A Contribution to the Study of Pollination of Alfalfa *Medicago Sativa* (L.) by Bees *Osmia Cornuta* (Latreille, 1805) (HYMENOPTERA: MEGACHILIDAE) in the Donbass Region

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Abstract. *O. cornuta* is a monovaltine polylectic bee, which in natural conditions prefers to forage, visiting fruit and berry crops, or, if they are missing, other plant species. With the aim of defining whether a bee under study is able to mate and build a nest in the summertime, detecting temperature conditions of the first emergence of individuals and specifying the duration of a lifecycle of breeding stock during the period of active flight, we conducted an experiment on the field of alfalfa *Medicago sativa* (Linnaeus, 1753) in agricultural limited liability company “Timiryazevskoe” in Amvrosievskiy raion, in the summertime (the third decade of June – the first decade of July). The data were obtained after artificially prolonged cold diapause of cocoons. For prolongation of diapause, refined cocoons of imagoes were placed in the fridge. After removal of cocoons from the fridge, incubated breeding stock was transported to the experimentation area and placed in previously prepared artificial nesting constructions. For nesting of future offspring bundles of single-channel reed were used. When nesting constructions had been installed, we started to observe emergence of male imagoes, followed by emergence of female imagoes. The article provides information about seasonal and circadian activity, lifecycle of bees *O.cornuta*. The received data could be used to plan artificial breeding of *O.cornuta* with a view to pollination of entomophilous agricultural plants on open ground during the summer period.

1. Introduction

Alfalfa *Medicago sativa* (L.) is one of the most important entomophilous crops that are grown in the Donbass region. This plant contains a large amount of protein and digestible fiber. It is added to the diet of cattle and goats in the form of herbal flour, granules, haylage for increased productivity. It facilitates the search for natural feed protein in stockbreeding [1-4].

The main contribution to pollination of alfalfa is made by wild bees, first and foremost, because this species is an optional cross-pollinating plant of entomophilous type, and is characterized by specific structure of corolla, pistil and stamen, which are not easily accessible for pollination by the honey bee *Apis mellifera* (L.) [5-10].

Honey bees gather nectar not through a corolla throat, but from the side, through a slit between a wing and a banner. That’s why in many countries people breed the alfalfa leafcutting bee *Megachile*
rotundata (F.) for a more qualitative process of cross-pollination, because this species can gather nectar through a corolla throat [11-18].

Information concerning possibility of pollination of this flower by spring species of mason bees, in particular O. cornuta, could be found in the work of V.M. Hukalo. For these goals he prolonged the time of diapause of bees, placing their cocoons in the fridge with specified temperature [19].

The main aim of the study is to investigate possibilities for prolongation of diapause in O. cornuta for subsequent pollination of alfalfa and to study circadian and seasonal dynamics of their flight during pollination of this plant in summer.

The findings of the study could be used to plan artificial breeding of O.cornuta with a view to pollination of agricultural plants on open ground during the summer period.

2. Materials and methods

The study of seasonal and circadian dynamics of flight, phenology and foraging behavior of O. cornuta was undertaken from June 19 till July 24 2021, on the field of alfalfa (a variety «Свиточ»), with total area of 31,556 ha in agricultural limited liability company “Timiryazevskoe” in village Kuteinikovo, Amvrosievskiy raion, Donetsk People Republic (Fig.1.)

![Figure 1. Geographical location of the investigated area under research.](image.png)

For a longer period of diapause cocoons of imagoes (n= 272) were kept in the fridge (Samsung RL56GREIH). Placement of cocoons in the cooling chamber was implemented in February, 2021.

For conducting the research an insectarium was constructed with total area of 12 m ² (3m x 4m x 2m); a net with a cell size of 1mm was used. Beforehand four-side edged wooden bars 45mm x 45 mm had been prepared, with a bar of 220 sm. in height (Fig.2.).

For covering the wooden framework we used 20 m of fiberglass net coated with polyvinylchloride to make it mechanically stable. Additional fixation of the net on the wooden frame was achieved with the help of the stapler gun STAYER 3150_z01 with the maximum length of a staple 14 mm. A door was installed on steel mortise hinges 100x75x2,5 mm.
Figure 2. An insectarium in the area under research.

Transportation of cocoons was carried out in the temperature controlled container “Arktika” at a temperature of + 7°C (Fig.3.).

Figure 3. Queen cocoons of O. cornuta.

Received data, after visual observation, were registered in the field book; moreover, photos and videos were made with the help of the electronic device (Xiaomi Redmi Note 9). Temperature measurement was performed with the help of the laboratory mercury thermometer TL №1. For nesting of future offspring bundles of single-channel reed were used. (Fig.4.)
Figure 4. Bundles of single-channel reed.

The bundles were placed in the beehive, constructed from foamed polyvinylchloride sheet, 4 mm thick and 2m x 3m in size. Parameters of nesting cavities offered for settlement, varied: diameter was from 5 mm to 11 mm, length was from 100 mm to 300 mm. Details of the construction were joined with the help of the glues «COSMO» CA/500.200 and «COSMO» SL - 660.220. This technology of constructing a beehive was used for the first time. (Fig.5.)

Figure 5. A beehive for nesting of bees O. cornuta.

3. Results and Discussion
In the course of the investigation a possibility of prolongation of diapause of bees O. cornuta by placing cocoons of imagoes in the fridge (t +5 °C) for four months has been confirmed. During the experiment placement of cocoons in the fridge was implemented in February, while their removal was carried out in June.

After the end of the artificially created diapause, during first two hours after removal of cocoons from the fridge with t +30 °C and air humidity 47%, active emergence of male imagoes was observed. After it emergence of female imagoes happened within five hours in the first day of the third decade.

Active flight of males was noted during first 4-5 days after their emergence from cocoons, and by the end of June flight of males in the area under study had been over. Thus in case of prolonged diapause flight of males lasted 10 days. It should be mentioned for comparison that in case of normal diapause flight of males lasts at least 24 days [20].

The maximum activity of females was registered during first 7-9 days. From the beginning of July it started to decline and finally stopped on July 12 (Fig.6.). Thus in case of prolonged diapause the period of flight of females was also reduced (22 days) as compared with normal diapause (33-35 days) [20].
3.1 Foraging activity
Foraging activity in females *O. cornuta* on flowers of alfalfa (Fig. 7) was noted right after mating and colonization of hollow reed pipes (Fig. 8).

**Figure 6.** Nest-building activity of *O. cornuta* on the field of alfalfa *Medicago sativa* (L.) with nests in bundles of reed.

**Figure 7.** Foraging activity of a female (A) and male (B) *O. cornuta* on the field of alfalfa *Medicago sativa* (L.) at the moment of visiting.
Figure 8. Mating of a male and a female *O. cornuta* (19.06.2021).

Females actively collected pollen from flowers of alfalfa, correctly penetrating a corolla throat of a flower.

3.2 Phenological development

It has been found out that the duration of preimaginal phases from egg to pupa in *O. cornuta* lasts 84 days (3.5 months) from the end of June till the second decade of September. With natural diapause, this period is at least 115 days [20].

Oviposition began in the third decade of June and came to an end in the second decade of July. Formation of larvae of younger ages lasted 23 days, prepupal stage – 17 days, formation of mature larvae in cocoons – 18 days. Adult imagoes in cocoons emerge in the second decade of September.

Thus the longest period of development of a new generation on the stage “egg – pupa” was development of a larva, namely transformation of a larva of younger ages in a mature larva in a cocoon (50 days), while the shortest period was the process of emergence and mating of imagoes – 8 days (Table 1.)

| June | July | August | September | October | November |
|------|------|--------|-----------|---------|----------|
| 1    | 2    | 3      | 1         | 2       | 3        | 1         | 2    | 3    |
| [♀,♂] |     |        |           |         |          |           |     |      |
| ♀♀   | ♀♂   |        |           |         |          |           |     |      |
| E     | E     | E     |           |         |          |           |     |      |
| L     | L     |        |           |         |          |           |     |      |

Table 1. The phenogram of development of the bee *O. cornuta* in Kuteinikovo.
3.3 Circadian activity of a bee O. cornuta

Observation and recording of the results of the study were held from 8 AM till 11 PM on June 21, 2021. In the course of the observation of circadian activity in females a gradual increase in the number of individuals was noted in the period of time between 3:32 PM and 4:32 PM. Their peak number was registered in the period of time between 5:32 PM and 6:32 PM. A decline in activity was observed between 7:32 PM and 10:32 PM. (Fig.9.)

In the course of the observation of circadian activity in males a gradual increase in the number of individuals was noted in the period of time between 10:32 AM and 4:32 PM. Their peak number was registered in the period of time between 5:32 PM and 6:32 PM. A decline in activity was observed between 7:32 PM and 9:32 PM. (Fig.10.)

Thus the period of the maximum activity in males and females during the day coincides, while the period of gradual increase in the number of individuals differ. The decline in activity of males is shorter by one hour in comparison with that of females.

Figure 9. Circadian activity of females O. cornuta in the area under research.
3.4 Seasonal abundance

The seasonal abundance of males and females in the area under research is represented on the diagram (Fig.11.). In the first day of the experiment (June 19, 2021) at 1 PM emergence of males (3 individuals) out of the incubated queen cocoons was registered; in 3 hours, at 4 PM females (2 individuals) emerged. On June 21 the number of males was higher compared with that of females.

The number of females remained at the stable maximum level from June 25 till June 26. After it the number of individuals decreased and achieved a null-value on July 14.

Thus the increase in the number of males and females stopped on June 25. The period of males’ activity lasted 10 days, that of females lasted 23 days. The peak of males, maximum seasonal activity was 2 days, that of females was 4 days.

4. Conclusion

As a result of the study we have ascertained the possibility of the emergence of imagoes of the bees O. cornuta after artificially prolonged diapause, as well as their active flight, mating, foraging behavior, building of nests in the summer time (June – the first decade of July).

Besides, in comparison with spring (in case of normal diapause), flight time of males and females was slightly reduced (flight time of males was reduced approximately thrice, that of females was reduced by half). In the same time high foraging activity of females on the flowers of alfalfa has been
observed. In the isolated area of the field females collected pollen only from the flowers of alfalfa. In sealed nests the new generation of the species under study was obtained.

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