Insemination of bee queens with the use of an electric ejaculator for drone

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Abstract. Intensive crop production involves the widespread use of honeybees as organized pollinators. In this regard, there is a need to have a large number of bee colonies for pollination by the beginning of flowering (in May-June) of entomophilous agricultural crops in a short time. Providing a sufficient number of bees is difficult when the queen bees naturally fly around, even if they are reared in nursing families due to weather conditions (wind, low temperature, etc.). To achieve this goal, instrumental insemination of queen bees is used. The effectiveness of this technique and the productivity of a specialist are directly dependent on the technical perfection of the equipment used. In instrumental insemination, the collection of semen from drones is laborious. We have developed at the level of the invention (patent of the Russian Federation No. 2635691, application No. 2017100773 dated 01/10/2017) and tested a stimulator for eversion of the endophallus. The electric ejaculator is an alternating current source, the strength of which does not exceed 5 μA with the ability to regulate the output voltage from 0 to 220 V. Research carried out in the apiary of the Small Innovative Enterprise of the Kuban State Autonomous Institution "ZHIVPROM" Reduces operator time by 60%. Comparative analysis of the sperm quality of drones at the age of 10, 17, 24 days shows that drones of the age of 24 days produce better quality sperm. Its indicators are the best concentration, density and mobility up to 90%. The study of the egg production of queen bees by laying eggs indicates that the maximum egg production is achieved with instrumental insemination using an electric current for evverting the endophalus and collecting sperm. The use of an electric ejaculator with a separately removed handle reduces the time for obtaining drone seed and allows the operator to independently select it without involving an assistant, which reduces labor costs and increases the efficiency of conducting the industry.

1. Introduction

One of the most important sectors in agriculture is beekeeping, the products of which are useful and functional for humans. The pollination function of bees is extremely important, which brings incomes several times greater than from the sale of bee products (propolis, bee bread, honey, wax) [1].
Beekeeping is of great economic importance due to the fact that more than 150 species of entomophilous agricultural crops (fruit and fodder crops, buckwheat, sunflower, rapeseed, clover, alfalfa, etc.) require cross-pollination. Bee pollination increases productivity, improves the quality of seeds and fruits [2].

The number of bee colonies in different geographic zones develops in different ways. The northeastern and northern regions lack the number of colonies required for pollination of crops. Their number is more even in the southeastern regions [3].

The southern region of Russia is the basis for the production of purebred bees of the gray mountain Caucasian breed. The preservation of this breed in Russia is ensured by the State Institution Krasnopolyanskaya Experimental Beekeeping Station [4].

Under natural conditions, significant difficulties in the selection of bees are associated with the control of the mating of queen bees with drones of the desired origin, where insemination of the queen bee can be carried out by a drone of undesirable genetics. The method of instrumental insemination solves this problem and is an effective way to control mating [5].

Improvement of this technique is necessary due to the complexity of taking semen from drones with high labor costs and a large number of drones to take the required sperm dose. However, early insemination of queen bees makes it possible to obtain breeding material with specified economically useful traits, to have fertile queen bees for the production of bee packages and to reduce the cost of maintaining a nucleus farm, and also makes it possible to create sperm banks to preserve the valuable gene pool of bees. With the instrumental method of insemination of queen bees, control is provided for both the amount of sperm injected to the queen bee and the state of the drones themselves, while it becomes necessary to study the possibility and expediency of instrumental insemination of bees using an electro-ejaculator for sperm selection from drones [6].

Instrumental insemination of queen bees has become absolutely necessary in selection and breeding work and in the study of heredity in bees. Today this method of controlled mating is supported even in industrial beekeeping [7].

The unreliability of methods for controlling natural mating explains the need for the earliest possible development of the method of instrumental insemination. Knowledge of the heredity of queen bees helps to solve many genetic and physiological problems associated with the characteristics of bees and allows progress in breeding bees that are resistant to diseases and have higher honey productivity [8].

The most accurate method of controlling the mating of queen bees with drones of a selected origin is the method of instrumental insemination, which provides an opportunity for individual breeding selection. Both the effectiveness of instrumental insemination and the productivity of a specialist are directly dependent on the technical perfection and high quality of the equipment used. The method of instrumental insemination of queen bees began to develop successfully in the 1930s. The technique and equipment were rapidly improving during this period. Modern technology and equipment begin with the work of Watson, who used a microsyringe fixed in a manipulator [9].

Instrumental insemination is slowly being introduced into the practice of domestic beekeeping for a number of objective reasons. The main reason is the lack of high-performance equipment. The method of taking semen from drones remains quite complicated and laborious, which complicates the high-quality insemination of queen bees in large numbers [7].

The Research Institute of Beekeeping has developed, tested and is putting into practice a device for electrostimulation of drones when taking a seed. Observations have shown that the operator needs approximately 600 drones to inseminate 20 queen bees. Outside the family, drones live for about one hour and then die. In paternal families, 20-30 drones are usually selected, with this number 2-3 bees can be inseminated. Before sperm collection, the labeled drones are given the opportunity to perform a cleaning flight to prepare them for surgery. The drones are forced to vibrate with their wings, holding them in the
hand by slightly squeezing and shaking the drone's abdomen, achieve complete eversion of the genitals and ejaculation. All this takes a lot of time when filling the syringe with semen.

2. Research methods and materials

The studies were carried out in the apiaries of the Small Innovative Enterprise of the Kuban Agrarian University and the Krasnopolyanskaya beekeeping station. During the research, instrumental, analytical and statistical research methods were used.

3. Results and discussion

The process of optimization of artificial insemination of queen bees is associated with the feasibility of improving the quality of obtained purebred queens. When using instrumental insemination in 2017, the Kuban State Agrarian University with a population of 145 bee colonies to optimize the method of instrumental insemination was tested by R.D. Rib's electro-ejaculator for obtaining sperm from drones and assembled at the Department of Energy of the Kuban State Agrarian University. The device has been improved with a handle for the electrodes, which makes the ejaculation process of drones more convenient. (patent for invention of the Russian Federation No 2635691) [10].

The electro-ejaculator is an alternating current source, the strength of which does not exceed 5 μA. The output voltage regulation range is from 0 V to 220 V. When using a special voltage converter as a current source, batteries from a flashlight can be taken. For contact with the drones, the electrodes are located on a separate handle and are made of copper wire, which allows you to change the distance between them depending on the size of the drones. The device turns on the network, after which the required voltage is set. The safety of working with the drone electric ejaculator is ensured by a low and non-hazardous current strength.

The efficiency of sperm collection from drones is determined by the degree of eversion of the endophallus, while the greatest effect is achieved with its complete eversion. The research results at different voltage values are presented in table 1.

| Voltage, V | Number of drones pcs. | Number of drones with complete eversion of the endophallus, pcs. |
|-----------|------------------------|---------------------------------------------------------------|
| 150       | 50                     | 27                                                            |
| 160       | 50                     | 31                                                            |
| 170       | 50                     | 41                                                            |
| 180       | 50                     | 44                                                            |
| 190       | 50                     | 46                                                            |
| 200       | 50                     | 49                                                            |
| 210       | 50                     | 49                                                            |
| 220       | 50                     | 50                                                            |

As follows from table 1, with increasing voltage, the number of drones with a complete eversion of the endophallus grows, and at a voltage of 220 V, its complete eversion is observed in all the studied drones.

The timing of the time spent on everting the endophallus manually and when using an electric ejaculator indicates a significant reduction in the time for the operation. If with the manual method the time spent was 6.97 + 0.12 s, then it took 2.62 + 0.10 s to turn out the genitals in the drone under the influence of an electric current, that is, 4.35 s less. Thus, the costs of the laboratory assistant for the operation have decreased by more than 60%.
The difference in eversion of the endophallus depends on the age of the drones. Some individuals, when the endophallus is everted, do not secrete a seed, which entails further manipulations in the form of squeezing sperm out of the endophallus. Considering that electro-ejaculation is possible with the appearance of sperm at the end of the endophallus and depends directly on the age of the drones and the strength of the current influenced on them, we conducted an experiment on eversion of the endophallus with a voltage of 200 V and 220 V. Drones aged 10, 17, 24 days were used for the experiment. The experimental group with the use of an electric ejaculator, the control group without the use of an electric ejaculator. From the data in table 2, it can be seen that there was an increase in the eversion of the endophallus when using a voltage of 220 V in drones at 24 days of age. Thus, the use of an electric ejaculator will increase labor productivity, and therefore improve the economic state of the beekeeping industry when using insemination of bees. Experimental data are contained in table 2.

**Table 2. Efficiency of the electro-ejaculator at different ages of drones and voltage 200 V - 220 V. (n = 50).**

| Group   | Voltage 200 V | Voltage 220 V |
|---------|---------------|---------------|
|         | Number of drones at the age of 10 days | Number of drones at the age of 10 days |
|         | No sperm | With sperm | No sperm | With sperm | No sperm | With sperm |
| Experimental | 20 | 16 | 22 | 28 | 13 | 37 |
| Control | 16 | 12 | 23 | 18 | 19 | 25 |

To determine the quality of sperm, we formed two groups of drones aged 10, 17, 24 days. In the control group, semen sampling was carried out without the use of an electric ejaculator, and in the experimental group with the use of. The voltage was 220 V. The semen was collected with a special syringe used in the system of the apparatus for instrumental insemination. The quality of the semen was assessed according to three indicators: concentration (number of sperm), density, mobility in percent. The results are presented in table 3, which allows us to conclude that the use of an electro-ejaculator does not have a negative effect on sperm quality.

Higher quality semen was found in drones at the age of 24 days. Its indicators are the best concentration, density and mobility up to 90%. Therefore, in order to reduce the time required for obtaining sperm for insemination, an electric ejaculator should be used with a voltage of 200 V and above. With this voltage, the eversion of the endophallus occurs completely with the release of sperm, and no additional time is required to obtain it from incomplete eversion of the endophallus.

Accounting for the egg production of queen bees during instrumental insemination of queen bees, without the use of electric current and with the application, was checked on the 14th day after replanting in the bee colony (table 4).
Table 3. Sperm quality of drones 10, 17, 24 days old (n = 10).

| Drone age | Sperm quality indicators | Control (without electric ejaculator) | Experienced (with an electro-ejaculator) |
|-----------|--------------------------|---------------------------------------|----------------------------------------|
|           |                          | Concentration, mln / μl                | Density | Mobility % | Concentration, mln / μl | Density | Mobility % |
| 10 days   |                          | 2.08±0.3                             | Average | 40%        | 2.05±0.4                          | Average | 70%        |
| 17 days   |                          | 4.70±0.2                             | Thick   | 70%        | 4.60±0.3                          | Thick   | 80%        |
| 24 days   |                          | 5.08±0.3                             | Thick   | 90%        | 5.07±0.4                          | Thick   | 90%        |

According to the results of our research, it was found that the egg production of queen bees in the control group was 936.35±20.46 pcs / day, and with the instrumental in group 2, (without the use of an electric ejaculator for drones) 1026.32±20.31 pcs / day. The maximum egg production rate of 1235.25 ±6.38 pcs / day was recorded during instrumental insemination of queen bees in group 1, where an electric current was used to collect sperm.

Before the main honey harvest, the bee family needs to build up a large family, so the main supplier of bees is the queen bee. The more eggs it lays in the cells, the stronger the bee colony will be by the beginning of the main honey collection.

Table 4. Egg production of instrumentally inseminated queen bees on the 14th day, using an electric current to collect semen from drones. (n = 10).

| Eggs laid per day, pcs. | Natural mating | Instrumental insemination |
|------------------------|----------------|---------------------------|
|                        |                | Using an electric drone   | No electric drone |
|                        |                | ejaculator                | ejaculator       |
| Control group          | 1 group        | 1235.25±6.38              | 1026.32±20.31    |
| 936.35±20.46           |                |                           |                 |

Table 5 shows the results of brood rearing in colonies with queen bees after instrumental insemination before the main honey harvest.

The inseminated queen bees were checked for the amount of sealed brood on the 20th day after replanting.

Table 5. Results of rearing the printed brood before the main honey harvest. (n = 10).

| Groups          | Indicators                  | Sealed brood |
|-----------------|-----------------------------|--------------|
| Control         | Without using an electric ejaculator | 2.5±0.2      |
| Control         | With an electric ejaculator  | 3.0±0.4      |

From the data in table 5, it can be seen that, after instrumental insemination using an electro-ejaculator, the queen bees laid eggs more actively, which was reflected in an increase in the development of bee colonies for honey harvest. Thus, in experienced families, the number of bees for honey harvest was 0.5 more streets, which is 4250 pieces of bees. The intensity of brood rearing using electric current has increased. Oviposition in instrumentally inseminated queen bees begins 3-4 days earlier, which makes it possible to enhance the development and growth of bees for honey collection. Queen bees, instrumentally inseminated with semen obtained using an electro-ejaculator, laid fertilized eggs on the 3rd day.
The productivity of bees was assessed by the amount of collected honey and beeswax. Table 6 shows the indicators of the collected bee production during natural mating in the control group, and when using the method of instrumental insemination in the experimental group.

Table 6. Productive indicators of bee colonies (n = 10).

| Groups                | Indicators                               | Marketable honey, kg | Wax, g     |
|-----------------------|------------------------------------------|----------------------|------------|
| Control               | With natural mating                      | 21.70±0.85           | 604.60±4.97|
| Experimental          | At instrumental insemination             | 29.40±0.99           | 687.60±4.20|

The results of table 6 show that stronger colonies formed with the use of instrumentally inseminated queen bees are distinguished by better performance indicators of honey (+35%), wax (+13%). Thus, with instrumental insemination, the amount of commercial honey and wax produced was greater than with natural mating, which had a positive effect on the overall economic component of beekeeping.

Figure 1. Sampling from a drone.  
Figure 2. Electric ejaculator.

In our opinion, it is advisable to use an electro-ejaculator in specialized breeding bee farms and breeding nurseries in order to reduce the time and costs for the production of instrumentally inseminated queen bees.

4. Conclusions
Instrumental insemination of queen bees makes it possible to obtain the required number of bees in a short time, which is extremely important to ensure full pollination of agricultural entomophilic crops, since bees are currently their main organized pollinators. The efficiency of insemination and the productivity of a specialist are directly dependent on the technical perfection of the equipment used. In instrumental insemination, the collection of semen from drones is laborious. We have developed at the level of the invention (patent of the Russian Federation No. 2635691, application No. 2017100773 dated 01/10/2017) and tested a stimulator for eversion of the endophallus of drones. The electro-ejaculator is an alternating current source, the strength of which does not exceed 5 μA with the ability to regulate the output voltage from 0 to 220 V. Reduces operator time by 60%. Comparative analysis of the sperm quality of drones at the age of 10, 17, 24 days shows that drones of the age of 24 days produce better quality sperm. Its indicators are the best concentration, density and mobility up to 90%. The study of the egg production of queen bees by laying eggs indicates that the maximum egg production is achieved with instrumental insemination using an electric current for everting the endophalus and collecting sperm. The use of an electric ejaculator with a separately removed handle reduces the time for obtaining drone seed and allows the operator to independently select it without involving an assistant, which reduces labor costs and increases the efficiency.
of conducting the industry. To save the time spent on the process of instrumental insemination, the electro-
ejaculator with a separate handle for the electrodes allows you to reduce the time when receiving drone
seed. The inseminator can independently select the semen. Thus, the use of an electric ejaculator will
increase labor productivity, and, therefore, improve the efficiency of beekeeping.

Acknowledgement
This work was carried out under the grant of the Russian Science Foundation 19-76-10010, SSI NIIMMP.
Grant sponsors were not directly involved in the development, analysis, or writing of this article.

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