

BIOLOGICAL PROTECTION OF SOYBEAN CROPS AGAINST THE STINK BUG COMPLEX USING THE ENTOMOPHAGOUS *TRICHOGRAMMA EVANESCENS* WESTW

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Key words: releases, parasites, *Vanessa cardui*, generations, entomophagous *Trichogramma evanescens*, stink bug complex

INTRODUCTION

The control of harmful insects in modern plant protection concepts is one of the important links in food chains and trophic relationships, which would ensure a superior quality environment with the possibility of obtaining organic agricultural products.

The soybean crop is attacked by various species of pests, but the most important ones economically are: the fruit borer (*Helicoverpa armigera* Hb.), the cutworm (*Agrotis segetum* Schiff), the soybean pod borer (*Etiella zinckenella*), the black cutworm (*Amathes-C-nigrum* L.), the armyworm (*Agrotis ypsilon* Huf.), the heart and dart moth (*Agrotis exclamationis* L.), and the painted lady butterfly (*Vanessa cardui* L.) (Trotuș, Elena, Pochișcanu, Simona, Pomohaci, T., 2014; Kovalenkov B.G., Tyurina N.M., 2002).

In our research at the Institute of Genetics, Physiology, and Plant Protection in Chisinau, Republic of Moldova, the complex of pests was monitored using pheromone traps and light traps. The use of the entomophagous *Trichogramma* spp. is widely used in plant protection due to its quality in mass reproduction and its application in the field, in the fight against complex pests, including borers, moths, and whiteflies.

In 2019, the intense flight of the painted lady butterfly (*Vanessa cardui* L.1758) was noted in the Republic of Moldova. Until recently, this pest was considered occasional on soybean crops, but once it appears in the field, it can defoliate the plants, causing significant damage to this crop. As in the case of most legume crops such as soybeans, beans, peas, lentils, and decorative plants, they are preferred by this pest, as well as sunflowers.

The painted lady butterfly is one of the most interesting species of Lepidoptera due to its vibrant colors, and it migrates annually between North Africa and Europe (Stefanescu Constantin, Richard R. Askew, Jordi Corbera, Mark R. Shaw, 2012). The pest in question is the Painted Lady butterfly (*Vanessa cardui* L.).

The adult has a brownish-grey color with black antennae. The hindwings are orange with white and black designs, while the forewings are brown with two rows of white spots. The wingspan exceeds 50 mm. The adult feeds on the nectar of various spontaneous or cultivated flowering plants and on secretions left behind by different species of aphids. It is a migratory species that can fly in rain and wind.

The eggs have a barrel shape with vertical ridges and are laid singly on the upper and lower side of leaves. They are light green in color and turn gray before hatching. The hatching of the eggs and rapid development of the larvae are favored by high atmospheric humidity. After 3-5 days, the larvae (caterpillars) appear which begin to feed and cause significant damage.

The larvae are black and have numerous black and yellow spines and a yellow stripe on each side, reaching over 40 mm in length. The damage is caused by the larvae, which skeletonize or nibble the leaves, leaving only the main veins. During their development, the larvae molt three times, and after each molt, they consume more leaves. Using the attacked leaves, the larva weaves a silk shelter (nest), which protects it while it feeds.

The pupae are brown or gray and are attached to the leaves, hanging with their head down. The pest overwinters in the pupal stage. In 2019, this pest developed in a single generation in Romania, with a higher density than the economic damage threshold. The first generation appeared in mid-May and lasted until the end of June, then disappeared. The second generation did not develop, suggesting that the weather conditions were favorable for *Vanessa cardui's* development. The pest attacks over 300 species of plants and is polyphagous. The characteristic attack pattern is the skeletonization of the leaf blade, which is consumed entirely, leaving only the veins intact. The pest can be controlled with specific insecticides for combating Lepidoptera larvae, entomophagous organisms, and biological preparations. The high density of the pest is due to climate change, which favors the development of these species.

MATERIAL AND METHODS

Location of research: The research was conducted at the Institute of Genetics, Physiology and Plant Protection, under laboratory conditions and on experimental fields for soybean cultivation, the Laduta variety, covering an area of two hectares. The entomophagous *Trichogramma evanescens* Westw., collected from soybean crops was used.

To reduce the density of the *Vanessa cardui* L. butterfly pest, first, the egg density was monitored in the field, and then the entomophagous *Trichogramma evanescens* Westw. was applied during the development period of the first pest generation. In 2019, egg and larval counts were carried out at 10 points in the soybean field, with 200 plants per hectare. Based on the parasitized egg counts in the field, the biological efficacy of *T. evanescens* was determined. The release rate of the entomophagous in pest control was 100.000-300.000 individuals per hectare, depending on the pest egg density. The entomophagous was not released in the control group. In the second generation, this pest did not manifest itself (it disappeared).

In the years 2021-2022, the *Vanessa cardui* L. butterfly was present in very low numbers, and the egg density was lower than the economic damage threshold.

RESULTS AND DISCUSSION

To reduce the density of the harmful brick red butterfly (*Vanessa cardui* L.) in soybean crops, the density of eggs and larvae in the field was monitored, and two releases of the *Trichogramma evanescens* insect was carried out at the egg stage. Prior to releasing *Trichogramma evanescens* in the soybean field, exposure tests were conducted using eggs of the cereal moth glued onto cards, to collect *Trichogramma* sp. from nature (Table 2). The percentage of parasitization of *Sitotroga cerealella* Ol. eggs as a result of exposure varied from 1.0% to

8.8%. From May to September, the presence of the insect was observed in the field. The following species were identified from the specimens collected from the soybean crops: *T. evanescens*-60%, *T. dendrolimi*-10%, *T. pintoi*-15%, and *T. semblidis*-15%, which were maintained and multiplied for further research. The experimental soybean field at IGFPP is shown in Fig. 12.

During the first generation development period of the brick red butterfly (*Vanessa cardui* L.1758), the first release rate was 100,000 individuals of the insect per hectare (June 5, 2020), and the second release rate was 300,000 individuals per hectare (June 07.2020). Egg and larval density of the *Vanessa cardui* butterfly in soybean crops were monitored 9 times during this period. During the first generation development period, the number of *Vanessa cardui* eggs ranged from 61 to 2.287 per 200 plants per hectare, and the average density varied from 1.0 to 11.43 eggs per plant. This density is higher than the economic threshold for the complex of stink bugs. In the control group, the average density per plant during this period varied from 1.55 to 15.90 eggs per plant. (Table 1, Fig. 1, 2). In the control group, the *Trichogramma* insect was not released. Fig. 13 shows soybean is presented pods attacked by the stink bug complex in the control group in 2019.

During the development of the first generation of the pest, the density of *Vanessa cardui* larvae ranged from 104 to 1609 larvae per 200 plants per hectare, with an average density per plant ranging from 0.52 to 8.04 larvae. In the control group during this period, the average density per plant ranged from 0.55 to 9.90 larvae of different ages.

Before and after each release of *T. evanescens*, the eggs were collected from the plants and placed in separate test tubes. The biological efficacy of *T. evanescens* was determined by the evidence of the number of parasitized eggs in the field. Soybean pods attacked by a complex of the *Lepidoptera* complex bugs in the control group.

Table 1. The average density of *Vanessa cardui* eggs and larvae in soybean crop, 2019

Date	Sum of eggs	Average eggs/plant	Control	Date	Sum larve	Average larvae/plant	Control
28.05	183±3.3	1.00	1.55	28.05	104±3.2	0.52	0.55
29.05	218±4.3	1.09	1.70	29.05	123±3.3	0.61	0.70
30.05	266±4.5	1.47	1.75	30.05	129±3.5	0.64	0.75
05.06	313±4.7	1.33	2.73	05.06	124±3.1	0.62	0.63
07.06	605±3.3	3.02	3.75	07.06	217±3.8	1.10	1.75
10.06	2287±5.8	11.43	15.90	10.06	1609±5.1	8.04	9.90
11.06	1718±5.2	8.58	9.97	11.06	1431±4.4	7.15	7.98
17.06	454±3.5	2.24	4.90	17.06	374±4.3	1.87	4.90
27.06	61±2.3	0.30	3.50	27.06	115±3.3	0.57	2.50
Sum	6105	30.37	45.75	Sum/	4226	21.12	29.66
Average	678.33	3.37	5.08	Average	469.5	2.34	3.3

The total number of eggs collected from 200 plants per hectare during the period from May 28, 2019, to June 27, 2019, during the development of the first generation of *Vanessa cardui* pests was 6105. The entomophagous *Trichogramma evanescens* Wesw. was applied during the development of the crop to protect the soybeans. Out

of the total number of *Vanessa cardui* eggs, 4400 were parasitized by *Trichogramma evanescens*, with a parasitized egg percentage of 72%. Fig. 3-8 show different stages of development of the pest, the Painted Lady butterfly (*Vanessa cardui* L., 1758). Soybean pod bugs egg mass on soybean pods, 2019. Fig. 9, 10, 11.

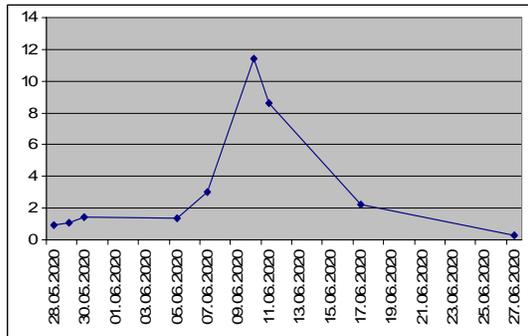


Fig. 1. Average density per plant of *Vanessa cardui* eggs in soybean crop, 2019

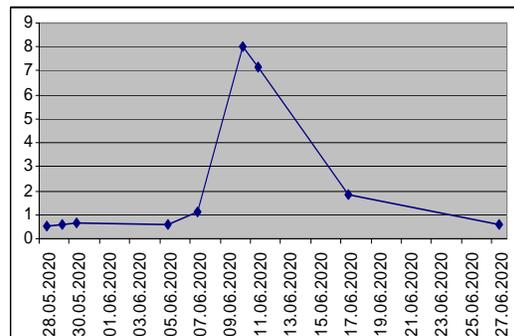


Fig. 2. Average density per plant of *Vanessa cardui* larvae in soybean crop, 2019

Development of the pest Brick moth (*Vanessa cardui* L. 1758)



Fig.3. Fresh *V. cardui* eggs on soybean leaves, 2019. (Photos by Gavrilita L.)



Fig. 4. Cocoon of *V. cardui* on soybean, 2019. (Photos by Gavrilita L.).



Figure 5. Appearance of *V. cardui* caterpillar in soybean culture, 2019. (Photos by Gavrilita L.)



Fig.6. Pupae of *V. cardui*, 2019 (Pictures by Gavrilita L.)



Fig.7. Adult *V. cardui* on soybean plant, 2019. (Photo by Gavrilita L.)

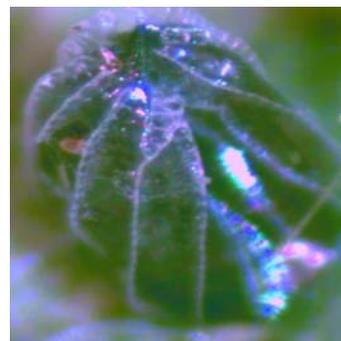


Fig.8. *V. cardui* egg parasitized by *Trichogramma* on soybean, 2019. (Photos by Gavrilita L.).



Fig. 9. Soybean pod bugs egg mass on soybean pods, 2019

Table 2. Percentage of *Sitotroga cerealella* Ol. egg parasitization after exposure for *Trichogramma* sp. collection in nature, in soybean culture, IGFP, 2019

Number of exposures	Date exposures	Number parasitized eggs	Percentage of parasitization
1.	22.05.19	0.06	0.6±0.02
2.	10.06.19	0.8	0.8±0.02
3.	12.06.19	10	1.0±0.2
4.	14.06.19	14	1.4±0.3
5.	15.06.19	25	2.5±0.6
6.	16.06.19	30	3.0±0.6
7.	17.06.19	43	4.3±0.7
8.	19.06.19	45	4.5±0.8
9.	24.06.19	60	6.0±0.9
10.	03.07.19	63	6.3±0.9
11.	06.07.19	70	7.0±0.9
12.	09.07.19	72	7.2±1.0
13.	15.07.19	76	7.6±1.0
14.	18.07.19	79	7.9±1.1
15.	23.07.19	82	8.2±1.1
16.	30.07.19	83	8.3±1.2
17.	05.08.19	85	8.5±1.3
18.	08.08.19	88	8.8±1.3



Fig. 10. Soybean experimental field, 2019



Fig. 11. Soybean crop in the biological variant, 2019



Fig. 12. Exposing eggs of the cereal moth for collecting *Trichogramma* from nature, in the soybean crop, 2019



Fig. 13. Soybean pods attacked by a complex of the *Lepidoptera* complex bugs in the control group, 2019

CONCLUSIONS

1. During the development period of the first generation, the total number of *Vanessa cardui* L. pest eggs varied from 61 to 2287 per 200 plants per hectare, with the average density per plant ranging from 1.0 to 11.43 eggs.

2. During the development period of the first generation of *Vanessa cardui* pest, the number of pest larvae was recorded, where the total number per 200 plants per hectare varied from 104 to 1609 larvae, with the average density per plant ranging from 0.52 to 8.04 larvae.

3. Out of the total number of 6105 *Vanessa cardui* eggs collected in the soybean crop, after two releases, the total number of parasitized eggs amounted to 4400, with a parasitized egg quantity of 72%.

The release rate of the entomophagous in pest control was 100.000/ha for the first release (05.06.20), and 300.000/ha for the second release (07.06.20), depending on the density of pest eggs. In the control group, no entomophagous was released.

ABSTRACT

Soybean crops are attacked by various pests, but the most economically important are the stink bug complex. In 2019, on the territory of the Republic of Moldova, a significant flight of butterflies from the *Nymphalidae* family, specifically Painted Lady (*Vanessa cardui* L., 1758), was observed. During the first generation of soybean crop development, the number of *V. cardui* L. eggs ranged from 61 to 2287 eggs per 200 plants per hectare. The average density per plant ranged from 1.0 to 13.0 eggs.

Out of the total number of *V. cardui* eggs (6105) collected in soybean culture, after two

releases of *Trichogramma evanescens*, the number of parasitized eggs was 4400-72%. The norm for the entomophage pest control release was 100,000 eggs/ha in the first release (05.06.20) and 300,000 eggs/ha of individuals (07.06.20) in the second release.

No entomophage was released in the control group. In the years 2020-2022, the Painted Lady butterfly was present in very low numbers, and the egg density was below the economic damage threshold.

ACKNOWLEDGEMENTS

Research was carried out within the project of the State Program 20.80009.5107.27 “Elaboration of the alternative methods based on environmentally friendly means and procedures for harmful arthropods control in different agricultural crops”, financed by the National Agency for Research and Development.

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