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EFFECT OF TEMPERATURE DEGREES AND CO_2 ON THE DEVELOPMENTAL DEGREE OF OVARIES IN HONEYBEE WORKERS

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ABSTRACT

In this study 4 treatments were applied on worker honeybees to study their stimulative effect on their ovaries development. Five groups of newly emerged Carniolan worker bees were used for this study. The first and second bee groups were exposed to low temperature at 4 °C for ½ and 1 houre, respectively, while the third and fourth groups were expopsed to 40 °C for 24 hour and Co_2 for 5 minutes, respectively. The remaining group was used as a control. The bees were put in small cages supplied with a piece of wax comb containing pollen and supplied with diluted sugar solution and water. After applying the treatments the cages were put in an incubator at 34 °C . After 30 days the cages were inspected and the remaining workers were dissected to detrmine the degree of ovaries development in the different treatments. The effect of ½- and 1-hour cold treatments were superior to CO_2 treatment. The CO_2 had no stimulative effect on ovary development. The results suggested further using of lower temperature or incresing the exposure duration for the current low temperature as a tool for obtaining drones from workers with specific characters.

INTRODUCTION

Worker honeybees are the sterile female of honeybee colonies. Although the queen honeybee normally lays all the eggs in a colony, worker bees begin to develop their ovaries when the queen and all brood are removed from the colony (Jay, 1970, 1972). The appearance of laying workers in the colonies is a negative incidence and a colony it contains have to be treated suitably to be clear from it. In recent years, however, drones produced by worker honeybees are used in breeding program. The array of male genotypes in a colony with laying workers is derived from the genotypes of both the queen mother and her mates, and therefore represents greater genotypes variability than the genotypes of drone produced by a single queen (Rinderer, 1986).

Many characteristics of interest in the colony (Honey production, for example) are expressed on the colony level. However, many important traits such as disease resistance, longevity and development time are measurable on single worker honeybee. Breeding from single workers provide the precision needed to detect and propagate genetic combinations that occur at very low frequencies, such as grooming behavior against the ectoparasite mite *Varroa destructor* (Harris and Harbo. 1991), but because a solitary

worker will not lay eggs, the problem is to get a specific worker to lay and then determine which eggs are hers.

The using of worker bees as a source of drones in breeding programs was used by Bienefeld et al (1999). He chosed some workers in some colonies which showed an intensive hygenic behaviour towords ecoparasite *Varroa* and crossed it with specific genetic lines of queen, and could concenterate the hygenic behaviour character in the next generation. He put the chosen workers with other workers in small nucli, which are isolated from the other colonies. He replaced the attendant workers every 8 days with newly emerged ones until the chosen workers begin to lay eggs. Many factors are known to effect the rate of ovary development of queenless groups of bees including worker age (Delaplane and Harbo, 1987), nutiration and degree of ovary development of other worker bees (Velthuis et al, 1965). Racial differences and rate of ovary development for worker honeybees have also been described (Ruttner and Hess, 1981). Hillesheim et al (1989) used the racial difference in the rate of ovary development for worker honeybees to selectively obtain eggs from single workers.

The objective of this work was to study the stimulative effect of low, high temperature and CO_2 on the developmental degree of worker honeybees ovaries.

MATERIALS AND METHODS

Five small cages were prepared to be filled with worker honeybees. In each cage a small piece of wax comb containing pollen was put inside. Pollen was needed for workers ovary development (Harris and harbo, 1991). Each cage was supplied with diluted suger solution and tap water. The cages were supplied on one side with movable glass pane and the other side was closed with mesh wire. Each cage was filled with not less than 200 newly emerged Carniolan worker honeybees, which were exposed to the treatments as follows:

Two CO_2 tratments in 2 consecutive days for 5 minutes, 4C° for ½ an hour, 4C° for 1 hour, 40C° for 24 hour, and a group was left without any tratments.

The cages were then put in an incubator at 34C° , and each cage was inspected at 3 days intervals for removing the dead bees and renewing the diluted sugar solution and the water supply. After 30 days the combs in the cages were inspected for searching on eggs and the remaining workers were dissected to determine the developmental degree of their ovaries. The ovaries were classified according to the shape of the most developed oocyte in the ovarioles into 5 classes :

Class 0: Ovary with no apparent eggs.
Class 1: Ovary with small round eggs.
Class 2: Ovary with small elongated eggs.
Class 3: Ovary with big elongated eggs.
Class 4: Ovary with sausage-shaped eggs.

After assigning the ovary of each worker to the suitable class , the number of bee in each class was recorded for each treatment, and the data

obtained were displayed in form of 2 X 2 tables and the degree of ovary development was tested for independence from the treatments under study using chi-square test by using Almo statistical package (Holm, 1997).

RESULTS AND DISSCUSION

The effect of different treatments on the development of worker honeybee ovaries is shown in table (1). In comparison with control, the effect of low temperature (4 C°) exposure was more effective, although not significantly different, than high temperature after 30 days from the beginning of the experiment.. In contrary, the effect of CO_2 treatment has no stimulative effect on the developmental degree of the ovary.

Within the first ovary class (Class:1) , it could be remarked that althougth the number of bees in the control was higher (18) than the other groups, the percentages of bees in remaining classes (Class 1 -- 4), with the different degrees of ovary developments, was less in the control 35% (10 /28) than in the other groups except the workers treated with CO_2, which shows the same percentage 35% (5/14).

Table (1): The ovary classes and the number of worker bees having these classes across the different treatments under study.

Ovary classes	Treatments and exposure periods				
	4C° 1/2h.	4C° 1h.	40C° 24h.	Co_2 5 min.	Control
0	11	4	13	9	18
1	5	1	5	2	0
2	6	4	5	2	6
3	2	3	5	1	3
4	4	0	1	0	1

The percentages of the developed ovaries were 60% (17/28), 55%(16/29) and 66% (8/12) for 1/2h.cold treatment, hot , and 1h-cold - treatment, respectively (Table, 2).

Table(2): A comparison of number of workers with (class 1:4) and without (class: 0) developed ovaries.

Ovary classes	Treatments and exposure periods				
	4C° 1/2h.	4C° 1h.	40C° 24h.	Co_2 5 min.	Control
0	11	4	13	9	18
1 -- 4	17	8	16	5	10
Total	28	12	29	14	28

The previous results idicated that more than 50% of the ½-hour,1-hour cold treated workers and hot-treated workers had developed ovaries, but less than 50% had developed ovaries in both control and CO_2-treated workers.

Although the difference was not significant, this indicate the stimulative effect of both cold and hot temperature on ovary development.

This result confirms that double CO_2 narcosis inhibits ovary activation in workers (Harris and harbo, 1990; Harris et. al. 1996). In contrast, Mackensen (1947) showed that double CO_2 narcosis of virgin queens actually accelerated ovary activation and egg laying.

Concerning ovary class 4, the highst percentage was observed in the cages treated with ½-hour cold temperature 14% (4/28) in comparison to 3% (1/29)for workers treated with hot temperature and control 3% (1/28). No workers with ovary class 4 were observed in the remaining treatments

The result may indicate the usefullness of of using both low and high temperature for stimulated high number of worker honeybees to activate their ovaries.This may be achived by using lower temperatur than that used in this work or increasing the exposure duration to the low and high temperature used in this study.

In bee colony certain group of workers become drone layers, these being genetically determined workers, which may have a higher number of ovarioles in comparison to other workers (Harris and Harbo, 1991). In this work all workers originated from one Carniolan bee colony, thus the difference between treatments under study can be ascribed to the treatments itself, as all the bees in all cages originated from one queen.

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تأثير درجات الحرارة و غاز ثانى أكسيد الكربون على درجة نمو مبايض شغالات نحل العسل

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فى هذا البحث تم دراسة التأثير التتبيهي لكل من الحرارة المرتفعة والمنخفضة بالإضافة لتأثير غاز ثاني أكسيد الكربون على نمو مبايض الشغالات، حيث جهز لهذا الغرض ٨ أقفاص صغيرة يحتوى كل منها على قطعة من قرص شمعي تحتوى على كمية من حبوب اللقاح مع تزويد هذه الأقراص بمحلول سكرى مخفف ومصدر مائي. تم تعبئة هذه الأقفاص بحوالي عدد ٢٠٠ شغالة نحل حديثة الخروج لكل قفص.

وقدتم تعريض النحل فى هذه الأقفاص الى ٤م° لمدة١/٢ ساعة ولنفس الدرجة لمدة ساعة ولدرجة حرارة ٤٠م° لمدة ٢٤ ساعة ولغاز ثانى أكسيد الكربون مرتين على يومين متتابعين لمدة ٥ دقائق، بينما تركت مجموعة من النحل بدون أي معاملة للمقارنة، ثم وضعت جميع الأقفاص فى الحضان على درجة حرارة ٣٤م° مع القيام بالفحص الدوري لها كل ٣ أيام لإزالة النحل الميت وتجديد كلا من المحلول السكري والماء.

بعد مرور شهر من تطبيق المعاملات تم فحص الأقراص الشمعية للكشف عن البيض كما تم تشريح ما تبقى من شغالات للكشف عن درجة نمو مبايضها. وقد أظهرت النتائج زيادة عدد الشغالات ذات المبايض النامية فى الأقفاص التي تعرض نحلها لدرجة ٤م يليها تلك المعاملة بالحرارة العالية (٤٠م°) بينما لم يتأثر معدل النمو فى الشغالات التي تم معاملتها بغاز ثاني أكسيد الكربون. وعلى هذا فاستعمال الحرارة المنخفضة (٤م°) يمكن من خلاله الحصول على شغالات ذات مبايض نامية وانه ربما يمكن الحصول نتائج اكثر ايجابية فيما بعد لو انخفضت درجة الحرارة عن ٤م° أو عن طريق زيادة فترة المعاملة لنفس درجة الحرارة، وذلك كوسيلة لتربية ذكور من شغالات ذات صفات محددة داخل طائفة نحل العسل.