INTERSPECIFIC COMPETITION OF *Trichogramma* sp. AT ITS MASS REARING

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Abstract. As a result of the laboratory experiments, it has been established that along the 4 generations in mixed populations of *T. evanescens* Westw. and *T. pintoi* Voeg., gradual substitution of *T. evanescens* by *T. pintoi* occurs. The mechanism of interspecific competition on grain moth eggs was established. In the field of cereal, technical and vegetable crops, *T. evanescens* is dominant species (up to 95-99%). *T. pintoi* represents a laboratory population, the preferred host of which is *Siotroga cerealella* Ol. In the presence of the two species of *Trichogramma* in the biotope, their quantitative ratio depends on the specific climatic conditions and resource of eggs of the preferred host Biological indices for *T. pintoi* are higher than those of *T. evanescens*. Therefore, in the laboratory conditions we substitute *T. evanescens* by *T. pintoi*, but in the field it is the opposite.

Keywords: biological indices, prolificacy, pests, *Trichogramma*, competition, biological efficacy.

Rezumat. Concurenţa interspecifică la *Trichogramma* sp. la înmulţirea în masă. În urma experimentelor de laborator s-a stabilit că, pe parcursul a 4 generaţii, în populaţiile mixte de *T. evanescens* Westw. şi *T. pintoi* Voeg., are loc substituirea treptată a *T. evanescens* de către *T. pintoi*. Totodată, s-a determinat mecanismul concurenţei interspecifice pe ouă de molia cerealelor (*Sitotroga cerealella* Ol.). În câmp *T. evanescens* reprezintă o specie dominantă în agrocenozele culturilor cerealiere, tehnice, legumicole şi leguminoase (până la 95-99%), iar *T. pintoi* reprezintă o populaţie de laborator, pentru care *S. cerealella* este o gazdă preferată. În prezenţa a două specii de *Trichogramma* în biotop, raportul lor cantitativ depinde de condiţiile concrete climatice şi resursa de ouă de gazdă preferată. Pentru *T. pintoi* indicii biologici sunt mai mari decât la *T. evanescens*. Din această cauză, are loc substituirea *T. evanescens* de către *T. pintoi* în condiţii de laborator, iar în câmp – invers.

Cuvinte cheie: indicii biologici, prolificitatea, gazdă, *Trichogramma*, concurenţa, eficacitatea biologică.

INTRODUCTION

Biological control plays an important role in integrated plant protection. Beneficial insects are rather important in modern biological control practice for reducing the density of pests.

The Institute of Genetics, Physiology and the Plant Protection of the Academy of Sciences of Moldova conducts fundamental and applied research with *Trichogramma* to settle technical issues, improve the quality of entomophage-and effectiveness in plant protection to obtain ecological safe products. To improve parasitoid quality and efficacy, it is necessary to select the right species best adapted to natural conditions of certain zones with certain species.

At the initiative of the academician Ion Popusoi, there was set up within the Institute of Genetics, Physiology and Plant Protection, the laboratory of *Trichogramma*, initially headed by Dr. Ala P. Adaskevici (1976-1977), then by Dr. Şoil M. Greenberg (1977-1992), later on by Dr. Vladimir A. Sleahtici (1995-1999) and finally by Dr. Lidia T. Gavriliţa (1999-2003).

In 2003, the Laboratory of *Trichogramma* was united with another laboratory and named „Phytopharmacy and Ecotoxicology" that has continued functioning till nowadays (2016). Thus the Laboratory of *Trichogramma* existed as a research unit in the Institute of Department Plant Protection within the Academy of Sciences of Moldova from year 1976 up to year 2013, i.e. 27 years and thus, *Trichogramma* has continued being studied for 40 years (1976 till present, 2016). Since its setting up, the Laboratory of *Trichogramma* has dealt with big and important issues. Along the period, *Trichogramma* studies in laboratory conditions led to important data, which were described in national and international scientific publications. These results have been presented at various conferences, symposia, round tables and exhibitions.

In mass rearing of *Trichogramma*, the numerical density of the initial colonies grows by tents, even hundreds of times leading to a depression of the number of inherited crossings and thus leading to sexual deregulation of the population, and to a lowering of the quality of *Trichogramma.*

Such factor as interspecific competition becomes particularly important at *Trichogramma* mass rearing and releasing into the field. At relative trophic specialization of *Trichogramma* competitive capacity impacts its efficacy (GAVRILIŢA et al., 1984; SOROKINA, 1984; MENCHER et al., 1980).

MATERIALS AND METHODS

During the rearing of the laboratory host, biological indices were determined for *T. evanescens* (prolificacy, hatching, rate of the females-reared on these eggs. Experiments were carried under laboratory and field conditions.

Experiments have been made in three climatic chambers SKP-1 at average daily temperatures of 15, 20 and 25°C and relative humidity of 50, 65 and 80%, the photoperiod lasting for 16 hours. Experiments have been effectuated according to Box-3 plan.

Collecting, identification, storage and accumulation of *Trichogramma* species were done according to DIURICI (2008). Rearing of the laboratory host – grain moth (*Sitotroga cerealella* Ol.), for *Trichogramma* production was done by ABAȘCHIN et al. (1979) authors' methods. Mathematical data processing has been done using variance analysis method after MENCER & ZEMSHMAN (1986).

RESULTS AND DISCUSSIONS

Five trial variants in five repetitions were conducted in the Institute of Genetics, Physiology and the Plant Protection of the Academy of Sciences of Moldova, using various reports on *Trichogramma* species under laboratory and field conditions: 1. *T. evanescens* – 100%; 2. *T. pintoi* – 100%; 3. *T. evanescens* + *T. pintoi* – 50% +50%; 4. *T. evanescens* + *T. pintoi* – 10% +90%; 5. *T. evanescens* + *T. pintoi* – 90% +10%. Experiments conditions are shown in Table 1.

Table 1. Box-3 (planned conditions).

Levels of factors	**T, °C**	**W, %**	***T. evanescens* : *T. pintoi***
-1	15	50	1 : 9
0	20	65	1 : 1
1	25	80	9 : 1

Laboratory experiments demonstrated that during 4 generations of *Trichogramma* development the *T. evanescens* species was gradually substituted by *T. pintoi* (Table 2, Fig. 1).

When the share ratio of *T. evanescens* and *T. pintoi* equals to 1:1 passed through Angoumois grain moth eggs (*Sitotroga cerealella* Ol.) after the fourth generation it was noticed that at T=15°C combined with different humidity values, *T. pintoi* share increased from 68.3 to 83.95% and *T. evanescens* share – from 16.1 to 31.7%; at T=20°C, these parameters ranged, from 76.0 to 95.0% and 5.0 to 24.0%, while at T=25°C – they ranged from 76.1 to 100% and from 0 to 23. 9%.

When shares of *T. evanescens* and *T. pintoi* equalled to 9:1, the substitution rate of *T. evanescens* was more reduced. However, at T=15°C in mixed batch, there remained from 42.9 to 45.0% of *T. evanescens* at T=20°C, respectively, from 38.9 to 45.0%, at T=25°C – from 31.5 to 38.35%. At the end of all experiment variants no *T. evanescens* were found, while at T=25°C, a similar situation was observed for the 3rd generation.

The mechanism of substituting one species by the other was explained by different responses of *T. evanescens* and *T. pintoi* to temperature and humidity regimes at mass rearing.

Lower prolificacy and sex index as well as longer duration of one generation of *T. evanescens* and *T. pintoi* was observed in all experiment variants. The obtained results demonstrated that the Angoumois grain moth is a more preferable laboratory host for *T. pintoi* and the introduction of even a small share of this species may lead to the substitution of *T. evanescens*. Experiments were made with 4 generations due to the fact that commercial biological laboratories recommended to rear no more than 4 generations of *Trichogramma* on the eggs of the Angoumois grain moth after passage on cabbage moth (*Mamestra brassicae*) eggs followed by field release. Substitution phenomenon of one species by the other may be explained by different reasons. Under the conditions of South-West region of the former USSR, *T. evanescens* was a dominant species (up to 95-99%) in the agrocoenoses of cereal, technical and leguminous crops. *T. pintoi* was the laboratory population for which the Angoumois grain moth became a preferable host.

Hence, the number of *T. pintoi* females that refused parasitizing the eggs of the Angoumois grain moth equalled to 2%, while that of *T. evanescens* – 38%. *T. pintoi* reared on the eggs of the Angoumois grain moth has higher prolificacy and sex index and a new generation develops faster than that of *T. evanescens*. It has been found that the intensity of substituting of *T. evanescens* by *T. pintoi* was regulated by the response to temperature and humidity regimes at mass rearing (Tables 3, 4). Ecologically, *T. pintoi* is a more plastic species, while *T. evanescens* stronger responds to humidity fluctuations under the same temperature. Field experiments showed that cabbage moth eggs were more parasitized by *T. evanescens* – 54 to 60% than by *T. pintoi* – from 13 to 14%.

Table 2. Influence of temperature and humidity on the substitution process of *T. evanescens* by *T. pintoi* (after the 4th generation).

Temperature, °C	**15°C**						**20°C**						**25°C**					
Species ratio	1:1		1:9		9:1		1:1		1:9		9:1		1:1		1:9		9:1	
Species	*T. evanescens*	*T. pintoi*	*T. evanescens*	*T. pintoi*	*T. evanescens*	*T. pintoi*	*T. evanescens*	*T. pintoi*	*T. evanescens*	*T. pintoi*	*T. evanescens*	*T. pintoi*	*T. evanescens*	*T. pintoi*	*T. evanescens*	*T. pintoi*	*T. evanescens*	*T. pintoi*
Humidity, %	**Humidity 80%**																	
No. of individuals, %	16.1 ±0.5	83.9 ±3.2	7.0 ±0.5	93.0 ±3.9	45 ±2.0	55 ±2.2	5.0 ±2.2	95 ±3.8	0	100	45±2.0	55 ±2.5	0	100	0	100	38.5 ±1.5	61.5±2.0
Humidity, %	**Humidity 65%**																	
No. of individuals, %	29.5 ±0.8	70.5 ±3.7	9.0 ±0.3	91.0 ±4.2	44.0 ±2.2	56.0 ±2.3	7.0 ±2.3	93.0 ±4.8	0	100	43,0 ±2.0	57.0 ±2.6	5.0	95.0 ±3.5	0	100	35.0 ±1.5	65.0 ±2.2
Humidity, %	**Humidity 50%**																	
No. of individuals, %	31.7 ±1.8	68.3 ±3.0	22 ±0.9	78.0 ±3.8	42.9 ±3.1	57.1 ±2.6	24.0 ±2.8	76.0 ±3.3	6.0 ±0,5	94.0 ±4.9	38.9 ±2.4	61.1 ±2.8	23.9 ±1.8	76.1 ±3.8	8.0 ±0.8	92.0 ±4.9	31.5 ±0.8	68.5±2.8

Figure 1. Influence of temperature and humidity on the substitution of *T. evanescens* by *T. pintoi* (after the 4th generation).

Table 3. Influence of temperature and humidity on prolificacy and ratio of *T. evanescens* and *T. pintoi* species.

Temperature, °C	**Humidity, %**	**Prolificacy/female**		**Number of females, %**		**Period of generation development (days)**	
		T. evanescens	*T. pintoi*	*T. evanescens*	*T. pintoi*	*T. evanescens*	*T. pintoi*
15	80	17.6±1.0	19.7±1.8	58.4±2.8	60.2±2.9	27-28 ±1.3-1.9	25-26 ±1.2-1.6
	65	14.2±0.9	15.8±1.5	53.6±2.7	60.3±2.8		
	50	12.9±0.7	17.2±1.0	50.2±2.3	59.8±2.3		
20	80	19.7±1.4	25.7±1.9	60.2±2.9	62.9±2.9	19-20 ±1.2-1.7	16-17 ±1.1-1.5
	65	15.6±1.3	18.9±1.4	56.4±2.0	64.2±2.3		
	50	14.1±1.0	15.4±1.0	53.4±2.3	60.7±2.6		
25	80	29.0±1.8	33.5±2.4	63.0±2.9	62±2.3	12-13 ±1.1-1.5	10-11 ±1.0-1.4
	65	19.8±1.2	23.2±2.0	59.1±2.3	61.5±2.7		
	50	16.7±1.0	20.2±1.8	55.4±2.2	59.8±2.3		
DEM		2.54	3.40	4.3	4.55	1.40-1.8	2.20-2.6

Field experiments

Experiments were conducted in one farm from Grătiești on cabbage to control *Mamestra brassicae* at a density of 10 eggs/m^2. Temperature and humidity were taken into accountduring the research experiments. After field experiments *Trichogramma* species were identified by their morphological structure, male genitalia and share of *Trichogramma* species that hatched from parasitized pest eggs. No releases were made in the check.

After the first release of mixed populations of *T. evanescens* and *T. pintoi* (50 : 50%), the share was 91.05% for *T. evanescens* and 8.95% for *T. pintoi* (Table 5). After the second release, the respective shares was as follows – 94.5% and 5.5%. After the first release of the mixture of *T. evanescens* and *T. pintoi* (10:90%) egg laying of the cabbage moth was parasitized at the level of 85.6% by *T. evanescens* and at the level of 14.4% by *T. pintoi*, after the second release – 88.8 and 11.2%, respectively. After the first and second releases of *T. evanescens* and *T. pintoi* mixed populations (90:10%) analyses showed that it is *T. evanescens* that actually controlled the pest in the field while *T. pintoi* was not found.

It was established that under natural conditions species competition was lower. Fluctuations of temperature and humidity, localization of host eggs in time and space, as well as a more intensive accumulation of species that are not specific for this biocoenosis (for example *T. pintoi*) contribute to the aforementioned situation. However, if further *Trichogramma* release is not made, the dominant species is restored. In case of the presence of two species in the biotope, the quantitative share will depend on the specific climatic conditions and the number of preferable host individuals.

Table 4. Ratio of *Trichogramma* species in *Mamestra brassicae* parasitized egg laying (%) after field release.

Experiment variant	**Percentage of *Mamestra brassicae* eggs parasitized by *Trichogramma*.**		**Ratio of *Trichogramma* species in *Mamestra brassicae* parasitized egg-laying (%), after field release**			
	Released on 23.06	Released on 19.08	Released on 3.06		Released on 9.08	
			T. evanescens	*T. pintoi*	*T. evanescens*	*T. pintoi*
T. evanescens (100 %)	54.3±2.7	60.4±3.7	100	0	100	0
T. pintoi (100 %)	13.8±1.4	12.6±0.8	0	100	0	100
T. evanescens + *T. pintoi* (50:50%)	40.8±1.9	39.0±1.8	91.1±2.4	8.9±1.0	94.5±3.4	5.5±0.5
T. evanescens + *T. pintoi* (10:90%)	14.2±1.0	23.3±1.1	85.6±3.9	14.4±1.8	88.8±3.3	11.2±0.7
T. evanescens + *T. pintoi* (90:10%)	42.4±3.5	49.9±3.0	100	0	100	0
DEM	2.33	2.45	3.67	3.56	4.32	3.78

CONCLUSIONS

In the laboratory experiments, it was established that along the 4 development generations of mixed populations of *Trichogramma*, gradual substitution of *T. evanescens* by *T. pintoi* occurs.

In the field of cereal, technical and vegetable crops, *T. evanescens* is a dominant species (up to 95-99%). *T. pintoi* represents a laboratory population, the preferred host of which is *Siotroga cerealella*. In the presence of the two species of *Trichogramma* in the biotope, their quantitative ratio depends on the specific climatic conditions and resource of eggs of the preferred host. Biological indices for *T. pintoi* are higher than the ones of *T. evanescens*, therefore, in the laboratory conditions we substitute *T. evanescens* by *T. pintoi*, but in the field it is the opposite.

It was established that under natural conditions, species competition was lower. Fluctuations of temperature and humidity, localization of host eggs in time and space, as well as a more intensive accumulation of species that are not specific for this agrocoenosis (for example *T. pintoi*) contribute to the aforementioned situation.

However, if further *Trichogramma* release is not made, the dominant species is restored. In case of the presence of two species in the biotope, the quantitative share will depend on the specific climatic conditions and the number of preferable host individuals.

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