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Short communication

REARING OF THE BEET WEBWORM *LOXOSTEGE STICTICALIS* (LEPIDOPTERA: CRAMBIDAE) UNDER LABORATORY CONDITIONS USING AN ARTIFICIAL DIET

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The beet webworm *Loxostege sticticalis* is an economically important polyphagous pest causing serious damage to many agricultural crops. The work is devoted to the development of the methodology of propagation of beet webworm on an artificial diet. The peculiarities of the beet webworm maintenance at all stages of development are described. The weight of *L. sticticalis* pupae obtained from the larvae fed on natural feed (leaves of the burdock *Arctium lappa*) and artificial diet was compared. The results showed that there were no decrease in pupal weight after transferring insects to the artificial diet and maintenance for 50 generations. This indicates a sufficient accumulation of reserve substances during larval feeding with the diet. We have developed the method of continuous rearing the beet webworm on artificial diet, which allows continuously obtaining insects in laboratory conditions.

Keywords: permanent culture, laboratory maintenance, substitute feed, polyphagous lepidopterans

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Introduction

The beet webworm *Loxostege sticticalis* L. (Lepidoptera: Crambidae) is considered one of the most dangerous pests throughout Eurasia, including northern China and the steppe zones of European and Asian parts of Russia, due to its high damage rate, wide polyphagia and migration patterns. It causes serious damage to such crops as sugar beet, soybean, alfalfa, sunflower, corn and cereals. To date, more than 50 families and more than 300 species of plants damaged by larvae of *L. sticticalis* have been registered (Xiang et al., 2013; Malysh et al., 2020).

The migration distances of *L. sticticalis* adults can reach several hundred kilometers, with long-distance flights in the continental system of atmospheric fronts being a characteristic feature (Melnichenko, 1936; Makarova, Doronina, 1994).

The beet webworm is a host for parasites, predators and pathogens that can contribute significantly to the regulation of its population. However, examination of the interactions of the beet webworm with its natural enemies requires continuous maintenance of this pest throughout the year, rather than studying it only during periods of mass reproduction, as is currently done. In addition, working with the beet webworm is further complicated by long periods of population decline, when the insect can hardly be found in the wild.

An important feature in laboratory rearing of the beet webworm is the ability to synchronize the development of larvae with high precision (within 1–2 h), which is very important for the study of physiological and biochemical parameters. This makes it a very convenient laboratory model for physiological, biochemical, microbiological and toxicological studies.

To date, there are very few works describing the maintenance of the beet webworm in laboratory conditions on artificial diet (Kaçar et al., 2023). In addition, this artificial diet is mainly composed of forage plant (Wu et al., 2012), which certainly makes it difficult to reproduce. Therefore, the aim of the present work is to develop a methodology for year-round maintenance of the beet webworm *L. sticticalis* on artificial diet consisting of available ingredients and to obtain a permanent laboratory insect line.

Materials and Methods

The laboratory line of the beet webworm *L. sticticalis* originated from 30 moths caught in the vicinities of the city of Krasnoyarsk in August 2021. The moths were kept at 26 °C and a long-day photoperiod (18:6 h) in 500 ml glass jars. For efficient collection of the eggs, the bottom and walls of the jars were lined with paper and covered with gauze. The moths were fed with 15 % honey syrup, which was abundantly applied to cotton balls, placed inside the jars. The cotton balls were replaced daily with fresh ones. Paper sheets with laid eggs (Fig. 1A) were collected every second day and placed in glass Petri dishes (11 cm in diameter) with a cotton ball moistened with distilled water to maintain a sufficient level of humidity (at least 60 %). Petri dishes with eggs were placed in a thermostat at 26 °C. Larvae began to hatch 2 days after egg laying.

Immediately after hatching, a portion (1.5 g) of the artificial diet (see below) was placed into the Petri dish. The feed was provided in the form of a stripe, placed along the inner edge of the Petri dish to cover about 1/6 of its circumference. The feed was replaced every second day. Up to 200 first-second instars were kept per Petri dish. After larvae molted to the third instar, the diet was placed in the center of the inner side of the

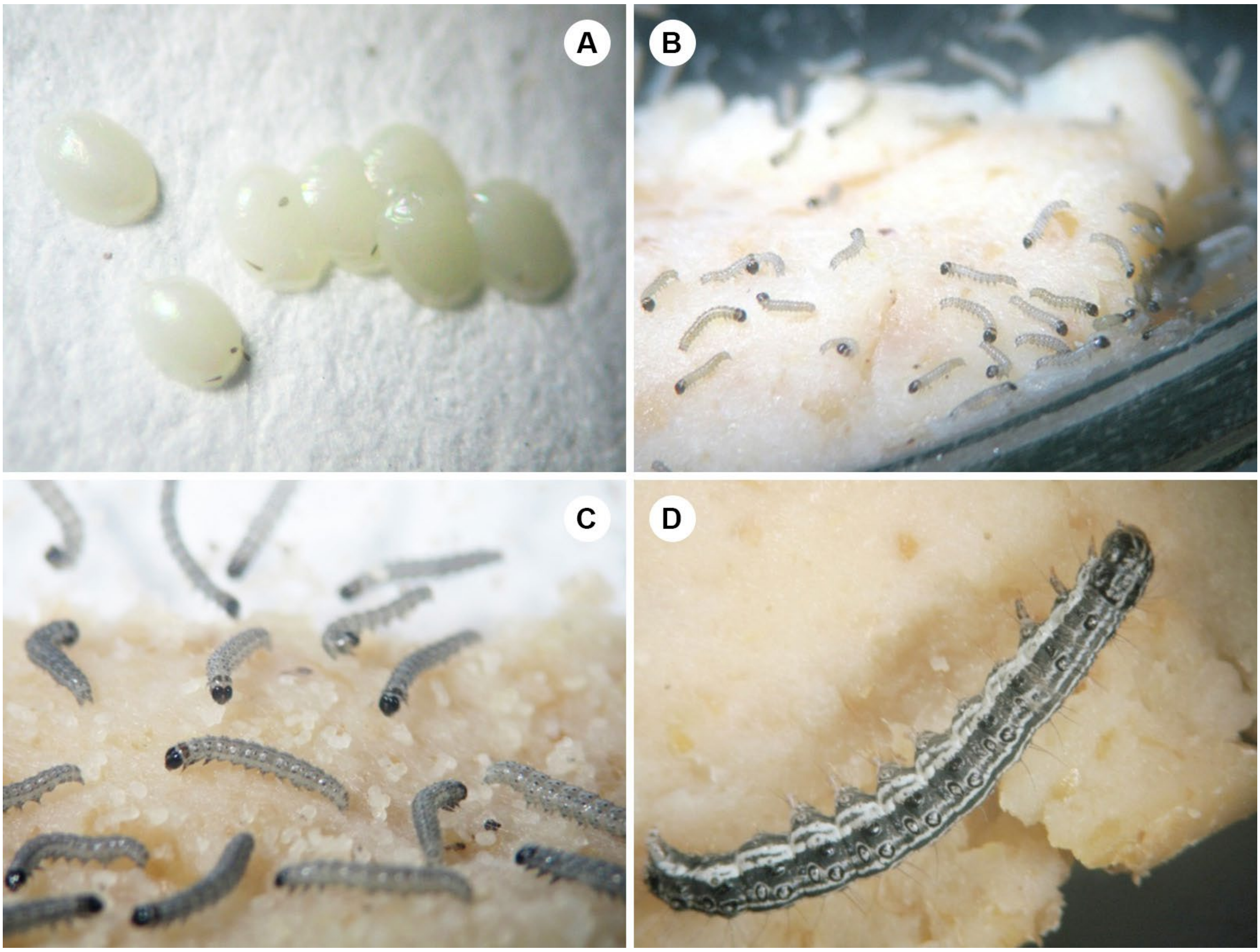

Figure 1. The eggs (A), first (B), third (C), and fifth (D) instar larvae of beet webworm *Loxostege sticticalis* reared under laboratory conditions on artificial diet

Рисунок 1. Яйца (A) и гусеницы первого (B), третьего (C) и пятого (D) возрастов лугового мотылька *Loxostege sticticalis* при выращивании в лабораторных условиях на искусственной питательной среде

Petri dish lid; and the number of larvae was reduced as not to exceed 100 individuals per dish. Fourth instars were kept at the density of 20 individuals per dish. After molting to the fifth instar, larvae were placed in 500 mL glass jars in groups of 50 to complete larval feeding and proceed to pupation. A portion of diet (7–10 g) was placed on the inner surface of the jar walls. Alternatively, fresh leaves of the burdock *Arctium lappa* were provided as feed throughout the larval development. Finely shredded paper (80 g/cm^2) was used as the substrate for pupation. The jars were capped with lids possessing fine metal mesh inserts for ventilation.

The artificial diet was developed based on methodology proposed for the European corn borer *Ostrinia nubilalis* (Frolov et al., 2019) and the cotton moth *Spodoptera littoralis* (Sayed et al., 2021). The composition of artificial diet was modified with the replacement of some ingredients, and the resulting formulation was tested on a laboratory population of beet webworm during three years of continuous maintenance. The major nutritive components of the diet were the yeast powder, soy flour, wheat bran, and corn meal, supplemented with agar for solidification. Other essential additives included alpha-tocopherol in sunflower oil and ascorbic acid. Benzoic acid served as a preservative (Table 1).

To prepare the ready-to-use diet, the agar was thoroughly mixed with distilled water melt on an electric stove, benzoic acid added, and brought to a boil. Then the mixture was cooled to 70–75 °C and thoroughly mixed with the dry nutritive components. Ascorbic acid was dissolved in 5 ml of distilled water, while alpha-tocopherol was added to sunflower oil. These ingredients were added to the main mixture after it was cooled to 50–55 °C. The diet was finally mixed to prepare a homogeneous mass, poured into Petri dishes for solidification at room temperature, and stored in the refrigerator for no more than 14 days.

Beet webworm pupae were kept in shredded paper at the temperature of 26 °C until the moths emerged (about 14 days). The latter were transferred to 500 mL glass jars in the groups of 10, at the 1:1 sex ratio. After emerging, moths needed additional nutrition, provided in the form 15 % honey syrup. Without feeding, the females remain sterile and males lose the ability to copulate repeatedly (Knorr et al., 1997).

For weight measurements, the pupae from each experimental group were collected and subdivided into males and females to evaluate separately. The laboratory scales Adventurer (Ohaus) were used to determine pupal weight. For statistical analysis, Student's t-test was exploited.

Table 1. Composition of the artificial diet utilized for feeding the beet webworm larvae

Ingredient	Quantity
Agar	3.8 g
Brewer's yeast powder	7.5 g
Soy flour	21 g
Wheat bran	8.5 g
Corn meal	15 g
Sunflower oil	1 mL
Alpha-tocopherol (added to the oil)	20 µL
Ascorbic acid	1.3 g
Benzoic acid	0.75 g
Distilled water	220 mL

Таблица 1. Состав искусственной питательной среды, используемой для кормления гусениц лугового мотылька

Ингредиент	Количество
Агар	3.8 г
Пивные дрожжи (порошок)	7.5 г
Соевая мука	21 г
Пшеничные отруби	8.5 г
Кукурузная крупа	15 г
Подсолнечное масло	1 мл
Альфа-токоферол (добавляется в масло)	20 мкл
Аскорбиновая кислота	1.3 г
Бензойная кислота	0.75 г
Дистиллированная вода	220 мл

Results and Discussion

The pupal weight is known to be the key parameter, as its value is determined by the "quality of life" throughout the larval stage, the amount of reserve substances accumulated in the fat body during larval development (Alekseev, 2000). The pupal weight plays an important role in the induction of diapause, which is an important adaptation that allows insects to avoid unfavorable environmental conditions (Liu et al., 2010). Thus, it can serve as an integral indicator of insect fitness and premature developmental success.

We compared weight of the beet webworm pupae reared on artificial vs natural feed. In females, the values were nearly the same, reaching 32±4.3 mg in the case of burdock leaves (number of examined pupae N=37) and 35±4.1 mg in the case of artificial diet (N=21). As for males, the pupae weighted 26.8±5.0 mg (n=37) and 33.6±3.2 mg (n=21), in the cases of leaves and diet, respectively (Fig. 2). And though there was a tendency of slightly higher values of pupal weight in insects reared on artificial diet, these differences were not statistically significant. Obviously, a sufficient amount of reserve substances was accumulated during larval development on both types of feed.

The beet webworm is polyphagous and can feed on a rather broad range of forage plants both in nature and laboratory. According to our observations, the burdock is among the preferred plant species. Its leaves are relatively thick, stay fresh after being cut for several days and can be stored in a refrigerator. During the vegetation season, it served as the forage plant to rear hordes of beet webworm larvae without loss of fitness over generations. It was therefore used as a handy model of the forage plant when artificial diet came into play. Interestingly, the pupal weight was not decreased after rearing insects on artificial diet for 50 generations. Therefore, all the necessary pre-requisites have been met to found and maintain the permanent laboratory culture of the pest.

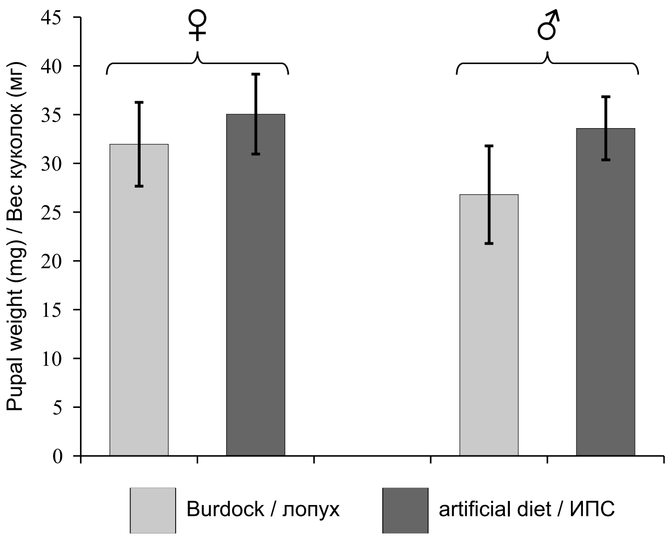

Figure 2. Pupal weight of the beet webworm *Loxostege sticticalis* reared on the burdock leaves vs artificial diet. Mean value ± confidence interval

Рисунок 2. Вес куколок лугового мотылька *Loxostege sticticalis*, выращенного на листьях лопуха или на искусственной питательной среде (ИПС). Средние значения ± доверительный интервал

Conclusion

Based on the obtained results, we can judge that the designed artificial diet is a worthy substitute for the natural feed and allows year-round maintenance of the beet webworm *L. sticticalis* in laboratory conditions. A stable laboratory line (50 generations) is obtained.

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Краткое сообщение

КУЛЬТИВИРОВАНИЕ ЛУГОВОГО МОТЫЛЬКА *LOXOSTEGE STICTICALIS* (LEPIDOPTERA: CRAMBIDAE) В ЛАБОРАТОРНЫХ УСЛОВИЯХ С ИСПОЛЬЗОВАНИЕМ ИСКУССТВЕННОЙ ПИТАТЕЛЬНОЙ СРЕДЫ

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Луговой мотылек *Loxostege sticticalis* – экономически важный вредитель, полифаг, наносящий серьезный ущерб многим сельскохозяйственным культурам. Работа посвящена разработке методики культивирования лугового мотылька на искусственной питательной среде. Описаны тонкости разведения лугового мотылька на всех стадиях развития. Проведено сравнение веса куколок лугового мотылька, выращенного на естественном (листья лопуха *Arctium lappa*) и искусственном корме. Результаты показали отсутствие снижения веса куколок при переводе насекомых на искусственную питательную среду и выращивании в течение 50 поколений, что свидетельствует о достаточном накоплении резервных веществ при питании гусениц. Таким образом, разработан метод лабораторного культивирования лугового мотылька на искусственной питательной среде и создана постоянная лабораторная культура, что позволяет круглогодично получать насекомых для исследовательских целей.

Ключевые слова: постоянная культура, лабораторное содержание, альтернативный корм, чешуекрылые полифаги

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