 regime. The egg laying delays were identical in the non-narcotized queens and those submitted to the narcosis (2 × 10 min) respectively 17.3±2.1 days (7) and 19.8±1.5 days (25) (Student ‘t’ = 0.8; df = 30; P>0.05). No significant difference was found between oviposition delays of non narcotized queens and those narcotized 2 × 5 min respectively 17.3±2.1 days (7) and 25±4.8 days (8) (Student ‘t’ = 1.4; df = 13; P>0.05). Chi-square analyses did not show significant differences between the proportions of queens laying eggs within 20 or 30 days (Fig. 4), respectively χ² = 2.1; P = 0.35 and χ² = 4.6; P = 0.09.

Discussion

Duration of narcosis. In bumblebees a 5 min exposure to CO₂ at 4 °C, repeated once, may be the minimum narcosis duration for a reliable stimulation of egg-laying. It is noticeable that some queens could start a brood in artificial conditions without being narcotized. In a larger sample of pre-diapausing non-narcotized

Table 3. Influence of photoperiod on oviposition delays of narcotized queens

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oviposition delays (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>medium</td>
<td>0-20 21-40 &gt;40</td>
</tr>
<tr>
<td>dark</td>
<td>18    18    25</td>
</tr>
<tr>
<td>Number of queens</td>
<td>photoperiod</td>
</tr>
<tr>
<td>L8:D16</td>
<td></td>
</tr>
</tbody>
</table>

| Number of queens | photoperiod | 20 | 12 | 1 |
| L8:D16               |               |    |    |    |
Fig. 1. Distribution of delays to initial oviposition of narcotized queens submitted to a post-narcosis temperature treatment (34 °C for 4 to 6 days) compared to a control.

Fig. 2. Distribution of delays to initial oviposition of queens narcotized either 5 days or 20 to 30 days after mating.
Fig. 3. Distribution of delays to initial oviposition of narcotized queens reared in a photoperiodic regime L8: D16, compared to control queens reared in the dark.

Fig. 4. Cumulative proportions of queens starting oviposition as a function of time since start of rearing at 28-29 °C.
queens (unpublished results) we observed that 30% of
queens founded a nest. Presumably their physiological
state was different from that of other queens which died
in identical rearing conditions. This latter category of
queens would probably have needed exposure to CO₂
to activate oogenesis. Rösel Rösel's (1985) narcosis of bumble-
bees lasted three times as long as ours and resulted in
oviposition delays of about one week instead of 19
days as shown in our study. In Locusta migratoria,
Nicolas (1979) showed that short exposures to CO₂
(one min every day) were able to stimulate oviposition
of females inhibited by a long photophase. The concentra-
tion of CO₂ should also be considered as a parameter
of the narcosis efficiency. According to Nicolas (1989),
variations in the concentration of carbon dioxide may
result in opposite physiological effects.

Age of queens. The timing of CO₂ narcosis after mating
did not affect the delays of egg-laying within the range of
5–30 days. According to Rösel Rösel (1985) narcosis
can be also applied to queens during hibernation to
terminate their diapause. Larrere et al. (1993) reported
similar experiments demonstrating that overwintering
queens submitted to a 10 min CO₂ narcosis repeated
once, emerged from the soil earlier than non-treated
diapasing insects.

Social stimulation. In our experiments each queen
was supplied with one worker bumblebee. The contact
between the queen and the worker proved efficient in
reducing egg laying delays as it has been demonstrated in
other studies. Engels et al. (1976) demonstrated that
honeybee queens submitted to a double CO₂ exposure
required social contacts with workers to initiate the
vitellogenin metabolism. In bumblebees various stimu-
ulating treatments have been used: brood cells con-
taining old larvae or pupae with some accompanying
workers (Rösel Rösel, 1985) or 4 to 5 honeybee workers
as recommended by Ptacek (1983, 1985) and van den
Eijnde et al. (1991). Oviposition may be influenced
by the kind of social stimulation to which narcotized
queens are submitted. Brood cell supply might have
accounted for the shorter egg-laying delays reported

Temperature. The short exposure of queens to 34 °C
after narcosis did not result in any significant modifica-
tion of egg-laying delays. Further experiments should
assess the influence of temperature on CO₂ efficiency
through exposures during narcosis, within the range
of 4 °C - 20 °C, so as to avoid lethal high tempera-
tures. In Drosophila, Nicolas (1989) reported a reduced
anaesthetic potency of CO₂ at high concentration if
the temperature was increased. On the contrary, if the
temperature varied between 27 °C and 35 °C, CO₂ at
low concentrations (1.5%) caused an increased produc-
tion of the juvenile hormone titre in honeybee workers
(Bühler et al., 1983).

Photoperiod. Exposure to artificial light (L8:D16)
had a remarkable influence on narcotized bumble-
bee queens. In other species a number of experiments
demonstrated the importance of photoperiod on ovipo-
sition: in Adalia bipunctata egg-laying onset was possible
with L13:D11 and inhibited by L12:D12 (Iperti
& Prudent, 1986). Aelia acuminata laid eggs earlier in
L18:D6 (Hodek, 1974). Myrmica rubra workers laid
eggs in L16:D8 and L:L more readily than in L8:D16
or in the dark (Hand, 1983). In Plodia interpunctel-
la L12:D12 or the dark was more efficient than con-
stant light (Mbata, 1985). More recent studies (unpub-
lished results) confirm the beneficial action of light-
dark regimes on overwintered B. terrestris queens:
L8:D16 stimulated oviposition more effectively than
L16:D8, L:L or D:D. In the natural cycle of B. terre-
stris, queens fly and forage for two to three weeks
before founding their nest and remaining in the dark. If
we consider that the prefoundling period in our region
lies from the beginning of February to mid-March the
day length varies from about 9 to 12h. Presumably
the photoperiod L8:D16 is relatively close to the nat-
ural regime. Difficulties remain owing to the different
spectrum of day-light and fluorescent lamps and to the
lack of knowledge on the wave length to which queen's
brain is susceptible. It is expected that further studies
will provide a better appraisal of light requirements of
bumblebees in climate room.

Physiological and side-effects of CO₂ narcosis. Insect
metabolism is influenced in various ways by carbon
dioxide exposure and by other environmental factors.
In Locusta migratoria short narcosis induces the same
effects as electric stimulation of the pars intercere-
bralis (Nicolas, 1979). Carbon dioxide concentrates in
the central nervous system and affects the amount of
neurotransmitters such as dopamine and octopamine
(Fuzeau-Braesch & Nicolas, 1981). In B. terrestris,
Larrere (1993) observed that neurosecretory cells of
diapasing queens resumed their activity 72 h after
CO₂ narcosis. In the honeybee, histological stud-
ies brought evidence of CO₂ effects on neurosec-
tory cells of brain, corpora allata and juvenile hor-
mone titres (Nicolas, 1989). In bumblebees Röseler and Röseler (1984) found that corpora allata of narcotized queens became enlarged and produced more juvenile hormone. Their fat reserves for hibernation were not built up and oogenesis had started. The same authors noted opposite results in queenright workers whose oogenesis was inhibited by carbon dioxide. Lum and Flaherty (1972) demonstrated that CO₂ narcosis reduced oviposition and hatchability in Plodia interpunctella Ebadi et al. (1980) noted adverse effects of CO₂ on honeybee longevity, and Pain et al. (1967) found a reduction of the secretion rate of the royal mandibular glands in narcotized honeybee queens. Pomeroy & Plowright (1979) found that CO₂ induced ejection of larvae by bumblebee workers in narcotized colonies. Röseler (1985) reported the emergence of some males among the first workers batch. On the contrary, Mackensen (1947) did not note any change in anaeasthetized honeybee queens performances.

Apart from its effects on oogenesis, carbon dioxide used on bumblebee queens may affect some other characteristics. They sometimes produce males instead of workers and their nests may be of smaller size than those of overwintered queens. These detrimental side-effects are worth investigating and a better knowledge of physiological modifications due to CO₂ narcosis would allow setting up new procedures, that will take into account important parameters, such as CO₂ concentration, narcosis timing, temperature, and light.

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References