

# ORIGINAL PAPERS

## REDUCING THE DENSITY OF DEFOLIATOR PESTS WITH THE HELP OF TRICHOGRAMMA IN THE PARKS FROM REPUBLIC OF MOLDOVA AND ROMANIA

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**Key words:** protection, efficacy, pests, pheromone, monitoring, biological indices, prolificacy

### INTRODUCTION

Till the ecological balance is restored, various artificial means of maintaining control over the mass multiplication of insects, must be used. In order for such means to be less destructive to the ecological system and be applied with minimum costs, it is necessary to acquire knowledge on dynamics of evolution of different populations, biotic limiting factors and methods to detect these phytophagous insects.

*Trichogramma* species are polyphagous using very different hosts like: Lepidoptera, Diptera, Coleoptera (BUENO & LENTEREN, 2002, GAVRILIȚA et al. 2, 2003; GAVRILIȚA, 2005). According to the author's DIURICI G. data 12 species of *Trichogramma* out of 14 known in Republic Moldova are present in the "Codrii" reservation, where the entomophagous required for the project were collected from (DIURICI, 2008). Data states that annual launches with *Trichogramma* worldwide amounts to a total area of 45 million hectares of agricultural crops (LENTEREN, 2000). Biological protection of parks, aims to prevent attack by pests and their control. A very important role on reducing the density of pests, during the 2 or 3 generation development, attributes to entomophagous insects. That is the reason why, at adult stage pheromone traps are installed, while at eggs stage, 2 – 5 launches of *Trichogramma* is performed with a norm of 200.000-300.000 individuals per hectare. The costs reduce by two or three times compared to the chemical protection. The integrated protection aims to minimize or avoid the use of chemical substances and substitute them with biological means of protection, without having harmful effects on humans and useful entomofauna, and maintain a proper health of the plant.

**RESEARCH GOAL:** *Elaboration and implementation of regulation proceedings of the harmful organisms' density, with the use of Trichogramma entomophagous, within the integrated protection, against defoliator pests in the parks from Republic Moldova and Romania.*

### MATERIALS AND METHODS

Collection of *Trichogramma* from the nature for the 2013 research, was carried out in Moldova and Romania at different species of plants and flowers. The experiments were performed in the laboratory "Fitofarmacie and Ecotoxicology" Institute of Genetics, Physiology and Plant Protection of the Academy of Sciences from Moldova. The collection, determination, maintaining and accumulation of the species of *Trichogramma* sp. were performed according to the author (DIURICI, 2008) methods, to be recommended in practice. Rearing the laboratory host grain moth (*Sitotroga cerealella* Oliv.), determining biological indices of *Trichogramma embryophagum* Hb., *Trichogramma evanescens* Westw. determination of the numerical density of the pest eggs, biological efficacy of *Trichogramma* were performed according to the traditional methods of the authors (ABAȘCHIN et al., 1979). Breeding of grain moth and *Trichogramma*, evaluation of biological effectiveness of entomophagous and the extent of fruit damage by pest and mathematical data processing were conducted according to the relevant procedures and guidelines for the mass breeding and use of *Trichogramma* (MENCER & ZIMMERMAN, 1986).

Investigation objectives were different species of *Trichogramma* (*T. evanescens*, *T. pintoi*, *T. embryophagum*) reared on host laboratory eggs – *Sitotroga cerealella*. The objects of the research in Chișinău's Botanical Garden, are the pests: *Lymantria dispar* Linnaeus, 1758, *Tortrix viridana* Linnaeus, 1758, *Grapholita funebrana* Treitschke, 1835, *Grapholita molesta* Busck, 1916, *Laspeyresia pomonella* Linnaeus, 1758, *Cameraria ohridella* Deschka & Dimic, 1986, *Helicoverpa armigera* Hiibner, 1809 that cause great damage to the trees and the decorative plants in parks and Botanical Gardens, the species which were monitored with the use of pheromone traps.

In combating these pests, in experiences the oophagous wasp *Trichogramma* was used, being widespread in all the areas where parasitize hosts met. Adult body length is 0.5 to 1.0 mm, lobed anterior wings and the interior ones narrow and short,

transparent, yellow-pale color, black ribs and fringes on the edges. Females lay their eggs in the eggs of hosts, with a prolificacy of 20 to 24 eggs, maximum 50. The egg is white color.

- collection method and mass multiplication of *T. evanescens*:

*Trichogramma* used in the experiments was collected from the field in two methods:

1. Collecting pests' eggs parasitized by *Trichogramma* from the nature.
2. Collecting moth eggs (*S. cerealella*) preliminarily launched in the field, parasitized by *Trichogramma* from the nature.

After collecting biological material, each parasite laid eggs were placed separately in a test tube, the material has had been accumulated for 2-3 generations, then *Trichogramma* species were identified for further researches.

## RESULTS AND DISCUSSIONS

Research conducted in Chisinau Botanical Garden, with *Trichogramma* was performed in two ways: in the first method, for efficacy determination of the entomophagous *Trichogramma* in the field, releases were made of small cards with grain moth eggs (*Sitotroga cerealella*) glued on and fixed at different species of plants and flowers. In the second method, precise identification of field natural presence of pest eggs was performed beforehand and then 4 launches with *Trichogramma* entomophagous were made.

At the Botanical Garden from Chisinau, on 05.28.2013 were exposed up to 20 cards, each with 100 grain moth eggs (*S. cerealella*), at three trees (three replicates) on each species of plant investigated, 60 cards in total: mulberry - *Morus alba*, Oak - *Quercus pedunculiflora*, Plum - *Prunus cerasifera*, Apple - *Malus domestica*, Chestnut - *Aesculus hippocastanum* and various flowers. In total 2000 eggs of *S. cerealella* at each plant species, based on the norm of 300,000 individuals per 1 ha with *T. embryophagum*. At flowers the launch was made, where *T. evanescens* Westw was released, based on the norm of 200,000 individuals per 1 ha of the area investigated. Such exposures with cards with *S. cerealella* at the same trees and releases with *Trichogramma* were performed the same on 28/05, 13/06, 10/07 and 30/07.

After each release, analysis was performed to determine the number of parasitized eggs. For this, all cards were collected from the 3 repetitions, then the number of parasitized eggs of *S. cerealella* by *T. embryophagum* and *T. evanescens* were counted from trees and flowers on each card separately. The dates of the records from the experiments were the following: 31.05, 17.06, 12.07, 02.08.

For the martor (where no *Trichogramma* launch was performed), one tree has been taken on which 30 cards were attached of 100 eggs of *S. cerealella* each to analyse the natural presence of *Trichogramma* and its capacity to parasitize the eggs. For each record afterwards, the numbers of parasitized eggs were counted. The results of the records done, and the number of parasitized eggs along with the percentage of parasitized eggs, are reported in the Table 1.

Table 1. The number of parasitized eggs of *Sitotroga cerealella* Ol. recorded from different species of trees and flowers in the Botanical Garden, Chisinau, 2013

Option	Specie	Percentage of parasitized eggs	Percentage of parasitized eggs	Percentage of parasitized eggs	Percentage of parasitized eggs
		1st launch, 28.05	2nd launch, 13.06	3rd launch, 10.07	4th launch, 30.07
After the launches	Mulberry	28.05	34.9	57.8	69.7±3.2
	Oak	23.25	35.9	56.3	67.6±3.2
	Flowers	30.6	37.9	60.0	74.4±3.8
	Apple	31.75	40.0	58.0	75.9±3.4
	Chestnut	25.05	35.8	53.1	69.05±2.2
	Plum	29.45	39.9	60.0	74.8±2.2
Control	Mulberry	1.4	2.8	5.8	6.9±1.4
	Oak	0.7	2	5.0	6.3±1.2
	Flowers	1.8	1.6	4.7	7.2±1.4
	Apple	1.3	2.4	6.6	7.0±1.2
	Chestnut	1.3	1.5	4.1	6.8±1.0
	Plum	1.3	2.2	4.5	6.1±1.0

From the data in the Table, it is revealed that on 31.05 when the first record was performed the highest rate of parasitization is present on cards taken from apple trees – 31,75%. The decreased percentage is recorded on flowers – 30.6%, plum – 29.45%, mulberry – 28.5%, chestnut – 25.05% and the least in Oak - 23.25%.

On the date of 17/06, for the second record, the percentage of parasitized eggs was as followed: apple - 40%, plum - 39.9%, the flowers - 37.9%, Oak - 35.9%, chestnut - 35.8% and the least in mulberry - 34.9%. On the date of 12/07, for the thirds record the percentage was: plum - 60%, flowers - 60%, apple - 58% mulberry - 57.85%, Oak - 56.25% and the least

were at Chestnut - 53.1%. For the fourth record, on the date of 02/08, the following data has had been accumulated: Apple - 75.9%, plum - 74.8%, flowers - 74.4%, mulberry - 69.7%, chestnut - 69.0, the least in Oak - 67.6%.

The following conclusion can be stated: the higher the percentage of parasitization, the higher the preference of the *Trichogramma* wasp to frequent

and nutrition and possibility to parasitize in nature the eggs of the harmful organisms for the plants, decreasing this was the loss of the vegetation surrounding. The results from control – number of eggs parasitized and the percentage of eggs parasitized (0.7 – 7.2%), in comparison with the variants where *Trichogramma* was launched, are very small presented in the Fig.1.

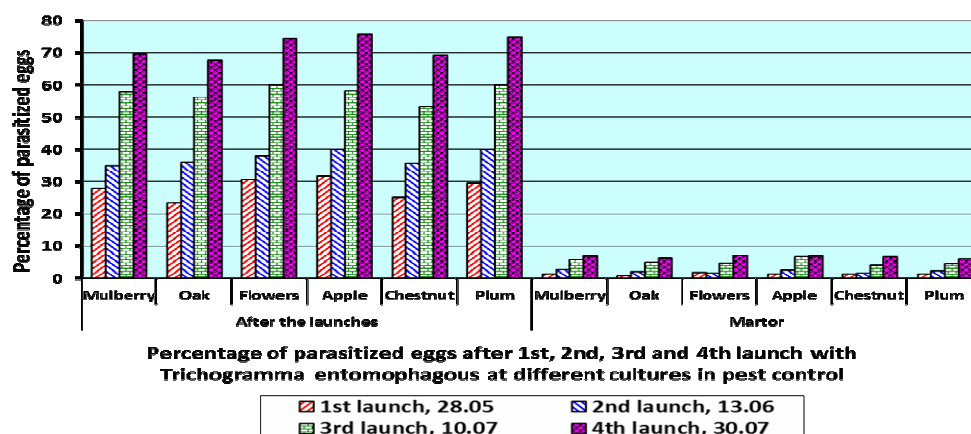


Figure 1. The percentage of parasitization of the *Sitotroga cerealella* eggs at different species of trees and decorative plants from parks, 2013.

#### Determination of biological efficacy of *Trichogramma* in defoliators' pests control from Botanical Garden from Republic of Moldova

In the Botanical Garden from Chisinau, Republic of Moldova according to the program of the project the density of pests eggs has been determined at different species of plants with a sample of 100 fruits + leaves, at 4-5 trees per specie. This information helped identify the terms and norms of launches with *Trichogramma*, used for pests' control. The results are represented in the Table 2.

The density of the nature pests' eggs at plum culture - Prunus Cerasifer for the pest *Grapholitha funebrana* varied from 100 to 145 eggs, for *G. molesta* varied from 90 to 158 eggs, at apple culture – Malus Domestica the pest's *L. pomonella* density varied from 126 up to 160 at 100 fruits and leaves.

Numerical determination of the density of pests' eggs was done starting from the very beginning of the vegetation period up to the end of it. To determine more precisely the effectiveness of *Trichogramma* in field, there were carried out launches on artificial background with eggs of grain moth (*Sitotroga cerealella*) for pest control in the parks.

In Chisinau, at Botanical Garden, 4 launches were conducted with *Trichogramma embryophagum* Hb spp., for pests' control at the eggs stage at different species: Mulberry - *Morus alba*, Oak - *Quercus pedunculiflora*, Plum - *Prunus cerasifera*, Apple - *Malus domestica*, Chestnut - *Aesculus hippocastanum* with the use of 300,000 individuals per 1 ha. At flowers, *T. evanescens* was released based on the norm of 200,000 individuals per 1 ha - the area occupied by the plants investigated.

Table 2. The density of the nature pests' eggs at different species of plants in the Botanical Garden, Chisinau, 2013

Culture	Pests	The number of records			
		1st Record 31.05	2nd Record 17.06	3rd Record 12.07	4th Record 02.08
Plum - Prunus cerasifera	<i>Grapholitha funebrana</i>	100.0	104±3.7	128±3.8	145±4.4
	<i>Grapholitha molesta</i>	90.0±4.0	94±3.4	120±4.2	158±5.0
Apple - Malus domestica	<i>Laspeyresia pomonella</i>	126.0±4.1	128±3.9	140±4.7	160±5.2
Flowers	<i>Helicoverpa armigera</i>	100.0	110±3.6	186±4.8	180±5.2

In control the entomophage was not launched. For the record, the pests' eggs parasitized by *Trichogramma* were collected from the nature. The results of the records – number of parasitized eggs and the percentage of them are represented in

the Table 3. Form the table we can analyze that the percentage of eggs parasitized by *T. embryophagum* at plum culture against the pest *G. funebrana*, which ranged from 27.0% to 68.6%, likewise against *G. molesta* ranged from 25.0% to 64.5%.

Table 3. The parasitization of the pests' nature eggs at different species of plants from Botanic garden, Chisinau, 2013.

Cul ture	Pests	Number of launches and records							
		1st launch, 28.05		2nd launch, 13.06		3rd launch, 10.07		4th launch, 30.07	
		Total number of eggs, parasited	Percentag e of parasitatio n, %	Total number of eggs, parasite d	Percentage of parasitatio n, %	Total number of eggs, parasite d	Percentage of parasitatio n, %	Total number of eggs, parasite d	Percentage of parasitatio n, %
Plum - Prunus cerasifera	<i>Grapholitha funebrana</i>	27.0	27.0	39.5	38.0	71.0	56.0	100	68.6±3.3
	<i>Grapholitha molesta</i>	22.5	25.0	34.5	36.7	60.0	50.1	102	64.5±3.0
Apple - Malus Domestica	<i>Laspeyresia pomonella</i>	28.0	22.2	48.5	37.9	79.0	56.3	105	65.4±3.2
Flowers	<i>Helicoverpa armigera</i>	27.0	27.0	35.2	32.0	128.3	69.0	132	73.3±3.2
Control									
Plum - Prunus cerasifera	<i>Grapholitha funebrana</i>		0.7		2.0		4.1		6.3±0.8
	<i>Grapholitha molesta</i>		0.4		2.0		3.8		5.7±0.6
Apple - Malus Domestica	<i>Laspeyresia pomonella a</i>		0,7		2.3		3.8		6.8±0.8
Flowers	<i>Helicoverpa armigera</i>		1.2		2.8		4.9		7.8±1.0

At flowers, the biological effectiveness of *T. evanescens* against the pest *H. armigera*, ranged from 27.0% to 73.3%. At apple culture, the effectiveness of *Trichogramma embryophagum* Hb. used against the pest *Laspeyresia pomonella*, after four releases, ranged from 28.0% to 65.4%. In control parasitized eggs of pests on crops named above, ranged from 0.4% to 7.8%. Collected *Trichogramma* is kept in diapause for further researches.

#### Determination of biological efficacy of *Trichogramma* in defoliators' pests control from in Romania

In Romania, Craiova at Botanical Garden experiments were started on 07/24/2013 at the following plant species: Apple - *Malus domestica*, Plum - *Prunus cerasifera*, mulberry - *Morus alba*, Oak - *Quercus pedunculiflora* and different flowers. There were set 20 little cards with 100 eggs each of *Sitotroga cerealella*, in three repetitions, then *Trichogramma embryophagum* Hb. was released at trees and *T. evanescens* Westw at flowers. The norm

of 300.000 individuals was maintained at trees and at flowers the norm was 200.000 individuals per ha. The cards were collected on 26/07/13.

For control one tree has been sampled on which 20 little cards with 100 eggs of *S. cerealella* on each were set. For evidences all the cards have been collected and counted the number of parasitized eggs on each card. The *Trichogramma* was not launched in control.

In the Romanescu Park experiences were started on 07/25/2013, for the following species of plants: Plum, Flowers, Chestnut, Oak, mulberry tree. Collection of cards was performed on 27/07/2013 (Table 4).

At ICAS Alley, Craiova experiences were mounted on 07/25/2013 at Chestnut tree and the collection of cards was performed on 7/26/2013 and 7/27/2013. The Youth Park (Lunca Jiului) experiences were mounted on 25/07/2013 at the following plant species: mulberry, plum, flowers, oak, chestnut. Collecting the cards was performed on 27/07/2013.

Table 4. Percentage of *Sitotroga cerealella* eggs parasitized by *T. embryophagum* Hb. and *T. evanescens* exposed at different forest plants and flowers in Craiova, Romania, 2013

Location	Culture	Percentage of parasitation	Control %
Botanic Garden, Craiova	Apple	69.90±3.8	6
	Plum tree	61.05±3.0	4
	Flowers	74.95±3.9	4
	Mulberry tree	65.19±2.8	2
	Oak	46.02±1.8	3
Romanescu Park, Craiova	Plum tree	57.42±1.9	3
	Flowers	72.17±3.2	4
	Chestnut	61.63±2.4	2
	Oak	36.28±1.8	2
	Mulberry tree	36.28±1.7	2
ICAS Alley, Craiova	Chestnut	68.28±2.8	3
Youth Park, Craiova	Mulberry tree	57.00±2.6	1
	Plum tree	64.01±2.8	3
	Flowers	53.35±1.9	2
	Oak	54.01±1.8	3
	Chestnut	44.69±1.3	3

The results of the experiments from Craiova, Romania (Botanic Garden, Romanescu Park, Youth Park), for the number of parasitized eggs of *S. cerealella* by *T. embryophagum* H. and *T. evanescens* and the percentage of parasitation at the trees where *Trichogramma* was launched and where it wasn't, are represented in the Table 4 and Figure 2. The most eggs of *S. cerealella*, were parasitized at apple culture with a percentage of - 69.7%, 71.9%, 70.0% on each repetition and an average of 70.50%, at flowers the percentage is 74.95% in the first launch, 72.17% after the second, 53.35% after the third and 66.82% is the average. At plum culture the information is as followed: 61.05%, 57.42%, 64.01% and the average is 60.83%. At chestnut we have observed the following data: 61.63%, 68.28%, 44.69% and average of 58.20%. At mulberry - 65.19%, 36.28%, 57.00% average 52.82%, the mulberry and oak - with averages 36.28%. In witness parasitized eggs were reported by 1-6%.

During the whole research period the species of *Trichogramma*, collected from the nature, were identified, maintained and accumulated for ulterior

plant protection and pests' control. *Trichogramma* was collected in the field using two different methods: collection of the pests' eggs parasitized by *Trichogramma* from the natural ambient, and placement of *S. cerealella* eggs in the field for their parasitation by *Trichogramma*. After the biological material collection, each parasitized clutch of eggs was then separately set in a test tube. The material was accumulated for a period of 2 to 3 generations, then the species of *Trichogramma* were identified (Table 5).

Table 5. *Trichogramma* species collected from the Botanical Garden and parks from Republic of Moldova and Romania, 2013

No	Culture	Species collected from Moldova	Species collected from Romania
1	Plum tree	<i>T.embryophagum</i> <i>T. dendrolimi</i> <i>T. evanescens</i>	<i>T.embryophagum</i> <i>T. dendrolimi</i>
2	Apple	<i>T.embryophagum</i> <i>T. dendrolimi</i>	<i>T.embryophagum</i> <i>T. dendrolimi</i>
3	Oak	<i>T.embryophagum</i> <i>T. pintoii</i>	<i>T.embryophagum</i> <i>T. pintoii</i>
4	Chestnut	<i>T.embryophagum</i>	<i>T.embryophagum</i>
5	Flowers	<i>T. evanescens</i> <i>T. pintoii</i>	<i>T. evanescens</i> <i>T. pintoii</i>
6	Mulberry tree	<i>T. dendrolimi</i>	<i>T. dendrolimi</i>

The collected species were then reared in laboratory conditions on grain moth eggs of *S. cerealella* Oliv. At plum the collected species were: *T.embryophagum*, *T. dendrolimi*, *T. evanescens*. At apple the species were: *T. embryophagum*, *T. dendrolimi*. At oak trees: *T. embryophagum*, *T. pintoii*; at the chestnut: *T. embryophagum*; at mulberry: *T. dendrolimi*. At the flowers the collected species of *Trichogramma* were: *T. evanescens*, *T. pintoii*.

For conducting the researched with different species of *Trichogramma*, the samples were kept in different rooms. Keeping the species together may result to a mixture of the species and experiments' failure. The species from Rep. of Moldova and Romania formed a stock of individuals which are kept in diapause at the Institute of Genetics, Physiology and Plant protection from Republic of Moldova.

In the figures below (Figure 3 to 6) are represented the images of the researches conducted within the bilateral project amongst Republic of Moldova and Romania.

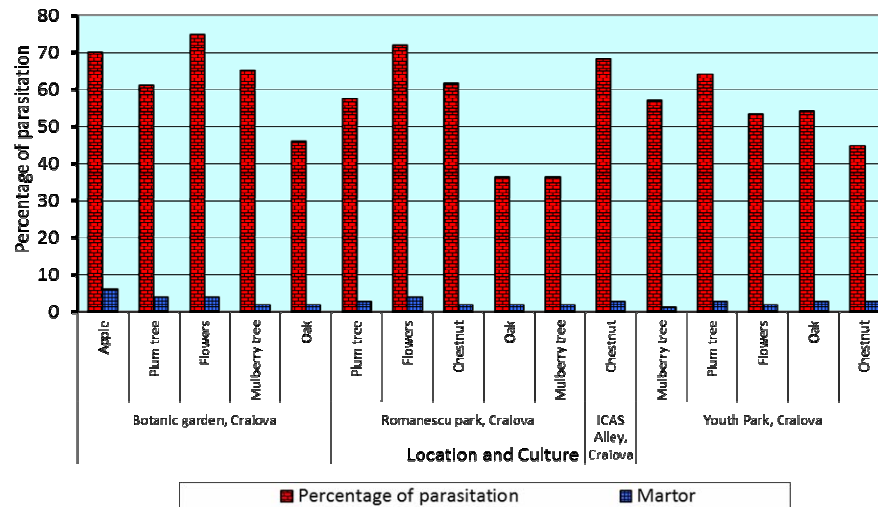


Figure 2. Percentage of *Sitotroga Cerealella* eggs parasitized by *T. embryophagum* H. and *T. Evanescens* exposed at different forest plants and flowers in Craiova, Romania, 2013



Figure 3. Cards exposure with eggs of *Sitotroga cerealella* on apple leaves for further collection of *Trichogramma*, 2013 (original)



Figure 4. Launch of *Trichogramma* in little envelopes in the parks. (original)



Figure 5. Eggs of the pest *Laspeyresia pomonella* on apple leaves, parasitized by *Trichogramma*, 2013 (original)



Figure 6. Fresh eggs:  
1. *Cameraria ohridella* on chestnut leaves  
2. *Helicoverpa armigera* on flowers, 2013.  
(original)

## CONCLUSIONS

During the 2013 years, there have been organized expeditions in various areas of Rep. of Moldova and Romania with the goal to collect population of *Trichogramma* to fulfill the bilateral joint project.

During the project were monitored and registered defoliating insect species such as: *Tortrix viridana*, *Helicoverpa armigera*, *Grapholitha funebrana*, *Grapholitha molesta*, *Lymantria dispar* L., *Laspeyresia pomonella* and determining the outbreaks (hazardous areas) using pheromone traps in the Botanical Gardens and parks from Republic of Moldova and Romania.

Launches with *Trichogramma* spp (reared on eggs of *Sitotroga cerealella* in the laboratory conditions) were performed for biological control against pests (defoliators), was determined the biological effectiveness of *T. embryophagum* and *T. evanescens* in the parks, which varied from 67% to 75,9% on artificial background (with the use of eggs of *S. cerealella*) and from 64,5% to 73,3% on natural background. In Romania these indices were from 36,3% to 75%, on average.

The species collected from Republic of Moldova and Romania formed a stock of individuals which are kept in diapauses at the Institute of Genetics, Physiology and Plant Protection from Republic of Moldova for fulfilling the bilateral joint project and for further researches.

As a final result there will be elaborated recommendations for: "Biological pest control and monitoring of the biologically active substances and entomophague (*Trichogramma*) for density reduction of the defoliating insects in the parks from Republic of Moldova and Romania".

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## ABSTRACT

During the year 2013 under the bilateral project of the Institute of Genetics, Physiology and the Plant Protection Institute of ASM, together with scientists from Romania from Forest Research and Management Institute, Craiova Research Station and partners from University of Agricultural Sciences and Veterinary Medicine from Timisoara (Romania) were conducted a series of studies. There were monitored and mass captured Lepidoptera insect species (*Tortrix viridana* L., *Helicoverpa armigera* Hb., *Grapholitha molesta* Br., *Grapholitha funebrana* Tr., *Laspeyresia pomonella* L., *Cameraria ohridella* Deschka & Dimic, *Lymantria dispar* L.) using pheromone traps in Botanical Garden and public parks of Republic Moldova and Romania. Monitoring and mass capturing of the males of main pest species using pheromone traps allow us to apply other biological methods of plant protection.. The biological efficacy of *T. embryophagum* Htg. and *T. evanescens* Westw, varied in different plant species (oak, mulberry, chestnut, plum, apple, flowers) from 64.5 to 73.3% in Moldova and from 36.3 to 75, 0 % of Romania.

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