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RESEARCH ON THE INFLUENCE OF TEMPERATURE AND HUMIDITY ON THE EX-SITU DEVELOPMENT OF QUEEN LARVAE

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Abstract

At it is known, the development of larvae from worker bees or queen bees is identical during the first two days of the larval stage. Morphological differences appear starting from the third day of larval development. The food administered to the larvae ex-situ, at regular intervals, forms a dry film upon contact with the larva, under conditions of temperature and humidity similar to those in the hive. The food drying phenomenon is due to the temperature-humidity variation, in the hive these parameters are regulated and monitored by the nurse bees. In the study, different combinations of temperature and humidity values in the controlled environment were tested, so as to increase the number of queens obtained, by increasing food intake. As a conclusion, at a temperature between 35-36ºC and a relative humidity between 75% and 85%, it is possible to obtain 20-25% queens that reach maturity from total number of two days old age larvae from the start of study.

Key words*: bees, controlled environment, ingestion, larvae, metamorphosis.*

INTRODUCTION

The experiences of growing bee larvae in the incubator, by administering diluted royal jelly, have been carried out in the last decades with quite good results (Human et al., 2006).

The problem to be solved is that of raising queens (queens) from worker larvae.

Most attempts have run into reduced larval growth due to reduced ingestion and controlled environmental factors, like temperature and humidity.

It is known that the larvae that develop into workers or queens are identical. Differentiation into the worker or queen bee occurs from the second day of larval life.

In the natural environment, in the colony of bees, during the first two days of the larva, they receive an identical food, consisting of royal jelly and honey. Starting from the second day, the worker larvae receive food consisting of royal jelly, diluted with honey in a large proportion.

It is obvious that the type of food decisively influences the development towards one or another type of bee. The queen larva receives the same type of food in the following days until the pupa stage. Basically, the queen larva floats on a layer of royal jelly.

Feeding is done by peristaltic, circular movements inside the cell. It is very important that the viscosity of the royal jelly allows these movements. The viscosity of the royal jelly must not increase beyond a certain limit because it makes it impossible for the larva to feed (Hanser, 1980).

In the natural environment, in the bee colony, the queen larva is fed small amounts of royal jelly, continuously administered by the nurse bees. The brood growth is directly influenced by the ability of the bee nurses to secrete royal jelly (Surlea (Surlea-Stoica) et al., 2022).

A queen larva is known to receive up to 1600 feeding visits.

The queen larva is fed *ad libitum*, the drying of the royal jelly being prevented by frequent feedings, up to the tipping phase.

In *ex situ* growth, these parameters are difficult to achieve (Tautz et al., 2003).

MATERIALS AND METHODS

Apis mellifera carpatica larvae were used in the experiment. The age of the larvae was precisely determined by the technique of isolation of the queen on the empty comb (Figure 1).

Figure 1. Two-day-old larvae after transvasation (Original photo)

All eggs laid between 10:00 and 12:00 are considered to be of the same age.
After 5 days - 3 days in the egg stage and 2 days in the larval stage, the larvae were transferred from the honeycomb to the 9 mm diameter grow-out beakers made of wax.
Previous experiments have shown that the diameter of the beaks does not influence the differentiation of larvae into worker or queen bees.
The space for the ex situ growth of the larvae was an oven with controlled temperatures and humidity, of our own construction.
The oven was equipped with a source of heat generation with a thermostat with a degree of variation of ± 0.1ºC and a fan to recirculate the air inside.
The research was carried out using three variants of temperature (T) - humidity (UR) combinations, respectively:
- Variant 1 (V1): T = 35 ± 0.5ºC and RH = 75%;
- Variant 2 (V2): T = 35.5 ± 0.5ºC and RH = 80%;
- Variant 3 (V3): T = 36ºC and RH = 85%.

The feed used was royal jelly diluted with a nutritious, vitaminized solution.
Food was administered at 2-hour intervals, throughout the larval phase, with the help of a dosing pipette. Food drops were deposited on the side wall of the beaks, imitating the feeding of nurse bees.
In this way, no temperature differences are created between the food administered and that existing in the barrel.
For each variant, 50 two-day-old larvae were introduced into the oven.
The larvae were introduced when the temperature-humidity ratio stabilized at the values specific to each work variant.
The duration of the research was 21 days, starting from the premise that the queen completes her development in 16-17 days, and the worker bee in 21 days.

RESULTS AND DISCUSSIONS

Although incompletely understood, the growth mechanism of bee larvae to obtain viable ex situ queens is influenced by the temperature-humidity relationship (Stabentheiner et al., 2010).
The three experimental variants had different results as a result of the influence of temperature and humidity in the ex-situ development of queen larvae.
In Variant 1, in the first 2 days after the start of the experiment, 23 larvae died of starvation due to dehydration of the food.
Of those that remained alive, only 12 removed excrement and began weaving the cocoon.
Eight larvae passed the metamorphosis phase, but died in the imago phase - unpigmented bees.
During the study period, in Variant 2, out of 50 larvae, 7 larvae died of starvation, and 26 removed excreta and wove the cocoon. 18 larvae completed metamorphosis and entered the imago phase, and 15 larvae completed full development at 17 days of age.
Of these, 4 presented morphological characters intermediate between queen and bee, with predominantly queen morphological characters (weight, shape of the mandibles, shape of the metatarsus).
Variant 3 offered the best results, respectively 6 larvae died during the larval period, through

starvation; 44 larvae eliminated excrement, wove the cocoon and completed metamorphosis; 27 larvae completed the imago phase and full development. Of these, 15 larvae completed full development in 17 days and 12 larvae in 16 ± 0.5 days.

As a result of the research carried out, three factors must be controlled for the success of ex situ rearing of queen larvae: food, temperature and humidity.

As for food, it has been found that the administration of fresh royal jelly at regular intervals is not a solution, as it dehydrates very quickly, leading to the death of the larva by starvation (Hanser, 1980).

Maintaining the growth temperature at 35 ± 0.5ºC as is done in the bee family, ensures larval growth up to day 3 - 4 larva, followed by high mortality (80% of larvae).

Keeping the humidity at around 75%, similar to the conditions in the bee family, did not influence the transition to the metamorphosis phase.

The stages and periods of development of queen larvae until the emergence of viable individuals are as follows:

1. Egg stage - 3 days.
2. The larval stage - 4.5 days.
3. Extended larval stage - 2 days.
4. Metamorphosis 1-2 hours.
5. Imago stage and pigmentation, full development - 7 days.

According to the centralized results presented in Table 1, Variant 3 provided the best results; 54% of the hatched larvae completed development, but not all had queen morphological characters. Only individuals that finish development in 16-16.5 days are considered to be queens.

Table 1. Results obtained in the larval growth variants

Variant/ Larvae number	Development stage				
	1	2	3	4	5
V1	50	27	12	8	0
V2	50	43	26	18	15
V3	50	44	44	44	27

Regarding the viability of the larvae, variant 3 also proved to be the best, offering temperature and humidity conditions favorable to the growth and development of the larvae (Figure 2).

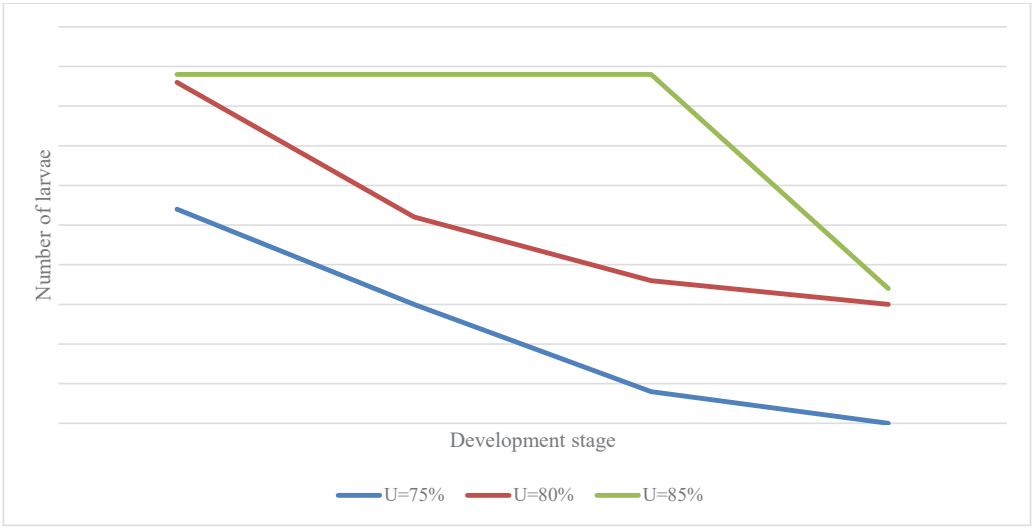

Figure 2. Larvae viability in the 3 experimental variants

Through the research carried out, an attempt was made to establish what are the suitable conditions for the ex situ growth of queen larvae.

The use of temperature and humidity parameter values in *ex situ* growth, similar to those of the bee family, (T =35 ± 0.5ºC and RH = 75%), in V1, does not lead to obtaining individuals with

specific queen characteristics. The larvae die in the various stages of development, through starvation, due to the dehydration of the food and the impossibility of ingesting it.
The viscosity of the feed is high and prevents the peristaltic movements of the larvae. They can no longer feed themselves or ingest a small amount of food.
Under natural conditions, the queen larvae are fed at short intervals, continuously, in the form of a drop of royal jelly and honey, deposited on the wall of the snout very close to the larva.

Figure 3. Larva during feeding (Original photo)

Larvae feed for long periods (Figure 3), interrupted by short periods of inactivity when molting occurs.
These periods of inactivity induce dehydration of the food around them, a phenomenon counteracted by nurse bees by searching for the larva and depositing fresh, excess food.
It is very important for the larva to ingest as much food as possible, until the excrement phase, when it stops feeding, in order to ensure the protein and energy necessary to complete metamorphosis and complete development.
The metamorphosis of bee larvae occurs with a high consumption of protein and energy, when a restructuring of the body is practically carried out (Figure 4).
The use in ex situ growth of royal jelly harvested from queen buds is not a solution because it dehydrates very quickly. Diluting the royal jelly, using different recipes, causes the larva to ingest food that is poorer in components that ensure development.
In Variant 1, the larvae die in various stages of development, by starvation, due to the dehydration of the food.
Variants 2 and 3 highlight the importance of the temperature-humidity relationship that influences larval development.
Temperature variations of ± 0.5ºC, measured during the experiments, did not influence the development.

Figure 4. Larvae after metamorphosis (Original photo)

The lowest degree of dehydration of the food was achieved at the value of relative humidity of 85%, in the larval phase, until the elimination of excrement.

Figure 5. Queen with development completed in 16.5 days (Original photo)

After removing excrement and weaving the cocoon, the humidity value can drop to 75%, without influencing the phase of metamorphosis and imago.
Organisms that have completed their development as close as possible to 16 days after laying the egg (natural queen development cycle), have characters similar to queens (Figure 5).

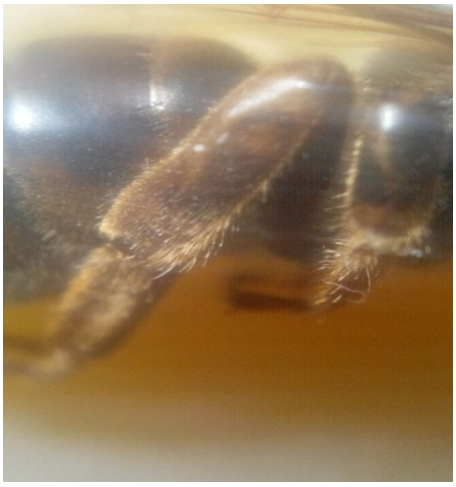

Figure 6. Metatarsus from a queen with intermediate characters (Original photo)

Organisms that exceed this term also have intermediate characters between the queen and the worker bee (shape of the mandible, shape of the metatarsus, shape of the thorax, etc.) (Figures 6 and 7).

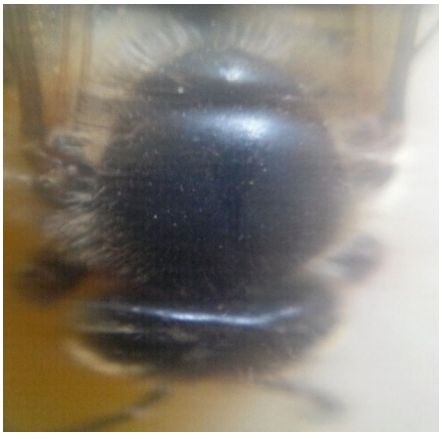

Figure 7. Queen-specific thorax (Original photo)

In organisms that finished development in 16.5 days, the lack of claws on one or more legs was observed (Figure 8), a phenomenon that also manifests itself in queens raised in bee families during periods of poor picking (Chuda-Mickiewicz & Samborski, 2015).

Figure 8. Clawless intermediate form (Original Photo)

Queens obtained in ex situ rearing were introduced into mating nuclei.
Out of 7 queens, 4 mated naturally and started laying eggs. The performances of the bee families, in which these queens were introduced, were similar to the apiary average, but no queen lasted more than one season.

CONCLUSIONS

The best results were obtained when the growth temperature was 36ºC and the relative humidity was 85%, resulting in 12 queens with a development period of 16-16.5 days and morphological characters, predominantly queen, about 24% of the larvae. These temperature and humidity values, correlated with the feeding of royal jelly diluted in various proportions with nutrient solutions, can increase the number of queens obtained *ex situ*.

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REFERENCES

Chuda-Mickiewicz, B., & Samborski, J. (2015). The quality of honey bee queens from queen cells incubated at different temperatures. *Acta Sci. Pol. Zootech.*, *14*(4), 25–32.

Hanser, G., (1980) *Breeding of queen bees in the laboratory,* Translated from German by Erika Dumitrașcu. Bucharest, RO: Apimondia-Verlag Publishing House.

Human, H., Nicolson, S. W., & Dietemann, V. (2006) Do honeybees, *Apis mellifera scutellata*, regulate humidity in their nest? *Naturwissenschaften*, *93*(8), 397–401. DOI: 10.1007/s00114-006-0117-y.

Surlea (Surlea-Stoica), C., Dinita, G., Maftei, M., Marin, I., & Nicolae C.G. (2022). Study on the viability of the young in the species *Apis mellifera* according to the secretory capacity of royal jelly. *Scientific Papers. Series D. Animal Science*, *LXV*(2), 274-278.

Stabentheiner, A., Kovac, H., & Brodschneider, R. (2010). Honeybee colony thermoregulation regulatory mechanisms and contribution of individuals in dependence on age, location and thermal stress. *PLoS One*., *5*(1), e8967. DOI: 10.1371/journal.pone.0008967.

Tautz, J., Maier, S., Groh, C., Rossler, W., & Brockmann, A. (2003). Behavioral performance in adult honey bees is influenced by the temperature experienced during their pupal development. *Proc. Natl. Acad. Sci. USA*, *100* (12), 7343–7347. DOI: 10.1073/pnas.1232346100.