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Invertebrates of Siberia, a potential source of animal protein for innovative food production. 4. New method of protein food and feed products generation

Беспозвоночные Сибири как перспективный источник животного белка для инновационного производства продуктов питания. 4. Новый способ получения белковой пищевой и кормовой добавки

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Ключевые слова: Insecta, Coleoptera, Scarabaeoidea, ксилосапрофаг, Сибирь, выращивание.

Abstract. Terrestrial invertebrates are raised for producing biomass rich in animal protein, that could be used for feed or food products. A new method proposed here involves the non-stop use of invertebrates occurring in Siberia raised on cellulose-rich feed substrates to be developed under conditions of small or medium farms. Two saproxylic beetle species, green rose chafer *Cetonia aurata viridiventris* Reitter, 1896 and the European rhinoceros beetle *Oryctes nasicornis* (Linnaeus, 1758) are proposed as model species for this study. Biochemical analysis of model species larvae showed a high content of nutrients and reduced calorific value in the respective biomasses of *Cetonia aurata* and *Oryctes nasicornis*, namely 20.8 and 25.9 % protein, 0.44 and 0.14 % fat, 0.27 and 0.23 % carbohydrates and calorific values of 99 and 96 kcal. Diverse contents of vitamins and minerals are also found, with the highest content of Mg 288 mg/100 g in *Cetonia aurata* and 251 mg/100 g in *Oryctes nasicornis*, and P 450 mg/100 g in *Cetonia aurata* and 400 mg/100 g in *Oryctes nasicornis*. Principles of feed substrate preparation, synchronization of beetle life cycles, and technological requirements for raising beetles on a farm are discussed. The duration of the life cycle decreased from 2 years in natural habitats to 7 months under laboratory conditions.

Резюме. Наземные беспозвоночные выращиваются для получения животного белка, либо с целью получения деликатесов (улитки ахатины), либо как побочный продукт шелководства (куколки *Bombyx mori*) и, как правило, представлены теплолюбивыми видами. Предлагаемый новый метод позволяет наладить непрерывное производство беспозвоночных, обитающих в Сибири, на целлюлозосодержащих субстратах в условиях фермерских хозяйств. В качестве модельных выбраны два вида ксилосапрофагов: бронзовка *Cetonia aurata viridiventris* Reitter, 1896 и жук-носорог *Oryctes nasicornis* (Linnaeus, 1758). Нутриентный анализ личинок выявил высокое содержание питательных веществ при небольшой калорийности: 20,8 и 25,9 % белка, 0,44 и 0,14 % жира, 0,27 и 0,23 % углеводов при калорийности 99 и 96 ккал. Отмечено разнообразное содержание витаминов и минералов с повышенным содержанием магния: 288 мг/100 г и 251 мг/100 г, и фосфора: 450 мг/100 г и 400 мг/100 г соответственно. Принципы подбора субстратов и синхронизация жизненного цикла жуков с условиями искусственного выращивания подробно обсуждаются в статье. Весь цикл развития сократился с 2-х лет до 7 месяцев.

Introduction

Currently, interest in protein production from terrestrial invertebrates is increasing [Oonincx, Poel, 2011; Van Hius et al., 2013; Zielińska et al., 2015; Derrien, Boccuni, 2018; Gorbunova, Zakharov, 2021; Shah et al., 2022; Tshernyshev et al., 2022]. In parallel with well-known companies worldwide, several new developments have appeared in Russia during this time [Gorbunova, Zakharov, 2021], namely: Entoproteh, in Moscow, utilizing organic waste material by the black soldier fly; Zooprotein, in Lipetskaya Oblast, utilizing livestock waste material by insect larvae mainly of the fly *Lucilia caesar*; Inagrobio, in Yaroslavskaya Oblast, utilizing reared larvae of the fly *Musca domestica* within processed livestock waste material, and also small private enterprises, such as T-RexFood, in Moskovskaya Oblast, which produces feed for domestic animals on the basis of rearing the tenebrionid beetle *Zophobas*, crickets, cockroaches and other insect species on organic substrates.

As a rule, selected southern insect species are chosen as a source of food protein. Four species, locust *Locusta migratoria* Linnaeus, 1758, the yellow mealworm beetle *Tenebrio molitor* Linnaeus, 1758, house cricket *Acheta domesticus* (Linnaeus, 1758) and lesser mealworm *Alphitobius diaperinus* Panzer, 1797 are approved by the European Commission (EC) for sale, farming and novel food consumption [EEC, 2004; EFSA, 2015, 2022; Lahteenmaki-Uutela, Grmelova, 2016; Van Peer et al., 2021; EEC, 2023]. There is a preference for using southern species due to their fast life cycle but rearing them in Siberian farms is difficult due to their specific requirements of warm temperatures, additional illumination, and special composition of feed.

Northern Asia is rich in forests and necessary timber works, but in the southern regions crops and legumes are mainly cultivated. From these two sources enormous quantities of organic waste in the form of timber (including sawdust, branches, bark) and agricultural material (including straw) are produced. This waste is rich in cellulose, lignin, hemicellulose and associated material, much of which is not used in industry. However, in Siberia, insect species occur which successfully develop in cellulose-rich remains in natural habitats. Two species, green rose chafer *Cetonia aurata viridiventris* Reitter, 1896 developing in decaying grassy and woody remains and the European rhinoceros beetle *Oryctes nasicornis* (Linnaeus, 1758) developing in decaying timber have been experimentally chosen for the generation of animal protein [Skriptcova et al., 2023; Tshernyshev et al., in litt.]. Biochemical study of these saproxylic beetle larvae, respectively showed a high level of protein in terms of wet matter (20.8 % in *Cetonia* and 25.9 % in *Oryctes*), presence of carbohydrates (0.27 and 0.23 %) and fat (0.44 and 0.14 %) under low mean calorific values (99 and 96 %). The biomass was rich in phosphorus (450 and 400 mg/100 g) and magnesium (288 and 251 mg/100 g), and contained significant levels of Fe, Se, Zn, Mo, Mn and Cu. Amongst vitamins, significant levels of B1 (2.5 and 3.4 mg/100 g), B2 (5.5 and 7.1 mg/100 g), E (0.59 and 0.74 mg/100 g) and A (0.0337 and 0.0414 mg/100 g) are revealed. Thus, the biomass of model species of larvae may be characterized by their nutrient composition (i.e. supplied with relatively more nutrients than calories) [Drewnowski, Fulgoni, 2023].

Material and methods

To generate an experimental line of non-stop rearing of saproxyllic beetles, a common species in forest-steppe of Southern Siberia, the rose chafer *Cetonia aurata viridiventris* Reitter, 1896 (Coleoptera: Scarabaeidae), was selected. Adult beetles were collected in suburbs of the cities of Tomsk and Novosibirsk in July 2023. During midsummer beetles occur in high numbers, and withdrawal of 100 specimens from different natural localities had no impact on local populations quantity.

The experimental line for rearing the rose chafer has been organized in laboratory room of the Tomsk University; its small space made it possible to raise a complete life cycle of the beetle, and to determine the minimal requirements for breeding under laboratory conditions, but scaling to industrial production should be treated under farm conditions.

Recording parameters of rearing by standard detectors included weighing larvae by an electronic balance (8068-series, Jewelry scale, China), and temperature and humidity were measured by means of an electronic thermohygrometer (FY12 with liquid-crystal display and remote sensor, China).

The present work is registered in ZooBank (www.zoobank.org) under LSID urn:lsid:zoobank.org:pub:C8B309C3-29B9-4B88-BA1B-CD02C7C4F7BD.

Results

Rearing cycle of the saproxylic beetle *Cetonia aurata* viridiventris Reitter, 1896 for biomass production

An experimental procedure developed for rearing model species larvae consists of four stages as follows: ***breeder***, to keep imago for egg nesting; ***incubator***, for egg maturation; ***larvae rearer***, for larvae development; ***pupae rearer***, for pupae maturation. The feeding substrate used for cetoniid larvae is fermented sawdust in combination with plant remains, and for *Oryctes* beetles it is fermented sawdust. Feeding substrates are enriched with nutrient precursors, which accumulate particular nutrients in the biomass of larvae. The average temperature of rearing is c. +25 °C, illumination is only needed for the imago stage, and the average humidity of the feeding substrate for larvae is c. 70 %. Decreasing the temperature to +15 °C is not fatal for these larvae in comparison with southern or tropical species.

Breeder, to keep imago for copulation and egg nesting

The first stage of the experimental line is directed towards maintaining the imago for successful copulation and subsequent oviposition (Fig. 1). The 40 x 30 x 30 cm breeder container for the experiment is made of transparent glass, the bottom of which is covered with a feeding substrate for larvae to attract females laying eggs. Since females normally dig into the substrate before nesting, it should be at least 10 cm in depth and appropriate for successful feeding of larvae newly bursting from eggs. These parameters for the substrate need to satisfy the females to start oviposition. To maintain stable microclimatic conditions and provide suitable places for beetle aggregation for further copulation, the breeder is provided with live plants which foliage and stems lack glandular hairs such as *Chlorophytum, Senseviera* or *Chavortia*, popular in domestic flower growing. The feeding substrate, given to adults in separate small Petri dishes, is easily found by beetles and eaten. In nature, the rose shafer imago feeds on the flowers of different plants such as lilac, rose and peony, often rendering the inflorescence unsightly. A feeding substrate containing sugar which is necessary for metabolism, in combination with honey to attract beetles, is a good substitution for flowers under laboratory conditions.

The breeder is designed for 25 pairs of imagos, which are kept for 12 hours under the daylight lamp, followed by 4 hours under combined illumination with an ultraviolet lamp. The breeder is provided with sensors for temperature and humidity, the former is stabilized at c. 25–26 °C by means of an infra-red electric mat, and the latter inside the breeder is c. 65–80 %.

Composition of feeding substrate for imago: apple fragments greased with thin layer of honey.

Composition of feeding substrate for egg nesting: mixture of sod soil (40 %) and ground cellulose-contained organic waste as sawdust of deciduous and coniferous timber (60 %).

Every four days, the breeder is inspected for the presence of eggs in the substrate.

Incubator

Each female laid 15 eggs during one oviposition, these were taken from the breeder and transferred to the substrate surface in the incubator; such numbers of eggs can be laid by the female during its life. A plastic container 15 x 20 x 6 cm provided with a press-on cap is used for the incubator, which is designed for 100 eggs, the surface of its substrate making it possible to monitor egg maturation and larvae exclusion (Fig. 2). Sensors for temperature and humidity are provided inside the incubator for environment parameter control.

Eggs are incubated for 8 days under a temperature of 29 °C and humidity of 70 % or higher. Under a temperature of 24 °C, incubation is prolonged to 14 days. After exclusion larvae are transferred to feeding containers of the larvae rearer.

Composition of feeding substrate for egg incubation: mixture of sod soil (40 %) and grinded cellulose-contained organic waste as sawdust of deciduous and coniferous timber (60 %). The incubator is permanently ventilated and inspected every 2 days.

Larvae rearer

This stage relates to the rearing of larvae for biomass production, and the selection of pre-pupae for continuation of the beetle life cycle.

The larvae rearer is composed of a plastic container 35 x 25 x 15 cm provided with press-on perforated cap, and sensors for temperature and humidity. Groups of such containers are housed in shelves. Illumination and warming are absent, but the larvae are maintained at the laboratory temperature of 23 °C, while in nature they are developed deep inside the substrate under temperatures of 15 to 18 °C. The bottom of the larvae rearer is filled to a depth of 10 cm with feeding substrate. Each container is designed for 10 larvae.

The larvae are arranged in separate containers according to four age stages, namely: L1 — newborn (< 1 cm), L2 — initial growth (1 to 2 cm), L3 — median growth (2 to 3 cm), and L4 — pre-pupation (> 3 cm). At the final stage the larvae are dark yellow, and non-active, having prepared a cocoon for pupation. At stage L3 larvae are eliminated for biomass production. Larvae of stage L4 are transferred to pupae rearer for further rearing. The larvae are fed over 6 weeks at a temperature of 23 °C and humidity of 70 %. By lowering the temperature to c. 15–18 °C, a level more typical of natural habitat conditions, elongation of larvae was recorded over a period of 12 weeks. Moisturizing the substrate is provided once a week with a sprayer if necessary.

Composition of feeding substrate for larvae nutrition: mixture of sod soil (40 %) and ground cellulose-contained organic waste as sawdust of deciduous and coniferous timber types (60 %).

Every 2 weeks following the second week when larvae have started feeding, 80 % of the substrate is substituted by newly prepared substrate. At the 11th week, the containers with prepupae are monitored every 2 days, and generated cocoons are transferred to the pupae rearer.

Pupae rearer

A plastic container 25 x 17 x 10 cm is provided with a press-on cap filled with a wet substrate, together with temperature and humidity sensors and an infrared mat for warming, as a breeder for pupae. Each breeder is designed for 20 pupae. Costs for environment moisturizing and warming, and substrate provision are optimal under this quantity for minimal duration of incubation.

Composition of feeding substrate for pupae: moisturized mixture of sawdust.

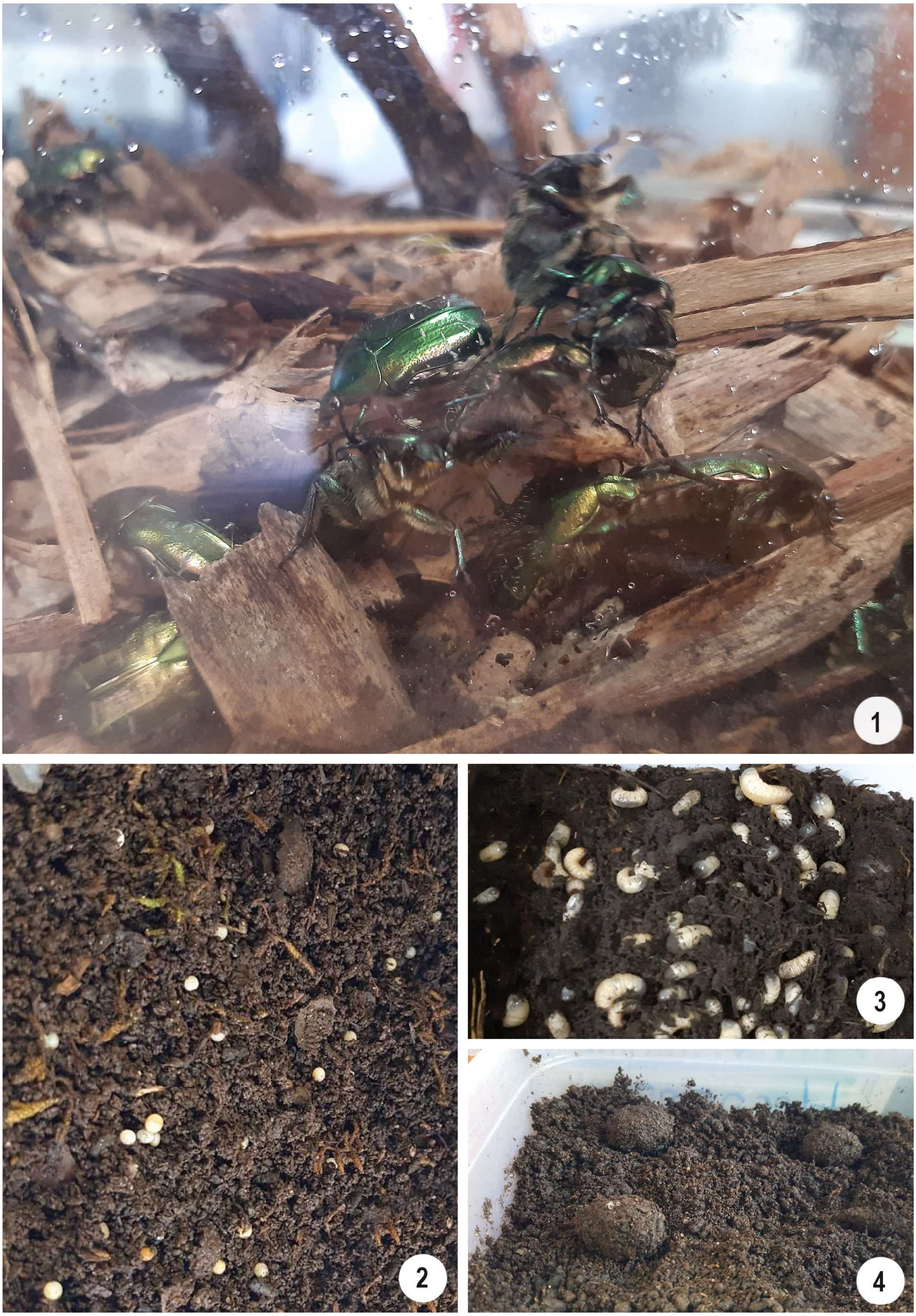
The incubation of pupae lasts for 25–26 days at a temperature of 28 °C. Twice a day following the 20th day of incubation, each container is inspected and emerged imago transferred to the breeder.

The duration of a complete cycle of normal beetle development decreased from 2 years to 7 months.

Discussion

For many years, saproxylic scarabeids have attracted attention as effective destructors of plant remains [Mico et al., 2011], the source of supplementary human consumption [Assielou et al., 2015; Kim, Park, 2016; Meyer-Rocho et al., 2018; Meutchieye, 2019; Oyewale, Makinde, 2023], and even as pests of palm trees [Doane, 2013; Pradiptra et al., 2020; Moore, Siderhurst, 2022; Marshall et al., 2023].

Species of the genus *Cetonia* Fabricius, 1775 are widespread, their adults occur in high number on blooming plants, and their larvae are common in decaying plant remains and decomposing timber. Due to their remark-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able numbers and availability explains why they have been considered for human consumption. For example, in Cameroon local species of *Cetonia* are consumed as dried adult beetles and their larvae roasted in oil [Meutchieye, 2019]. In Korea, *Cetonia pilifera* Motschulsky, 1860 [Kim, Park, 2016], *C. aurata* (Linnaeus, 1758) and *Protaetia brevitarsis* (Lewis, 1879) [Meyer-Rocho et al., 2018], are considered as promising food sources and used in cooking, but not reared at an industrial scale.

To date, methods for rearing flower chafers have not been developed. However, recipes of feeding substrates and indexes of environment requirements are generated for raising beetles in zoological gardens, as in the case of the noble chafer *Gnorimus nobilis* (Linnaeus, 1758) [Ellegaard, Bach, 2020]. A brief description of raising *Cetonia aurata* (Linnaeus, 1758) in the laboratory is used for educational purposes by A. Bikseleev [2015].

Amongst representatives of the genus *Oryctes* Illiger,1798 more detailed studies have been undertaken on *O. rhinoceros* (Linnaeus, 1758), a pest of coconut and oil palms in South-East Asia. The damage caused by this beetle was registered long ago, as, for example, coconut palms in Samoa since 1910 [Doane, 2013]. Detailed studies of the impact of this pest have been undertaken in the Solomon Islands [Tsatsia et al., 2018; Marshall et al., 2023], Indonesia [Pradiptra et al., 2020] and other territories of South-East Asia. The tangible damage allowed one to consider different methods of control such as gathering beetles by local people in oil palm plantations in Asahan Regency, North Sumatra Province of Indonesia [Pradiptra et al., 2020], and the use of harmonic radar for the detection of the beetle breeding sites [Moore, Siderhurst, 2022]. Furthermore, the large and fleshy larvae of this beetle represent a good source of animal protein and therefore used in rural areas of Nigeria as a needed food product [Oyewale, Makinde, 2023]. The larvae of the other rhinoceros beetle, *Oryctes owariensis* (Palisot de Beauvois, 1806), are also considered as good alternative to conventional protein food in Africa [Assielou et al., 2015]. For this species was precisely studied full life cycle under laboratory conditions in Nigeria [Ukoroije, Bawo, 2019]. Thus, commercial breeding of *Oryctes* Illiger species seems to be justified and may be profitable business, and it is for this reason that the pest *Oryctes rhinoceros* (Linnaeus) method of rearing was studied under laboratory conditions [Schipper, 2009]. *Oryctes* Illiger species are characterized by a long larval stage in the life cycle depending on environmental conditions. However, under artificial conditions the duration of larval development may be significantly reduced mainly due to stable conditions of rearing; for example, an enhanced feeding substrate and decreasing temperature in the final stages larval rearing from 28–25 °C to 3 °C resulted in a fast metamorphosis to the pupal stage [Schipper, 2009], the general period of larval development being reduced from 27 to 6 weeks. However, the selected strain of female beetles lived for 4 weeks longer and was able to lay 29 % more eggs [Schipper, 2009]. This experiment demonstrated the improvement in the rearing effectiveness of *Oryctes* Illiger rearing effectiveness improvement, which could also be applied to *Oryctes nasicornis* (Linnaeus, 1758), a representative of Siberian fauna.

The produced biomass of saproxylic beetles is rich in nutrient composition which may be adjusted by increasing the required elements via enrichment of the feeding substrate by means of precursors [Tshernyshev et al., 2023]. The above is not a sophisticated scheme of rearing which would demand a significant expenditure to provide for the reproductive cycle of beetles, but it demonstrates perspectives for gaining a biomass rich in animal protein and required minerals and vitamins for human nutrition.

Conclusions

The proposed scheme for rearing the green rose shafer, *Cetonia aurata viridiventris* Reitter was successfully achieved in one year and is currently ongoing. Separation of the different stages of development of the beetle prevents the impact of older stages on younger stages resulting in damaged eggs, young larvae or prepupae. This method enabled 100 final stage larvae containing 1500 g of live weight to be raised by using of the following materials: 35 litres of distilled water and 300 litres of feeding substrate compiled from cellulose-containing organic waste (120 litres of timber sawdust and 180 litres of sod soil). The feeding substrate altered by the larvae could, most probably, be used to increase soil efficiency.

Undoubtedly, some procedures of rearing may be modified and the method optimized in scale to the level of farm production.

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Рис. 1–4. Ксилосапротрофный вид жесткокрылых бронзовка *Cetonia aurata viridiventris* Reitter, 1896 в цикле выращивания для получения биомассы. 1 — имаго в освещаемом прозрачном контейнере для копуляции; 2 — яйца, отложенные на питательный субстрат в инкубаторе; 3 — личинки разных возрастов на обогащённом прекурсорами целлюлозосодержащем субстрате; 4 — куколки в инкубаторе для выведения имаго.

Figs 1–4. Saproxylic beetle green rose chafer *Cetonia aurata viridiventris* Reitter, 1896 used in the raising cycle for biomass production. 1 — imago in the breeder as illuminated transparent container; 2 — eggs laid on surface of feeding substrate in the incubator; 3 — different larval stages on the feeding substrate enriched with precursors in the larvae rearer; 4 — pupae in the pupae rearer for imago breeding.

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