ORIGINAL ARTICLE

Determining the influence of the size of grain moth eggs on *Trichogramma evanescens* indicators

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Abstract

The aim of this work was the determination of the influence of the size of grain moth eggs on qualitative characteristics of *Trichogramma evanescens* (Hymenoptera: Trichogrammatidae) from the second to the seventh generations. The indicators of *T. evanescens* determine its ability to provide effective plant protection. Using selected large eggs of grain moth *T. evanescens* reproduction was carried out. As controls, eggs that had only been cleaned were used. These studies were performed with *T. evanescens* from second to seventh generations. The correlation between the size of grain moth eggs and indicators of *T. evanescens* cens such as the level of search ability, the level of regeneration of individuals, the relative number of females, the level of deformed individuals, the lifespan and the fecundity of females were determined. The influence of the size of grain moth eggs for the production of *T. evanescens* allowed for maintaining its first class quality from the second to the seventh generations. *Trichogramma evanescens* from grain moth eggs, which had only been cleaned, had first class quality only up to the fourth generation.

Keywords: fecundity, females, regeneration of individuals, search ability

Introduction

The use of chemical drugs against pests of agricultural crops leads to negative consequences for the environment, and, thus, for humans. The use of chemical drugs leads to decreased soil fertility and crop yields (Hamouz *et al.* 2015). Biological plant protection results in environmentally safe products and improves the ecological situation. Environmental safety leads to the development and introduction of organic production technologies. During recent years, there has been a tendency to increase the number of such enterprises (Vlasicova and Naglova 2015). However, the priority for most farmers is to increase the yield of crops, which today is mainly ensured by the use of new varieties and the wide application of mineral fertilizers and other chemical preparations (Buranova *et al.* 2016).

One element of organic production is the use of biological control techniques in biological plant protection. In Ukraine, the most widespread natural enemy is *Trichogramma*. As a result a significant amount of scientific work has been devoted to the study of characteristics of *Trichogramma* and the optimal conditions for its laboratory production.

In addition, a significant amount of scientific research has been done on using *Trichogramma* in biological plant protection and determining its effectiveness against a number of pests (Davies *et al.* 2011; Manandhara and Wright 2015). A number of scientific papers has researched the use of *Trichogramma* in combination with chemicals, determining their impact on the quality of *Trichogramma* (Preetha *et al.* 2010; Delpuech and Delahaye 2013; Parsaeyan *et al.* 2018; Thubru *et al.* 2018). It has been established that the action of chemicals leads to the death of *Trichogramma*, so their use should not be simultaneous. Therefore, studies that use *Trichogramma* in combination with bioinsecticides are more relevant (Mandour *et al.* 2012).

The influence of viral insecticides (Khan 2017) and plant extracts (Rampelotti-Ferreira et al. 2017) on qualitative indices of Trichogramma were also investigated. The influence of extreme weather conditions on the effectiveness of entomophages throughout the year, including climate variability, indirect temperature factors, seasonality of precipitation, and type of cultivated crop with adherence to geographical latitude (Henry and Day 2000; Wu et al. 2016) were also examined. The suitability of Tuta absoluta (Lepidoptera: Gelechiidae) eggs was also studied which were obtained in different combinations of males and females after X-ray irradiation and without it. The results of the research showed that the eggs of T. absoluta from irradiated females were parasitized by Trichogramma nerudai (Hymenoptera: Trichogrammatidae) and T. pretiosum (Hymenoptera: Trichogrammatidae) 60% less than eggs that were obtained from non-irradiated females (Cagnotti et al. 2016). The authors explain the reduction of parasitism of Trichogramma by referring to van Alphen and Jervis (1996), who stated that Trichogramma, during the search for host eggs, was influenced by many environmental and physiological factors, such as the egg size of the host, the smell, shape and internal quality.

Waage and Lane (1984) determined the effect of *Mamestra brassicae* L. (Lepidoptera: Noctuidae) egg size on fertility and sex distribution of *T. evanescens* Westwood (Hymenoptera: Trichogrammatidae). These studies determined how the *M. brassicae* eggs are parasitized when several wasps of *Trichogramma* were released per egg of the pest. It was found that at the beginning of egg-laying by *Trichogramma* females more males were hatched from eggs than females.

Greenberg *et al.* (1998) determined the role of *T. minutum* Riley (Hymenoptera: Trichogrammatidae) and *T. pretiosum* Riley (Hymenoptera: Trichogrammatidae), reared on *Helicoverpa zea* (Lepidoptera: Noctuidae) eggs, as parasitoids of the Mexican rice borer, *Eoreuma loftini* (Dyar) (Lepidoptera: Crambidae) and sugarcane borer *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae) under laboratory and greenhouse conditions. Research results showed that *T. minutum*, reared on *H. zea* eggs parasitized 52.5% of Mexican rice borer eggs *E. loftini*, compared to 50.3% for *T. pretiosum*. At the same time *T. minutum* on *H. zea* eggs parasitized 42% of sugarcane borer eggs, compared to only 12.6% by *T. pretiosum*. These results show the potential use of *Trichogramma* to control sugarcane stalk borers in a field.

It is known that the production process of *Tricho-gramma* has two stages: the production of the host and the direct production of *Trichogramma* on the alternative eggs. In Ukraine, the most widespread host of *Trichogramma*, which is used under laboratory and industrial conditions, is the warehouse pest – grain moth *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae).

Rooms for the production of grain moths and *Trichogramma* must be spatially separated (Fig. 1) to prevent uncontrolled contamination of grain moth eggs because the *Trichogramma* is constantly searching for eggs.

A characteristic feature of the production process of *Trichogramma* in the educational, scientific and production laboratory of the National University of Life and Environmental Sciences of Ukraine is the use of calibration of grain moth eggs in order to obtain better quality eggs for breeding *Trichogramma* (Golub *et al.* 2019). That is why establishing the effect of the size of grain moth eggs on the qualitative indices of

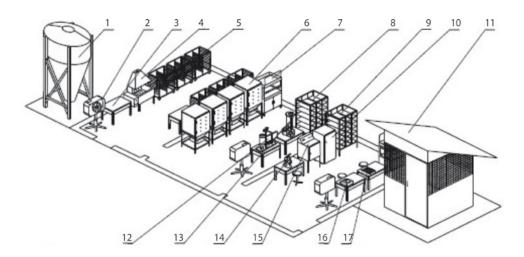


Fig. 1. Layout of technological equipment in the laboratory for the production of *Trichogramma*: 1 – grain hopper; 2 – humidifier; 3 – table; 4 – grain conditioner; 5 – grain rack; 6 – box for collecting grain moths; 7 – ventilation chamber; 8 – rack for collecting grain moth eggs; 9 – rack for containers with *Trichogramma*; 10 – *Trichogramma* and grain moth eggs storage chamber; 11 – insectarium; 12 – calibrator of grain moth eggs; 13 – cleaner of grain moth eggs; 14 – binocular; 15 – thermostat; 16 – scales; 17 – device for determining the searching capacity of *Trichogramma* (Marus and Golub 2014)

the entomological agent *Trichogramma* in the course of several generations is relevant.

Observing temperature regimes and humidity is important in *Trichogramma* production technology since temperature affects longevity and the ratio of sexes of *Trichogramma* individuals. Studies have shown that the optimum temperatures were in the range of 25 to 28°C (Oliveira *et al.* 2017).

To date, there are several ways to improve *Trichogramma* quality. One way is to use eggs from natural owners. Chinese scientists (Wanga *et al.* 2014) have been using oaken silkworms (*Antheraea pernyi*) as host eggs for the production of *T. dendrolimi* (Hymenoptera: Trichogrammatidae) for a long time. For maintaining qualitative indicators of the agent its introduction during diapause is also included. Russian scientists (Reznik and Samartsev 2015) studied the effect of the duration of day light and the change of temperature regimes on the output of *Trichogramma* from diapause and its quality indices.

Studies were made using grain moth eggs of approximately the same size. The ratio of males and females, tibia length and the fecundity of Trichogramma females (Pallewatta 1986) were determined. According to the research of others, 10 individuals of Trichogramma were used to parasitize 30 eggs of a grain moth. In this case the percentage of parasitized eggs of the grain moth was only 38%. The author explains this by the negative reaction of Trichogramma individuals on the eggs, in this case of the grain moth, while the Trichogramma itself was obtained from the eggs of other hosts. At the same time, hypothetically it may also be low quality Trichogramma which was not grown on a large enough number of host eggs. How the size of the grain moth eggs affects the quality of Trichogramma is not given in this paper.

An analysis of the results of existing scientific research allows us to conclude that a considerable amount of research is devoted to the improvement of the technology of *Trichogramma* production, since its efficiency and, accordingly, the productivity of agricultural products depend on qualitative indicators of *Trichogramma*. At the same time, the issue of the influence of the size of grain moth eggs on the qualitative parameters of *Trichogramma* over several generations has not been sufficiently studied. The aim of this work was the determination of the influence of the size of grain moth eggs on the qualitative characteristics of *T. evanescens* from the second to the seventh generations.

Materials and Methods

In order to establish the characteristic sizes of grain moth eggs, the work of the Plant Protection Department of the All-Union Research Institute of Biological Methods of Plant Protection was used as a basis, which states that according to the lengths of the body of females or their weight, the quality indices of the populations of grain moths are divided into four groups corresponding to different technologies of its breeding on barley grain (Table 1) (Golyshin 1983). The length of grain moth eggs most often varies from 0.48 to 0.63 mm, the width – from 0.20 to 0.35 mm. Groups 3 and 4 mainly include eggs, the width of which is more than 0.28 mm, and the length is more than 0.59 mm.

These studies have made it possible to establish a minimum volume of large grain moth eggs for the production of *Trichogramma* stock culture, which was 0.0247 mm³ (for grain moth cultivation regime with weak violations).

A pneumatic calibrator of grain moth eggs (Fig. 2) was used to separate the eggs of grain moths (Golub *et al.* 2019). Using it, the eggs of grain moths were divided into three fractions: conglomerates (stuck eggs of grain moth), which fell into the first container; large eggs – fell into the second container; small eggs – fell into the third container.

Reproduction of large selected eggs of grain moth *T. evanescens* was carried out. As controls, eggs that had only been cleaned were used. These studies were performed with *T. evanescens* from the second to the seventh generations. At the beginning, all indicators were determined on first generation *T. evanescens* using eggs that had only been cleaned. Starting from the second generation, breeding *T. evanescens* was carried out on large grain moth eggs obtained after cleaning and calibration and on grain moth eggs after clearing without calibration. All studies were conducted in three replicates.

Table 1. Indicators of Sitotroga cerealella populations based on Golyshin (1983)

Estimation of the grain moth cultivation regime	Female body length [mm]	Weight of females [mg]	Potential female fecundity (eggs) [units]	The average volume of eggs [mm ³]
Serious violations	less than 5.9	less than 5.2	less than 108	less than 0.0217
Significant violations	5.9–6.5	5.2-6.8	108–142	0.0231 ± 0.0016
Weak violations	6.5–7.1	6.8-8.4	142–179	0.0263 ± 0.0016
No violations	more than 7.1	more than 8.4	more than 179	more than 0.0279

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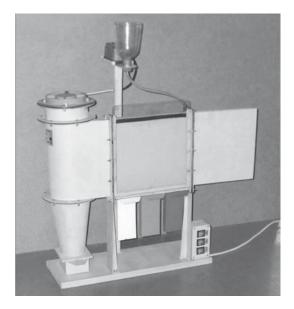


Fig. 2. A general view of the pneumatic calibrator of grain moth eggs

The level of search ability, as the ratio of the number of parasitized eggs to the total number of grain moth eggs in the labyrinth, was determined using a device called the labyrinth (Fig. 3), which has two compartments – the *Trichogramma* trigger compartment and the compartment for placing cards with grain moth eggs.

The compartments are interconnected by a twisted channel, the length of which is 3 m. It is believed that this distance is effective for the operation of Trichogramma under field conditions. To determine the search ability, 0.5 g of eggs parasitized by T. evanescens of each generation with its qualitative indicators were loaded into a test tube and closed with a cotton swab until Trichogramma began to appear. With the beginning of Trichogramma rebirth, the test tube was placed in the compartment of the labyrinth. The cards on which the grain moth eggs were placed, were put in the other compartment. Then the test tube was opened and left for 8 hours. After that, the cards with the of grain moth eggs from the labyrinth were removed and placed on a Petri dish. After 5 days, the search ability of Trichogramma was determined according to the ratio of the number of parasitized eggs to the total number of grain moth eggs in the labyrinth.

The level of regeneration of individuals, the ratio of the number of reborn individuals to the total number of parasitized eggs of the grain moth was determined using 500 parasitized eggs which were pasted on five cards. Each card was placed in a test tube. The test tubes were tightly sealed with cotton swabs and after the natural death of the *Trichogramma*, a count was made of the regenerated individuals.

The relative number of females, as the ratio of the number of females to the total number of reborn

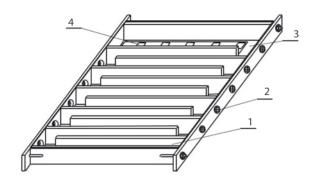


Fig. 3. Device for determining the search ability of *Trichogramma*:
1 - *Trichogramma* trigger compartment; 2 - ventilation window;
3 - compartment of infection; 4 - card with grain moth eggs

individuals, was determined on the basis of the previous experiment. Using a stereoscopic microscope MBS-10 in each of the five tubes the number of males and females was counted.

The level of deformed individuals, as the ratio of the number of deformed individuals to the total number of reborn individuals, was determined using a 0.5 liter glass jar with approximately 500 parasitized eggs of the grain moth by the *Trichogramma*. After the natural death of the *Trichogramma*, using a stereoscopic microscope MBS-10, a count was made of the deformed individuals (absence of wings or other deformations).

The lifespan and the fecundity of females were determined using 10 test tubes. An individual *Trichogramma* female was placed in each test tube. Also, a card with 100–150 grain moth eggs pasted on it was placed in each test tube. Everyday, for 5 days, cards with 100–150 grain moth eggs pasted on them were added to the test tubes. Then, after 5 days and after the natural death of the *Trichogramma*, using a stere-oscopic microscope MBS-10, a count was made of the parasitized grain moth eggs. The fecundity of females was determined for every test tube by summing up the number of parasitized eggs. Also, the females' lifespan was determined for every test tube by summing up the time to the natural death of the *Trichogramma*.

To combine quality indicators, a generalized indicator that determines the effectiveness of the *Trichogramma* was used. It included the most significant indicators, namely: the revival of *Trichogramma* from parasitized eggs, fecundity of females and search ability. This generalized coefficient of quality (y) was determined by the equation of multiple regression (Shelestova *et al.* 2004):

$$y = -0.28 + 0.0034r + 0.0084f + 0.0074s, \qquad (1)$$

where: y – generalized indicator of quality (rel. un.); r – the regeneration of individuals (%); f – fecundity of females, number of eggs deposited; s – search ability (%).

Quality class	Generalized <i>Trichogramma</i> quality criterion [rel. un.]
I	0.71-1.0
П	0.51–0.7
III	0.31–0.5

Table 2. Characteristics of Trichogramma quality classes

According to this indicator, the quality class of the *Trichogramma* batch was determined using the data given in Table 2.

The results of studies on determining the effect of the size of the grain moth eggs on the quality of the *Trichogramma* from the second to the seventh generations were statistically processed using a well-known Fisher criterion (*F*-criterion) that was used to compare variances of the two variation series with the tabular value of the Fisher test $F_T = 2.3$.

Results

The experimentally established optimal values of structural and technological parameters of the advanced pneumatic calibrator of grain moth eggs are given in Table 3.

Before calibration, the size distribution of grain moth eggs was: conglomerates – 33%; large eggs – 27%; small eggs – 40%. The results of determining the probability of selection of grain moth eggs in three containers of an advanced pneumatic calibrator are given in Table 4.

Thus, after testing, the probability of selection of large eggs in the second container was 58%, which

increased the number of large eggs by 31% compared to the fraction before calibration.

For the first generation of *T. evanescens* eggs that were only cleaned the level of parasitized eggs was – 82%, the level of search ability – 87%, the level of regeneration of individuals – 92%, the relative number of females – 63%, the level of deformed individuals – 2%, females' lifespan – 3 days and the fecundity of females – 35 eggs per female.

Trichogramma evanescens, which was cultivated on large eggs, had a stable level of search ability from the second to the seventh generations (Fig. 4), as opposed to *Trichogramma*, which was cultivated on eggs that had not been calibrated. Thus, from large eggs, stronger and more active individuals were cultivated which were able to search for the eggs of pests under natural conditions. The value of Fisher's criterion for the two variation series was F = 85.8, which is more than the tabular value of the Fisher test $F_T = 2.3$. This indicates the significance of the deviations of the values obtained for the search ability of large grain moth eggs compared to the search ability of grain moth eggs which passed only cleaning without calibration. The difference between the mean values of the search ability for the two variations was 80.1%.

For the level of regeneration of individuals of *Tri*chogramma (Fig. 5) from the second to the seventh generations the size of the eggs was not really significant, because the value of Fisher's criterion for the two variation series was F = 0.8, which is less than the tabular value of the Fisher test $F_T = 2.3$. This indicates the insignificance of the deviations of the values obtained for the level of regeneration of large grain moth eggs compared to the level of regeneration for grain moth eggs which passed only cleaning without calibration. The difference between the mean values of the level of regeneration for the two variations was 6.1%.

Table 3. Structural and technological parameters of the pneumatic calibrator of grain moth eggs

Parameters	Value	Parameters	Value
Separation chamber width	20 mm	Air flow velocity	3.8 m ⋅ s ⁻¹
Length of the container for conglomerates	110 mm	Height of separation chamber	198–199 mm
Length of the container for large eggs	30 mm	Height of the valve between the second	26.27.000
Length of the container for small eggs	90 mm	and third containers	26–27 mm

Table 4. Evaluation of the work of the advanced pneumatic calibrator of grain moth eggs

Fractions of grain moth eggs	Probability of selecting eggs [%]			
	container for conglomerates	container for large eggs	container for small eggs	
Conglomerates	78	16	2	
Large eggs	18	58	20	
Small eggs	4	26	78	

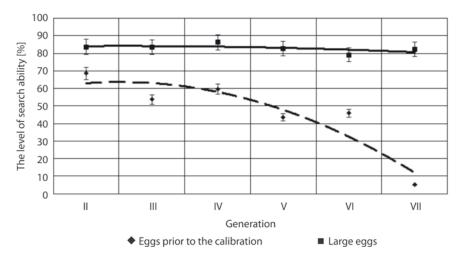


Fig. 4. The influence of the size of grain moth eggs on the level of search ability of Trichogramma evanescens

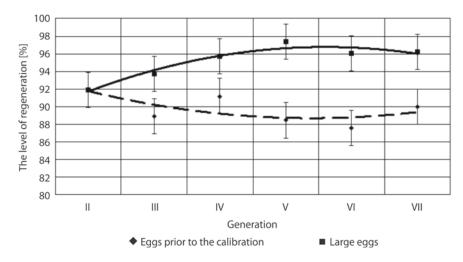


Fig. 5. The influence of the size of grain moth eggs on the level of regeneration of individuals of Trichogramma evanescens

Also, for the relative number of females (Fig. 6), from the second to the seventh generations the size of the eggs was not really significant because the value of Fisher's criterion for the two variation series was F = 0.6, which is less than the tabular value of the Fisher test $F_T = 2.3$. This indicates that the deviations of the values obtained for the relative number of females for large grain moth eggs in comparison with the relative number of females for eggs of grain moths which passed only cleaning without calibration were not significant. The difference between the mean values of the relative number of females for the two variations was 3.6%.

Investigations on the effect of the size of grain moth eggs on the level of deformed individuals showed (Fig. 7) that the difference between the mean values of the level of deformed individuals for the two variations was 50.7%. The value of Fisher's criterion for the two variation series was F = 3.5, which is more than the tabular value of the Fisher test $F_T = 2.3$. This indicates

the significance of the deviations of the values obtained for the level of deformed individuals for large grain moth eggs in comparison with the level of deformed individuals for grain moth eggs, which passed only cleaning without calibration.

The effect of the size of grain moth eggs on the females' lifetime of *Trichogramma* (Fig. 8), which was determined during the studies, showed that *Trichogramma* produced on large eggs had an average life expectancy of 3.5 days, and the *Trichogramma* that were produced on eggs that were not calibrated, had a lifetime of 2.6 days. Thus, the volume of the nutrient medium affects the females' life and, accordingly, allows the individuals of *Trichogramma* to carry out a longer search for eggs of pests. As a result, the efficiency of using *Trichogramma* increases. In this case the value of Fisher's criterion for the two variation series was F = 3, which is more than the tabular value of the Fisher test $F_T = 2.3$. This indicates the significance of the deviations of the values obtained

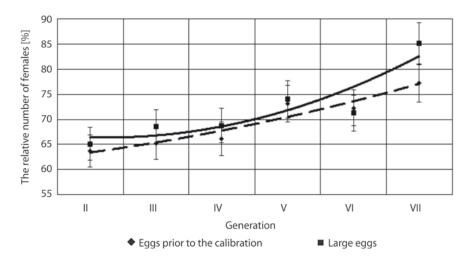


Fig. 6. The influence of the size of grain moth eggs on the relative number of females of Trichogramma evanescens

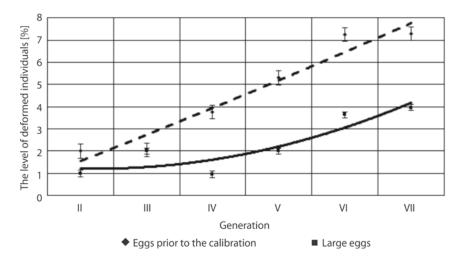


Fig. 7. The influence of the size of grain moth eggs on the level of deformed individuals of Trichogramma evanescens

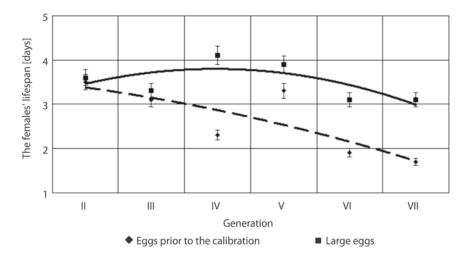


Fig. 8. The influence of the size of grain moth eggs on the females' lifespan of Trichogramma evanescens

for the females' lifespan for large grain moth eggs in comparison with the duration of females' lifespan for grain moth eggs which passed only cleaning without calibration. The difference between the mean values of the females' lifespan for the two variations was 34%.

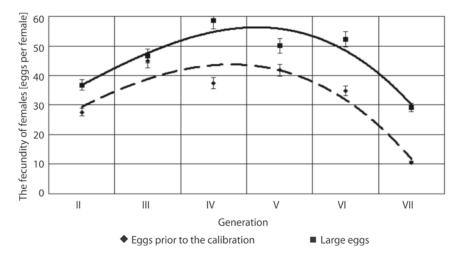


Fig. 9. The influence of the size of grain moth eggs on the fecundity of females of Trichogramma evanescens

The influence of the size of grain moth eggs on the fecundity of females of *Trichogramma* individuals (Fig. 9) from the second to the seventh generations was also not really significant, because the value of Fisher's criterion for the two variation series was F = 1.4, which is less than the tabular value of the Fisher test $F_T = 2.3$. This indicates the insignificance of the deviations of the values obtained for the fecundity of females for large grain moth eggs compared to the fecundity of females for grain moth eggs which passed only cleaning without calibration. The difference between the mean values of the fecundity of females for the two variations was 39.1%.

Thus, as a result of the research, it was found that the size of grain moth eggs from the second to the seventh generation had the largest positive effect on the level of search ability, the level of deformed individuals and fecundity of females. The influence of the size of grain moth eggs on the level of regeneration of individuals, the relative number of females and the fecundity of females of *T. evanescens* from the second to the seventh generation was not significant.

Discussion

These studies which were focused on the effect of *M. brassicae* eggs on the biological characteristics of *T. evanescens*, confirmed that pest egg sizes affected the *Trichogramma* tibia length both in males and in females. It was found that the lifetimes of males and females under laboratory conditions increased with increased size. A correlation was also established between the fertility of *Trichogramma* females and their size and the number of eggs laid by *Trichogramma* in one egg of the pest. The number of females and males in the progeny of a single *Trichogramma* female was

also determined. It was found that *Trichogramma* regulates the number of males in progeny (Waage and Ming 1984).

It is well known that *M. brassicae* eggs are almost 2.5 times larger than *S. cerealella* eggs. However, under production conditions, in most cases *Trichogramma* is bred on the eggs of a grain moth. *Trichogramma* rapidly degenerate under production conditions, so our research was aimed at determining the impact of the size of grain moth eggs on *Trichogramma* quality for several (seven) generations.

Indicators of *T. evanescens*, such as the level of search ability, the level of regeneration of individuals, the relative number of females, the level of deformed individuals, the females' lifespan and the fecundity of females were determined according to the well-known method of Shelestova *et al.* (2004).

In our research the size of grain moth eggs from the second to the seventh generations had the greatest positive effect on the level of search ability, the level of deformed individuals, life expectancy and fecundity of females. The positive influence of large grain moth eggs on the fecundity of females of Trichogramma was already pointed out by Telenga and Shchepetylnikova (1949). In studies by Medoni et al. (1980) the influence of the quality of grain moth eggs on the females' lifespan of Trichogramma was shown. There are also known studies on the influence of the size of grain moth eggs on other individual quality indicators of different entomophages. In our studies, the effect of the size of grain moth eggs on seven major Trichogramma indices was determined, namely: the level of search ability, the level of regeneration of individuals, the relative number of females, the level of deformed individuals, the females' lifespan and the fecundity of females. The studies were performed from the second to the seventh generations of Trichogramma reproduction.

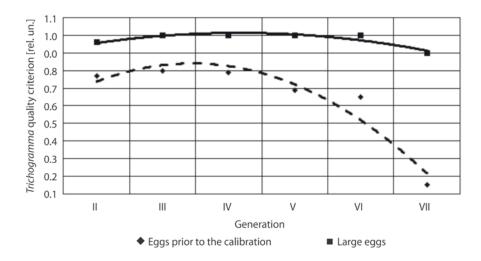


Fig. 10. The influence of the size of grain moth eggs on the Trichogramma evanescens quality criterion

We conducted research on the production of T. pintoi on small and large grain moth eggs for seven generations and T. evanescens on large grain moth eggs and on eggs without calibration for six generations. The effect of the size of grain moth eggs on the level of parasitized eggs of Trichogramma was also investigated. These studies showed that the level of parasitized eggs of Trichogramma, which was produced on small grain moth eggs decreased sharply from the third to the ninth generations. The Trichogramma that were produced on large grain moth eggs had a fairly high level of parasitized eggs for five generations for T. pintoi and six generations for T. evanescens. The influence of the size of grain moth eggs on the level of parasitized eggs by T. pintoi and T. evanescens was identical (Marus et al. 2020).

Our studies on the influence of the size of grain moth eggs on the relative number of females showed positive results. However, Oliveira *et al.* (2017) noted the influence of temperature regimes and humidity on the females' lifespan and the relative number of females of *Trichogramma*. It is also necessary to note the necessity and importance of conducting studies to determine the fecundity of females of *Trichogramma* depending on their lifespan.

Using formula 1 we found that *Trichogramma*, obtained with the use of a pneumatic calibrator, had first class quality from the second to the seventh generations. Without a pneumatic calibrator, the *Trichogramma* had first class quality up to the fourth generation, from the fifth to the sixth – second class quality, and from the seventh generation – below the third class (Fig. 10). Therefore, the calibration operation should be an integral part of the production process of the stock and industrial *Trichogramma* cultures.

Breeding on large grain moth eggs has made it possible to increase the number of generations of

Trichogramma with the corresponding qualitative indicators. It can also extend *Trichogramma* production and improve its efficiency. Further research in this direction should be directed to the study of the efficiency of using of *Trichogramma* for plant protection.

Conclusions

It was found that the size of grain moth eggs from the second to the seventh generations of *Trichogramma* had the greatest positive effect on the level of search ability, the level of deformed individuals and fecundity of females of *Trichogramma*. The influence of the size of grain moth eggs on the level of regeneration of individuals, the relative number of females and the fecundity of females of *Trichogramma* from the second to the seventh generations was not statistically significant.

Studies on determining the influence of the size of grain moth eggs have shown that *T. evanescens*, obtained with the use of a pneumatic calibrator, had first class quality from the second to the seventh generations. At the same time, without the use of a pneumatic calibrator, the *T. evanescens* had first quality only up to the fourth generation.

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