Studii și Cercetări Științifice. Biologie 2025, 34 (1), 29-34
Scientific Study & Research. Biology
Print ISSN 1224-919X Online ISSN 2457-5178

ESTIMATION OF THREE NUTRITIVE MEDIA FOR THE PROPAGATION OF THE SPECIES *PLODIA INTERPUNCTELLA* (HÜBNER, 1813) - AS A HOST FOR THE ENTOMOPHAGIC *BRACON HEBETOR* (SAY, 1836)

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KEYWORDS

Plodia interpuncteella
Bracon hebetor
Nutrient medium
Larva
Pupa
Imago

ABSTRACT

Experimental investigations were carried out under controlled laboratory conditions with the aim of elucidating the particularities of the ontogenetic development cycle of the species *Plodia interpunctella* (Hübner), an important economic pest at a global level. The experiments aimed to estimate the influence of three distinct types of nutrient substrates, both in macerated and non-macerated form, on the biological parameters of this species, including the duration of larval stages development, survival rate and reproductive efficiency. These investigations were designed to highlight the impact of nutrient media compositions on the eco-physiological performance of individuals, thus contributing to a better understanding of the trophic adaptation mechanisms of this phytophage under laboratory conditions with the perspective of including it in the technological process as a host for the multiplication of the entomophage *Bracon hebetor*.

INTRODUCTION

Entomophages constitute an essential component of the biocenosis of agroecosystems, having a central role in regulating phytophage populations through specific trophic mechanisms. Beneficial insect species are predators or parasitoids, which voraciously consume other insects and, therefore, play an essential role in shaping ecosystems, maintaining ecological balance through predator-prey or parasite-host interactions (Hardy et al., 2023).

The detailed study of their ethology, including their parasitism strategies and ecological adaptability, is essential for optimizing their efficiency in the bioregulation of agricultural pests. Behavioral, physiological and ecological analysis of entomophagous insects allows for a thorough understanding of how they influence the dynamics of pest populations, facilitating the implementation of sustainable methods of phytosanitary protection. The taxonomy of entomophagous insects provides a fundamental understanding of their evolutionary adaptations, providing insights into their morphological, physiological and behavioral traits that contribute to their effectiveness in attacking their hosts or prey (Heimpel et al., 2017).

Host insect species use various mechanisms, both physiological and behavioral, to mitigate the impact of parasitism (Russo et al., 2024). The significant role of entomophagous insect species, as natural enemies, controlling populations of harmful insects has been exploited in pest control strategies and has attracted considerable interest in the scientific community. Scientific research provides valuable insights into the development of new tools to improve biological control programs. Elucidating both the behavioral aspects of laboratory host insects under controlled conditions facilitates the multiplication of viable entomophagous. Biotechnology for the production and multiplication of entomophagous insects represents, globally, a fundamental strategic area in the implementation of modern biological control methods in contemporary agroecosystems.

The development of advanced and efficient systems for the multiplication of these beneficial organisms is essential for reducing the use of chemical pesticides and maintaining the ecological balance in contemporary agroecosystems. Among the entomophagous species with major importance in biological control is the

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https://doi.org/10.29081/scsb.2025.34.1.04

entomophagous *Bracon hebetor* (Hymenoptera: Braconidae). It stands out for its ability to parasitize the larvae of over 260 species of harmful lepidopterans, especially those belonging to the families Pyralidae and Noctuidae (Russo et al., 2024). This species is an effective biological regulator, being widely used worldwide to combat pests at the larval stage, the moment when the hosts are most vulnerable to parasitoid attack. For the multiplication of the laboratory host it is very important to select the nutrient medium on which it can develop. The laboratory "Phytopharmacy and Ecotoxicology" (Institute of Genetics, Physiology and Plant Protection, Republic of Moldova) maintains the species *Plodia interpunctella* as an alternative laboratory host. This species is known as a pest of dried fruits. The larvae of this species have also been recorded on nuts and cereals, contaminating a wide range of products (Glibciuc et al., 2024).

The purpose of the investigations is to estimate the influence of three nutrient substrate variants on the biological and reproductive parameters during the ontogenetic cycle development of the species *Plodia interpunctella* as a host for the reproduction of the entomophagous *Bracon hebetor*.

MATERIALS AND METHODS

In order to estimate the efficiency of the nutrient medium intended for the multiplication of the Plodia interpunctella species, a series of experiments was carried out aimed at selecting the most suitable components and methods of their preparation (in macerated and whole form). The following food products were tested in the experiments: walnut kernels, buckwheat grains and rice grains. In order to optimize the nutrient medium and ensure better homogenization, the following components were additionally added: glycerin (which serves as a humectant and stabilizer), dried apple (as an additional source of carbohydrates), corn flour (for the supply of starch and fiber), as well as agar-agar. These components were included in various combinations and proportions, in order to determine the optimal conditions for the development of the ontogenetic cycle of the *Plodia interpunctella* species (Table 1).

Table 1. Composition of nutrient media, tested under controlled laboratory conditions, for the multiplication of the species *Plodia interpunctella*

Variant	Components and their rate per 1 kg of nutrient medium
Macerated / non-macerated components	Walnut kernel (300g) + dried apple (390g) + corn flour (200g) + glycerin (100ml) + agar-agar (10g)
Macerated / non-macerated components	Rice (300g) + dried apple (390g) + corn flour (200g) + glycerin (100ml) + agar-agar (10g)
Macerated / non-macerated components	Buckwheat (300g) + dried apple (390g) + corn flour (200g) + glycerin (100ml) + agar-agar (10g)

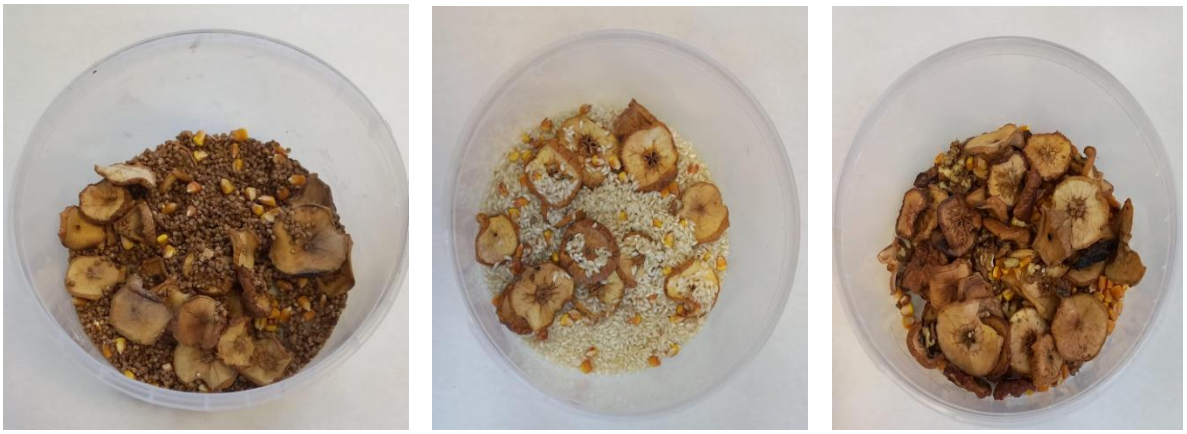
This experimental approach allowed the evaluation and adjustment of the nutrient medium according to the biological needs of the studied species, in order to maximize its efficiency as a host for the entomophagous *Bracon hebetor*. The biological indices of the *Plodia interpunctella* species were determined throughout the entire ontogenetic cycle, monitoring all stages of development. The experiments were carried out under controlled laboratory conditions, using specific equipment, such as "NITEH" type climate chambers and "BRUVE" type thermostats, which ensured a constant temperature between 27-28°C and a relative air humidity of 65-70%. For the multiplication and development of *Plodia interpunctella* larvae, glass vessels with a volume of 2 liters were used, in which the previously prepared nutrient medium was placed. The larvae received nutrient medium according to their needs, and the experimental conditions were monitored to ensure stability of the necessary parameters.

Each variant of nutrient media and propagation methods were tested in four replicates to ensure the precision of the data obtained and the reproducibility of the experimental results. Each replicate was treated as an independent experiment, and the data obtained were statistically analyzed to determine the influence of different environmental factors on the development of the species *Plodia interpunctella* (Figure 1).

For the preparation of the nutrient media intended for the development of the *Plodia interpunctella* species, a balanced ratio between the components was maintained, so as to ensure optimal conditions for the development of the larvae. Depending on the proportion of ingredients used, the nutrient medium must maintain its malleability, an essential characteristic to allow the larvae to move easily within the nutrient medium. Larval mobility is a strictly necessary factor for adequate nutrition of the larvae, since free movement on the surface and inside the nutrient medium facilitates uniform access to the resources necessary for development. In addition to this aspect, the nutrient medium must ensure an appropriate nutritional content, providing the elements necessary for the optimal growth and development of the *Plodia interpunctella* species.

These elements include proteins, lipids, carbohydrates and micronutrients, which are essential for larval development, metamorphosis and reproduction. Thus, maintaining the malleability of the nutrient medium is directly related not only to its physical texture, but also to its ability to provide an efficient and evenly distributed

nutrient source. In this way, the components of the nutrient media, such as flour, dried fruits, glycerin and agar-agar, are selected and combined in a way that guarantees both the necessary nutritional value and the appropriate texture for the beneficial development of the larvae, thus contributing to the success of multiplication.



a) b) c)

Figure 1. Variants of media tested for the multiplication of the species *Plodia interpunctella* as a host for the multiplication of the entomophagous *Bracon hebetor* (a. buckwheat grains; b. rice grains; c. walnut kernels)

RESULTS AND DISCUSSION

In the experiments carried out, some methodological elements were also developed so that the multiplication of the host species would be efficient, optimizing the nutrient media to provide both nutritional values and favorable conditions for development and reproduction. Thus, the nutrient media, developed according to the previous specifications, were subjected to a hardening process before use. This process was carried out by exposing the nutrient media in the drying cabinet at a temperature of 70°C for 60 minutes. The purpose of this hardening consists of both sterilization and uniformization of the nutrient media, eliminating any microorganisms and ensuring optimal conditions for the multiplication of the host species. Subsequently, after the nutrient media were hardened and prepared accordingly, they were distributed in boxes with a volume of 2 liters, to ensure adequate space for the development of the larvae and the conduct of the experiments under controlled conditions. In each box, one male and one female of the species *Plodia interpunctella* were introduced. Four variants were tested, depending on the age of the imago stage at the time of release into the boxes (24, 48, 72, and 96 hours). Each variant was tested in four repetitions. This experimental approach allowed the age of the imago stage to be highlighted and the influence on the reproduction and development rate of the offspring to be estimated, providing essential data for optimizing the growth and development conditions of the host species. Each variant was monitored throughout the entire ontogenetic development cycle of the species. The results obtained were essential both in identifying the optimal parameters for the multiplication of the *Plodia interpunctella* species, and in improving the nutritional media developed and tested.

Subsequently, the hatching process of larvae from eggs laid on the developed nutrient media was carefully monitored, dynamically recording the hatching of larvae and assessing the hatching rate depending on the variant of the nutrient media used. The recordings were made at intervals of 24, 48, 72 and 96 hours after egg laying, following the evolution of hatching. The results obtained were compared with each of the developed nutrient media. Throughout the development of the larval stage, changes were fixed and highlighted daily to follow the progress of their growth and development. Indices such as the survival rate and the time required to reach each age in the larval development process were evaluated, which are essential factors for determining the efficiency of the nutrient media. The purpose of these daily monitoring was to identify any differences between the tested nutrient media variants and to select the optimal variant, which ensures rapid development of the larvae. The collected data were compared and statistically analyzed to determine the nutrient medium variant that effectively supported the development of the *Plodia interpunctella* species, providing the most favorable nutritional conditions. These results are reflected in Table 2.

Following the analysis of the data obtained, it was found that the age of individuals at the imago stage plays an essential role in laying the maximum number of eggs. It is further reflected in the number of larvae obtained.

Thus, females of the *Plodia interpunctella* species aged between 24 and 48 hours lay a number of eggs from which the number of hatched larvae is significantly lower than that of females aged between 72 and 96 hours (by about 25%). Comparing the number of larvae obtained from females aged 24 hours with that obtained from females aged 96 hours, a considerable difference is observed (by about 45%). This significant difference indicates that females

aged between 72 and 96 hours are at an optimal stage of reproductive maturity, when they can lay a maximum number of eggs, thus ensuring a greater number of larvae. Thus, it was demonstrated that for the efficient multiplication of the *Plodia interpunctella* species, it is optimal to use females and males between 72 and 96 hours old, as they ensure a higher reproduction rate and, implicitly, a greater number of offspring.

Table 2. Estimation of biological parameters of the larval stage of the species *Plodia interpunctella* depending on the nutrient medium applied for multiplication

Age of imago stage at which eggs were laid (hours)	Number of larvae obtained on nutrient media					
	Walnut kernel		Buckwheat grains		Rice grains	
	Macerated	Non-macerated	Macerated	Non-macerated	Macerated	Non-macerated
24	31,6	24,6	22,3	19,6	18,3	15,3
48	53,6	45,6	29,3	27,3	24,0	19,3
72	92,0	76,6	35,0	31,6	29,3	28,3
96	104,6	90,3	44,3	38,3	38,0	30,3

In addition to the analysis of the influence of imago age, a comparison was also made between the numbers of larvae obtained depending on the variant of the nutrient medium used. The comparison of the obtained results demonstrated that only the nut kernel-based nutrient medium had a significant positive effect on the number of larvae obtained, compared to the other tested media. Thus, the number of larvae obtained on the nut kernel medium was significantly higher than that obtained on the buckwheat grain medium (approximately 2.5 times) and, respectively, compared to that obtained on the rice grain medium (approximately 3 times). These data suggest that the nut kernel provides a more adequate nutritional source for the development of *Plodia interpunctella* larvae than buckwheat or rice grains, due to its high content of fats and proteins, which are essential components for larval growth and development.

Further analysis of the experimental data, obtained from the evaluation of the preparation of the nutrient media, revealed an important methodological factor - the preparation of nutrient media by the maceration method of the components leads to a significant increase in the number of larvae obtained. Maceration, a process by which the nutrient components are crushed to facilitate their nutritional accessibility to the larvae, has been shown to have a positive impact on the efficiency of the nutrient media, thus improving the development conditions for the larvae of the species *Plodia interpunctella*. In the case of using the nutrient medium based on walnut kernels, the application of the maceration method led to obtaining a number of larvae approximately 15% higher compared to the medium prepared by other methods. Similarly, the nutrient medium based on buckwheat grains recorded an increase of approximately 11% in the number of larvae obtained, and the medium based on rice grains generated a number of larvae approximately 15% higher by applying the same method of maceration of the components. These results indicate that the maceration method optimizes the bioavailability of nutrients in the nutrient media, thus facilitating a faster and more efficient digestion of nutritional resources by the larvae. Therefore, it was found that the maceration method of the components is the most efficient method of preparing nutrient media for the multiplication of the laboratory host *Plodia interpunctella*, ensuring a maximum number of offspring and an accelerated development cycle.

Another important aspect observed was the duration of larval development depending on the nutrient medium used. Thus, on the nut kernel nutrient medium, prepared by the maceration method, larval development duration of approximately 25 days was recorded. This development period is considerably shorter compared to the nutrient media based on buckwheat and rice grains, where the larval development period was extended to approximately 40-45 days. This time difference reflects the superior efficiency of the nut kernel-based medium, which provides an optimal balance of nutrients and an adequate structure for rapid and healthy larval growth.

Monitoring the development of the ontogenetic cycle of the species *Plodia interpunctella* continued by evaluating the pupal stage. It was demonstrated that the duration of the pupal stage is relatively constant, extending over a period of about 7-8 days, regardless of the nutrient medium used. However, the number of pupae obtained varied depending on the composition and preparation method of the nutrient media on which the larvae were raised until the pupation stage. This phase was analyzed in detail, and the number of pupae was recorded for each experimental variant, depending on the nutrient media and preparation methods used. The results were presented in Table 3.

Table 3. Pupae rate of the species *Plodia interpunctella*, obtained depending on the nutrient medium on which the larvae developed

Pupae obtained from F_1 imago of different ages (hours)	Number of pupae obtained depending on the nutrient media					
	Walnut kernel		Buckwheat grains		Rice grains	
	Macerated	Non-macerated	Macerated	Non-macerated	Macerated	Non-macerated
24	30,0	22,3	17,3	15,3	17,0	14,0
48	52,0	40,0	24,6	19,3	23,0	18,6
72	92,0	71,3	32,6	28,6	28,6	27,3
96	95,6	75,0	43,3	36,6	33,6	29,6

Thus, nutrient media prepared by the maceration method proved to be superior, not only in terms of the number of larvae obtained, but also in their ability to support faster and more efficient larval development, leading to a greater number of healthy and viable pupae.

The detailed analysis of the experimental data obtained clearly confirms that the macerated walnut kernel-based nutrient medium is the most effective in supporting the development of *Plodia interpunctella* larvae, demonstrating the highest pupation rate (about 96%). This finding emphasizes the importance of the elaborated method of preparing the nutrient media, having a significant impact on the success of the ontogenetic development of the laboratory host. The method of preparing the nutrient medium proved to be a determining factor, since, on the nutrient medium with intact (non-macerated) walnut kernel, the pupation rate obtained was considerably lower, reaching only 85%. This significant difference demonstrates that the maceration process significantly improves the accessibility of essential nutrients for the larvae, thus optimizing the development conditions and accelerating the life cycle.

The same trends were confirmed for the other nutrient media tested. On nutrient media based on buckwheat grains and rice grains, prepared without maceration, the pupal rate obtained was also lower, recording a difference of approximately 5% compared to macerated nutrient media. These results highlight the fact that the maceration method of nutritional components improves the access of larvae to essential resources for growth and development, which is demonstrated by a higher rate of survival and development up to the pupal stage. It was also observed that on nutrient media with whole components, larval development proceeds at a slower pace compared to macerated media, indicating a prolonged longevity of the development period in these variants. This suggests that, in the absence of maceration, nutritional components are more difficult to digest and assimilate by larvae, which slows down the development process and may negatively affect the overall efficiency of the ontogenetic development cycle.

In the continuation of the monitoring of the ontogenetic development cycle of the species *Plodia interpunctella*, a detailed evaluation of the imago stage of the second generation (F_2) was carried out. Subsequent experiments also aimed to assess the ratio between males and females, depending on the nutrient media on which the larvae developed. This research direction was important to understand how the composition and preparation of nutrient media influence the sexual balance in the obtained population. This evaluation has important implications for the multiplication of the laboratory host, since an optimal ratio between males and females is crucial for ensuring an efficient reproductive cycle in the following generations. Unbalanced sex ratios could negatively affect reproduction, thus decreasing the yield of multiplication experiments. The results obtained from these experiments are presented in Table 4.

Table 4. Estimation of F_2 imago hatching of the species *Plodia interpunctella* from pupae obtained from larvae developed on different nutrient media

Hatching of F_2 imago from pupae obtained from imago of different ages (hours)	Number of F_2 imago hatched											
	Walnut kernel				Buckwheat grains				Rice grains			
	Macerated		Non-macerated		Macerated		Non-macerated		Macerated		Non-macerated	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
24	17,0	13,0	13,3	9,0	14,3	3,0	13,3	2,0	14,0	3,0	12,6	1,4
48	30,0	22,0	27,6	12,4	18,6	6,0	15,3	4,0	18,3	4,7	14,6	4,0
72	53,0	39,0	46,0	25,3	21,6	11,0	18,3	10,3	20,6	8,0	21,3	6,0
96	55,6	40,0	47,0	28,0	29,0	14,3	24,6	12,0	28,3	5,3	25,6	4,0

The analysis of the obtained data revealed a uniform hatching of the F_2 imago stage (100%), from the pupae developed on the macerated walnut kernel-based nutrient medium. These results suggest an excellent efficiency of the macerated walnut kernel-based nutrient medium in supporting the optimal development of the host *Plodia interpunctella*, ensuring a complete hatching of the adult stage. Regarding the sex ratio, it was determined to be 1.3♂:0.7♀, which indicates a slight predominance of males compared to females. This suggests that the macerated walnut kernel nutrient medium favors a relatively balanced reproduction and distribution of the sexes.

It was also observed that, even when the hatching of the F_2 imago stage was 100%, but from the pupae developed on the nutrient medium of intact walnut kernel, the rate of males was significantly higher, which led to a ratio between males and females of 1.8:0.5. This factor indicates a tendency to favor males if the nutrient medium is based on intact components, compared to the nutrient medium based on macerated components. These differences between the nutrient media suggest that it can significantly influence the distribution of sexes within the host population. At the same time, the pupae obtained on the nutrient media of buckwheat grains and rice grains also showed a hatching rate of 100%, but the ratio between males and females was much more unbalanced.

Thus, the ratio between males and females on the buckwheat grain medium was 3.0♂:0.3♀, and on the rice grain medium, the ratio was even more unbalanced, 4.3♂ : 0.2♀. These data suggest a strong predominance of males in both nutrient media, which may lead to inefficient reproduction and may limit success for multiplication purposes

or biological studies, given that females are essential for egg production and the continuation of the ontogenetic cycle.
In the context of monitoring the ratio between males and females, it was found that the macerated walnut kernel-based nutrient medium is superior in terms of maintaining a more balanced ratio between the two sexes. The number of females hatched on this nutrient medium was much higher compared to the nutrient media based on buckwheat grains and rice grains, which indicates that macerated walnut kernel promotes greater reproductive success and may represent an optimal medium for the multiplication of the species *Plodia interpunctella* as a laboratory host for the successful multiplication of the entomophagous *Bracon hebetor*.

CONCLUSION

It has been demonstrated that to optimize the multiplication process of the laboratory host *Plodia interpunctella*, it is necessary to use imagoes between 72 and 96 hours old, thus ensuring superior reproductive performance and a higher rate of viability of the offspring;
It was found that, thanks to the comparative testing of three types of nutrient media, the substrate containing walnut kernels was highlighted, which is the most favorable for the optimal development and multiplication of the *Plodia interpunctella* species, providing an adequate nutritional intake and favoring both the survival rate and the speed of development of individuals in all ontogenetic stages;
It has been demonstrated that larval development on the nutrient medium based on macerated walnut kernels takes place within a period of approximately 25 days, while on the other substrates tested, the duration of development extends significantly, reaching values of 40-45 days;
It was demonstrated that the hatching of the F_2 imago occurs uniformly in the case of pupae obtained from larvae developed on the macerated walnut kernel substrate, and the ratio between males and females was 1.3♂ : 0.7♀, which allows the multiplication of the *Plodia interpunctella* species as a laboratory cage for the propagation of the entomophagous *Bracon hebetor*.

ACKNOWLEDGEMENTS

The research was carried out within the framework of subprogram 011103 “Development of environmentally friendly means of reducing the impact of agricultural pests in the context of climate change”, funded by the Ministry of Education and Science.

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