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THE INFLUENCE OF THE BIOLOGICAL AGENTS ON THE DENSITY SUPPRESSION OF *GRAPHOLITHA FUNEBRANA TR.* POPULATION IN PLUM CROP

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INTRODUCTION

In order to protect crops there are annually applied a broad spectrum of pesticides. The treatments with pesticides cause environmental issues that occur not only through direct negative effects but also indirect, which will be visible throughout generations. In addition to control the harmful organisms, the chemical treatments cause reduced number of beneficial species but the phytophages obtain resistance against pesticides and therefore either increase the number of treatment or apply new chemicals more sophisticated and expensive than the previous ones. This situation can only be solved by developing methods based on natural mechanisms on controlling relations between harmful organisms and crops.

The need for protecting crops and the environment has stimulated investigations of highlight, preparation and implementation of alternative means which allow the replacement of chemical treatments with biological agents and biologically active substances. Taking into account the specific features of the mechanisms of action of biologically active substances, their application mode is radically different from chemical pesticides application both in form and in content. In Moldova the surfaces of plum orchards are steadily increasing (currently about 10 thousand hectares of which 50% are new). The increase of production per hectare can be obtained by applying the methods of integrated protection systems. The *Grapholitha funebrana* distinguishes among the species of pest by the important degree of harm. The chemical treatments do not bring the expected results because of the hidden mode of larvae life. For these reasons there have been conducted researches aimed at reducing the pest population by concomitant application of multiple biological agents, the results of which are shown in the present publication.

The purpose of the research was to develop a new technological process of plum crop protection from *Grapholitha funebrana* pest by concomitant application of pheromone traps and the entomophages *Trichogramma embryophagum and Bracon hebetor*.

MATERIALS AND METHODS

The method of plum crop protection from *Grapholitha funebrana* pest by concomitant application of multiple biological agents (pheromone traps - against imago stage, of the entomophage *Trichogramma embryophagum* - against egg phase and *Bracon hebetor* entomophage - against larval phase) was developed and tested in plum orchards of the Republic of Moldova ("AGROBRIO" Ltd, Bacioi). The plum orchard area constituted ha 5.4.

The composition of the synthetic sexual pheromone applied in traps for mass male trapping of *Grapholitha funebrana* composed of three components - [cis-8-dodecenyl acetate 96% + trans-8-dodecenyl acetate 4%] (10%) + synergist dodecenyl acetate (90%). The main component (cis-8-dodecenyl acetate) was obtained for the first time by the synthesis scheme modified by us [2]. The pheromone traps were displayed throughout the whole plum orchard as chess table, based on the consideration by 12 per hectare (Fig. 1).

The evidence of the males trapped was performed during the vegetation period within 7 days.

The collection of entomophage *Trichogramma* sp. was performed by the method of exposure of *Sitotroga cerealella* egg cards in the plum orchard. The determination, maintenance and accumulation of *Trichogramma* sp. species was performed according to standard methods [3, 4]. In the result of the analysis of parasitized eggs there was found that the most widespread species of *Trichogramma* sp. collected in plum are: *T. embryophagum* - 50.0%, *T. dendrolimi* - 30.0% and *T. evanescens* - 20.0%.

Fig. 1. The display of pheromone traps in plum orchard for mass male trapping of *Grapholitha funebrana* pest

The biological indices determined for *T. embryophagum* were: prolificacy - 27.0 eggs/female; eclosion - 86.0%; female share - 100%; static criterion of the quality - 23.2. For the determination of the rules for launching the entomophage *T. embryophagum* in reducing the *G. funebrana* pest population density different rules were applied: 450, 350, and 250 thousand/ha in sachets (Fig. 2).

Fig. 2. The method for launching the entomophage *Trichogramma embryophagum* in sachets in plum crop for parasitizing the eggs of *Grapholitha funebrana* pest.

The biological efficacy (E) of the entomophage in parasitizing the eggs of *G. funebrana* pest determined by the following formula:

$$\mathbf{E} = \frac{\mathbf{B_v} \times \mathbf{A_m} - \mathbf{B_m} \times \mathbf{A_v}}{(\mathbf{A_m} - \mathbf{B_m}) \times \mathbf{A_v}} \times 100$$

Where: $\mathbf{A_v}$ – the number of pest eggs detected in experimental variant; $\mathbf{A_m}$ – the number of pest eggs detected in control; $\mathbf{B_v}$ – the number of pest eggs parasitized in experimental variant; $\mathbf{B_m}$ – the number of pest eggs parasitized in control.

The digital maps of the spatial distribution of *G. funebrana* pest eggs in plum orchard were built using the BIOCLAS program.

For further investigations there were obtained under laboratory conditions more than 200 thousand imago of the entomophage *Bracon hebetor*. During investigations there was maintained and multiplied in mass the entomophage crop of *B. hebetor*. As a host served the larvae of *Galleria mellonella* L. species, which has been multiplied by the nutrient medium (to prepare 1 kg of nutrient medium is required 200 g of wheat bran + 130 g of wheat flour + 130 g of corn flour + 110 g of honey + 130 g of glycerol + 100 g of milk powder + 200g of merva).

During the growing season there were made 6 launches of the entomophage *B. hebetor* (both as imago, and as the stern), arising from the consideration by 30 thousand/ha. The evidences and assessing of the parasitizing degree of *G. funebrana* pest's larvae by the entomophage *B. hebetor* were performed after every 7 days of launching. The assessment of the parasitizing degree of *G. funebrana* larvae by the entomophage *B. hebetor* was performed by analyzing each 100 plum attacked in five repetitions (Fig. 3).

Fig. 3. The launch of entomophage *Bracon hebetor* in plum crop for the parasitizing of *Grapholitha funebrana* pest larvae

The obtained results were subjected to mathematical analysis in accordance with the program package Microsoft Excel.

RESULTS AND DISCUSSIONS

In the investigations made by us in previous years there has already been shown that in the result of the development of pheromone composition "[Z8-12Ac 97% + E8-12Ac 3%] (10%) + H-12Ac (90%)" and the application of the mass male trapping method it has permitted us to substantially reduce the population density of *G. funebrana* pest [1]. The purpose of the current researches was to develop a new technologic process of plum crop protection from *G. funebrana* by concomitant application both pheromone traps for mass male trapping and entomophages *Trichogramma embryophagum* and *Bracon hebetor* - for parasitizing the eggs and larvae of the given pest.

The mass male trapping of *Grapholitha funebrana*: The application of the appropriate method aimed to eliminate a maximum number of *G. funebrana* active pest population of males that are developing in the plum orchard that is being tested. To that effect there were uniformly distributed 65 pheromone traps, which by their action range have covered the entire surface of the adequate orchard. Thus there were created the necessary conditions to attract in the pheromone traps as most imposing number of the active males and their elimination of the population. The elimination of the active males will have direct influence on reducing the number of the coupled females. It should be noted that the density of *G. funebrana* pest population in the appropriate orchard was at a medium level. This was confirmed by the number of males trapped during the development of two generations - from 0.7 to 3.9 males/trap/7 days. Due to the uniform distribution of 65 pheromone traps on the surface of plum orchard was able to eliminate the pest population of about 2002 males (Fig. 4).

It was demonstrated that due to the application of the mass male trapping method it was managed to eliminate from the pest population of *G. funebrana* a large number of active males, the fact that has directly influenced in the reduction of eggs deposition by females. Thus, if on the control sector were in average about 30 eggs/100 plums, then on the experimental sector only 16 eggs/100 plums.

The launch of the entomophage *Trichogramma embryophagum*:

In the plum orchard where the method of *G. funebrana* pest control was developed by concomitant application of several biological agents, it has been demonstrated that in the control sector there were parasitized 6.5% eggs of the pest *G. funebrana* by entomophagous *Trichogramma* sp. In the experimental orchard, during the development of both generations of the pest *G. funebrana* there was determined the density of dynamic deposition of eggs. The analysis of obtained results has shown that on the average there were 16 eggs/100 fruits. By applying the BIOCLAS program there were built digital maps of distributing the pest eggs throughout the whole plum orchard to detect the outbreaks. Due to digital maps it was found that the females of *G. funebrana* pest have deposited eggs on fruit nonuniformly. Thus, at the beginning of the first generation the deposition of eggs went very slowly and with a rather low intensity. Towards the end of the first generation, the deposition of eggs by pest females has intensified and there began to appear some localized outbreaks mostly on the periphery of the orchard. Along with the beginning of the development of the second generation was further intensified the egg deposition, that by the end of generation to get a pretty strong character and a greater expressiveness of outbreaks (Fig. 5).

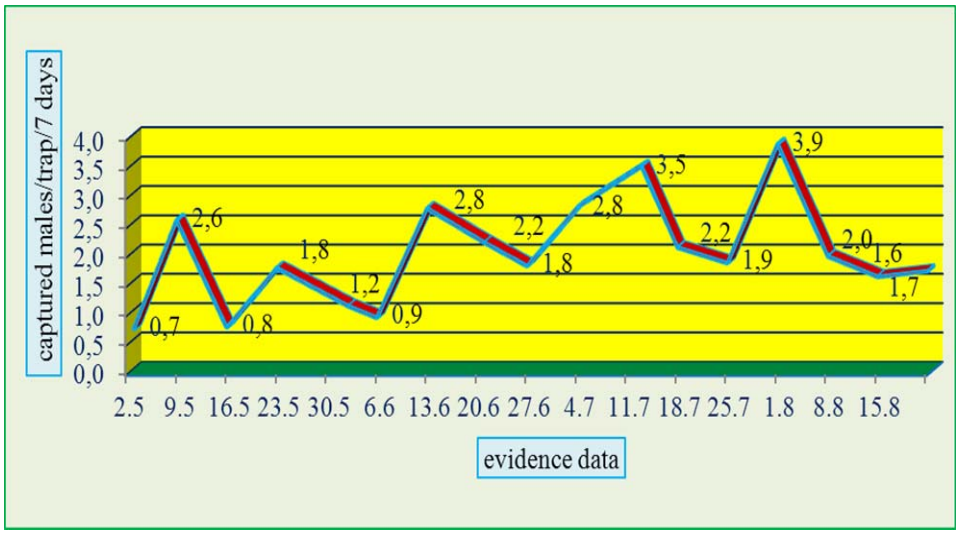

Fig. 4. Males of *Grapholitha funebrana* pest, captured in a pheromone trap during the development of two generations

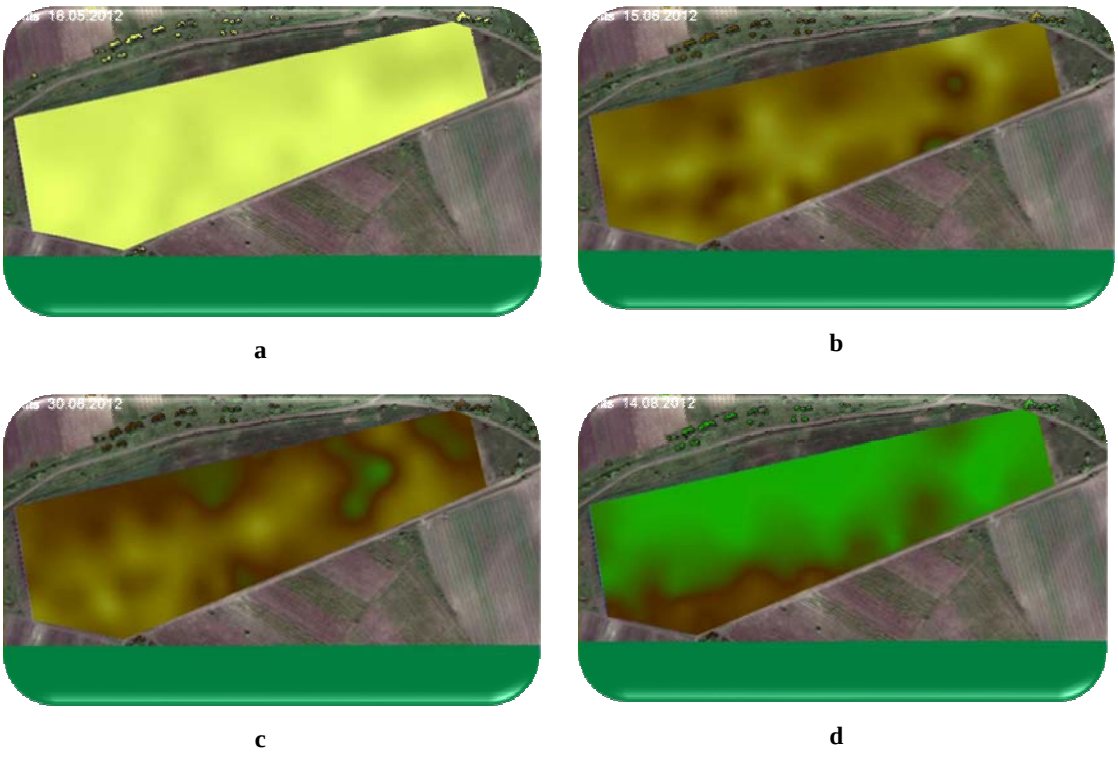

Fig. 5. The digital maps of the distribution of the *Grapholitha funebrana* pest clutch depending on the period of seasonal development of population in the plum orchard. (**a**. The beginning of the first generation development; **b**. The end of the first generation; **c**. The beginning of the second generation development; **d**. The end of the second generation development)

To assess the launch norms of the entomophage *T. embryophagum* there were tested three variants: Ist variant - 300000/ha; IInd variant - 200000/ha; and IIIrd variant - 100000/ha. As a result of testing it was found that the most optimal is conducting six launches (with the norm of 300000/ha), leading to the parasitizing on the average of about 69.0% of the eggs of *G. funebrana* pest, deposited on plums during the seasonal cycle of development (compared with 6.5% of parasitized eggs on sector without entomophage launching).

The launch of the entomophage *Bracon hebetor*:

For the conduction of planned investigations there were obtained under laboratory conditions more than 200000 imago of the entomophage *Bracon hebetor*. In laboratory conditions there has been demonstrated that the females of the entomophage *B. hebetor* react not in all *G. funebrana* pest larvae, but only to those who have already reached the age of IV-V. Experimentally it was found that the larvae in the IV age were in most cases only paralyzed, while the larvae that have already reached the age of V were attacked by entomophagous and they deposited the eggs in them. At the same time it was demonstrated that on the pest larvae of the age of V the development cycle of *B. hebetor* entomophage larvae extends over a period of about 11-13 days. During experiments it was also observed the fact that in a larva of the *G. funebrana* pest the development cycle until pupal stage can be finished by 1-3 larvae of the *B. hebetor* parasite.

In the experimental plum orchard there was made a dynamic analysis of the fruits attacked by larvae of the pest *G. funebrana*. It was found that in all the attacked plums only about 14.0% of the *G. funebrana* pest larvae have reached the age of IV-V.

In field conditions there has been demonstrated that the optimal norm to launch the entomophage *B. hebetor* against the larvae of *G. funebrana* pest are about 30000 imago/hectare. During the development of two generations of *G. funebrana* pest there were made 6 launches of the corresponding entomophage. The test results of the attacked fruits and the *G. funebrana* pest larvae have demonstrated that about 44.0% were parasitized by *B. hebetor* entomophage.

Thus, the purpose of development the technological process of plum crop protection from *G. funebrana* pest by concomitant application of multiple biological agents has been reached. In terms of production there has been demonstrated that the application of the method of mass trapping of *G. funebrana* male lead to the elimination of an essential number of active male population. Then, 6 launches of *T. embryophagum* entomophage lead to

parasitizing of about 69.0% of *G. funebrana* eggs deposited on plums during the seasonal cycle of development. Next, the performance of 6 launches of *B. hebetor* entomophage - causes the parasitizing of about 44.0% of the *G. funebrana* larvae. The analysis of the results achieved by the method developed on *G. funebrana* pest control has demonstrated that the biological effectiveness in plum protection is 97.2% (equal to the one obtained on chemical standard sector - two treatments with insecticides).

CONCLUSIONS

It was developed a methodological process of biological protection of plum;

It was demonstrated in terms of production, that the concomitant application of the method of mass male trapping, the launch of the entomophages *Trichogramma embryophagum* and *Bracon hebetor* reduces the damage caused to the plum by *Grapholitha funebrana* with about 97.2% (equal to the one obtained on the chemical standard sector - two treatments with insecticides).

ABSTRACT

In the result of the development of a new methodological process of biological protection of plum from *Grapholitha funebrana* pest by concomitant using both the pheromone traps for mass male trapping, and the entomophages of *Trichogramma embryophagum* and *Bracon hebetor* - for parasitizing the eggs and larvae of the given pest it was succeeded to reduce the damage caused to the plum with about 97.2% (equal to the one obtained on the chemical standard sector - two treatments with insecticides).

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