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MULTIPLICATION OF THE TRICHOGRAM ENTOMOPHAGUS IN EXTREME CONDITIONS

Lidia Gavrilita

Key words: *T. evanesces, hatching, larvae, treatment, females, extreme conditions*

INTRODUCTION

Pest control in modern plant protection concepts, is one of the important links in food chains and food webs, which would ensure a superior quality of the environment, with the possibility of obtaining organic agri-food products. The Copernicus Service, which monitors global temperature and other climate indicators, has found 2021 to be the fifth warmest year ever. The first phenomenon generates climate anomalies throughout the planet, especially in the tropics. Copernicus experts said that, in the last seven years, the average annual temperature of the Earth was 1.1 to 1.2 degrees Celsius above the levels before the industrial revolution, measured between 1850 and 1900.

Therefore, climate experts warn that mankind has managed to reach 73% of the way to the threshold of limiting the global average temperature to 1.5 degrees Celsius above pre-industrial levels. In fact, climatologists say that the planet must remain below this threshold in order to avoid the worst climate catastrophes.

In a bilateral project with Romania, research was carried out in order to detect changes caused by light water (DDW) in phenotypic and genotypic polymorphism in *Trichogramma* species, collected from different ecosystems in the Republic of Moldova and Romania (together with researchers in Romania). The main task of the research in this project was to develop and implement alternative methods of biological protection of maize and cabbage crops in controlling the pest complex *Trichogramma* spp. chemical treatments in integrated protection, conservation of useful insects, and avoidance of environmental pollution, obtaining ecological and qualitative agricultural production with minimum expenses.

No knowledge of the *Trichogramma* multiplication phenomenon, which moves on the water surface, persists. It was described only in other oophagous parasites, (totrypidae *Tiphodytes gerrifhagus* Marchae) on host eggs on the water surface (MARTIN, 1927), (AGAMI E., VOEJELE, 1988). Under natural conditions, *Trichogramma* may accidentally find itself in environmental conditions moving on water (rain, dew, irrigation, rice plants, growing in water, etc.). We have created conditions to challenge the aquatic movement behavior of Westwood *Trichogramma evanescens*. Research has been done to multiply the entomophagous *Trichogramma* in other extreme conditions with high temperatures.

MATERIALS AND METHODS

Research place in 2022: Fundamental research was conducted in laboratory conditions at the Institute of Genetics Physiology and Plant Protection in various crops.

1. **A number of experiments have been carried out to determine the percentage of parasitism of cereal moth eggs by *Trichogramma evanescens* Westwood in the aquatic environment**

In a flat glass vessel with a diameter of 40 cm and a height of 7 cm, water was poured into this vessel up to a height of 5 cm. 2000 fresh eggs of cereal moth were uniformly sprinkled on the surface of the water in the vessel, then 1000 eggs parasitized with *Trichogramma evanescens* were released, where the sexual intercourse being 50% of females and 50% of males by hatching, placed in three half-capsules, from which the entomophagous gently emerged and parasitized the moth eggs on the surface of the water.

The parasite-host ratio was (P:H) 1:4, ie 1 female had 4 moth eggs. The females, which hatched from the eggs, slipped on the surface of the water and looked for moth eggs. The evidence of the percentage of parasitism for 4 days showed that the number of parasitized eggs was 71%. We could see visually that the *Trichogramma* moves on the water, due to the surface tension, like on ice, very fast. For 3 hours of visual observations - for 3 days of hatching, it was found that the individuals hatched from the parasitized eggs and parasitized the fresh eggs from the grain moth.

2. **Determination of hatching percentage of larvae from moth eggs (*Sitotroga cerealella* Ol.) treated with water at high temperatures**

Cereal moth eggs aged 24-26 hours were treated at a temperature of 40-70℃. The hot water

treatment time was: 20 and 40 seconds each variant, situation, which can be in the warm years during the vegetation period. After egg treatment, the hatching percentage of the egg larvae in each variant was determined.

RESULTS AND DISCUSSIONS

Determination of the percentage of parasitism of cereal moth eggs by *Trichogramma evanescens* Westwood

Under laboratory conditions in the aquatic environment, a series of experiments were performed to determine the percentage of parasitism of *T. evanescens*.

The research given may be similar to the situation created in nature, after heavy dew, after rain, after irrigation, for rice plants, which grow in water, etc., when the leaves of the plants are wet and the pest's eggs are in the water. Our experience has shown that under watery (water) conditions *Trichogramma* can find the pest's eggs and parasitize them.In a flat glass vessel with a diameter of 40 cm and a height of 7 cm, water was poured into this vessel up to a height of 5 cm. On the surface of the water in the vessel were laid (uniformly sprinkled 2,000 fresh eggs of cereal moth, then launched 2000 eggs parasitized with *Trichogramma evanescens*, sexual intercourse being 50% females and 50% males hatching, laid in three half-capsules, from which the entomophagous one easily came out and parasitized the moth eggs on the surface of the water. The evidence of the percentage of parasitism for 4 zl showed that the number of parasitized eggs was 71% (Table 1). Observed visually, *Trichogramma* was moving on water due to surface tension, like ice, very fast. For 3 hours of visual observations - for 3 days of hatching, it was found, that individuals have hatched from parasitized eggs and started parasitizing fresh eggs of cereal moth. Percentage of hatching of larvae from moth eggs (*Sitotroga cerealella* Ol.) Treated with water at high temperatures.

Table 1. Percentage of parasitism of cereal moth eggs by *Trichogramma evanescens* Westwood in the aquatic environment

Repeats	The number of parasitized eggs	Percentage of parasitisation
I	1420	71,0±3.8
II	1500	75,0±3.5
III	1348	67,4±3.0
Sum/Average	4268	71,1±3.9

I. Variant: Cereal moth eggs, aged 24-26 hours, were treated at a temperature of 40-70 ℃. The time of treatment with hot water was: 20 and 40 seconds each variant.Cereal moth eggs aged 24-26 hours were treated at a temperature of 40-70°C. Time of treatment with hot water was 20 and 40 seconds at each variant, hatching rate varied from 91,0% to 90.4% at a temperature of 40℃. The higher the temperature, the lower the hatching rate. At a maximum temperature of 60℃, hatching was minimal 1-2%. (Table 2).

II. Variant. The moth eggs were stored in the refrigerator for a month at a temperature of 3-4℃, the relative humidity was 70%. Experimental cereal moth eggs were treated at temperatures from 50 to 80°C, the hot water treatment time was: 10 to 60 seconds in each variant. Egg larvae hatched in different variants after treatment. Situations, when at the surface of the soil, on the plants (the surface of the plants is large), the temperature, especially when it is dry, the plants wither, dry out. But what about the eggs of pests that are on the plants? Our experience has shown that the higher the temperature, the lower the number of hatching larvae in eggs. Hatching indices of larvae from moth eggs (*Sitotroga cerealella* Ol.) stored and treated with hot water are shown in the Table 3. The percentage of larvae hatching at a temperature of 50℃ on average was 24.83%, at a temperature of 75-80℃, this index was 2.66-5.66%.

Table 2. Determination of the optimum temperature for pr℃easing eggs from cereal moth (*S. cerealella* Ol.) for *Trichogramma* multiplication on them

Variants	Temperature, ℃	Time, sec	Hatching of larvae from eggs treated of moths ,%					Average
			I	II	III	IV	V	
1	40	20	91	92	91	90	91	91.0±2.2
		40	90	91	90	91	90	90.4 ±1.5
2	45	20	80	83	85	82	80	82.0±1.6
		40	80	81	83	80	79	80.6±2.2
3	50	20	60	58	56	54	52	56.0±2.5
		40	56	54	52	51	50	52.6±2.4
4	55	20	40	40	38	36	39	38.6±2.1
		40	30	33	30	30	32	31.0±2.0
5	60	20	2	4	3	3	1	2,.6±0.2
		40	1	1	1	1	1	1.0
6	65	20	0	0	0	0	0	0
		40	0	0	0	0	0	0
Martor	26		90	90	89	91	89	89.8±2.3

The percentage of parasitized eggs in nature and the ethology of insects depends on weather conditions. In Table 4, Fig. 1 it is shown the weather conditions, where there is a higher temperature difference in 2013-2021 than the multiannual temperature. Therefore, climate change in recent years has an impact on the ethology of insects, the last generation is more pronounced, more numerous, the development period of the generation, is 10-15 days longer. The diversity of the properties of the populations is reached on account of the natural collections of the initial colonies from a large number (not less than 1500-2000 parasitized eggs), in order to preserve the genofond of the population. This operation must be performed in the second half of the year (july to october). The effectiveness of the operation increases due to the field exposure of the cards with natural host eggs (fresh or sterile eggs from cereal moth). It should be noted that the term of use of fertile eggs parasitized by *Trichogramma* is limited to 2-3 days, and sterile eggs - 12 days. The collection of a large number of eggs parasitized by *Trichogramma* can be ensured on account of the organization in nature of the sources - artificial places (reservations) of concentration of *Trichogramma* in natural conditions.

The highest average monthly temperature (May-September) was in 2018, 2019, 2020). The lowest average monthly temperature (may-september) was in 2013, 2016).

The difference in average monthly temperature for the years 2013-2021 compared to the multiannual average monthly temperature (10 years) varied from 2.46-7.72^{0}C. Based on the diversity of temperature variation during the vegetation period, research and development of pests in 2-3 generations, there may be various weather conditions for the laying of pest eggs. Under natural conditions, pest eggs deposited on agricultural crops may accidentally hit the aquatic environment after rain, dew, irrigation, plants such as rice, which grows in water, in this case crop protection is more complicated, but with the entomophagous *Trichogramma* it has been shown that it is possible to move on *Trichogramma* water and parasitisation of host eggs.

The diversity of the properties of the populations is reached on account of the nature collections of the initial colonies from a large number (not less than 1500-2000 parasitized eggs), in order to preserve the genofund of the population. This operation must be performed in the second half of the year (july to october). The effectiveness of the operation increases due to the field exposure of the cards with natural host eggs (fresh or sterile eggs from cereal moth). It should be noted that the term of use of fertile eggs parasitized by *Trichogramma* it is limited to 2-3 days, and the sterile ones - 12 days. The collection of a large number of eggs parasitized by *Trichogramma* can be ensured on account of the organization in nature of the sources - artificial places (reservations) of concentration of *Trichogramma* in natural conditions. The reservation of the maternal culture - continuous system during the vegetation period, where the monitoring of phytophagous and *Trichogramma* sp.

Table 3. Hatching of larvae in eggs of cereal moth (*Sitotroga cerealella* Ol.) stored and treated with water at high temperatures, %

Variants	Temperature, ℃	Hatching of larvae from eggs, treated cereal moth % Treatment time, sec						Larvae hatching, %. Average
		10	20	30	40	50	60	
1	50	30	28	25	24	22	20	24.83±2.5
2	55	26	25	23	22	20	18	22.33±2.2
3	60	20	18	16	14	12	10	15.0±2.0
4	65	15	13	12	10	8	7	10.83±1.7
5	70	10	8	6	5	4	4	6.16±1.7
6	75	8	7	6	5	3	3	5.66±1.5
7	80	4	3	3	2	2	2	2.66±1.1
7	Martor	45						45±2.8

Table 4. Average monthly temperatures for 2013-2021 and multiannual average monthly temperature.

Years	Average monthly temperature in 2013-2021 and multiannual					Mediate by years	Difference
	May	June	July	August	September		
Multiannual	15.6	18.9	20.9	20.3	15.7	18.28	
2013	19.5	21.7	21.9	22.7	17.9	20.74	2.46
2014	16.0	24.0	23.0	28.5	25.5	23.4	5.46
2015	18.2	22.0	24.8	24.1	21.3	22.08	3.80
2016	15.7	21.3	24.0	24.0	20.3	21.06	2.78
2017	20.2	21.3	22.3	23.9	23.0	22.14	3.76
2018	23.0	26.0	29,0	29.0	23.0	26.0	7.72
2019	18.0	25.0	27.0	26.0	25.0	24.2	5.92
2020	18.0	19.0	28.5	29.0	28.0	24.5	6.22
2021	17.58	21.83	27.16	23.66	17.5	21.54	3.26

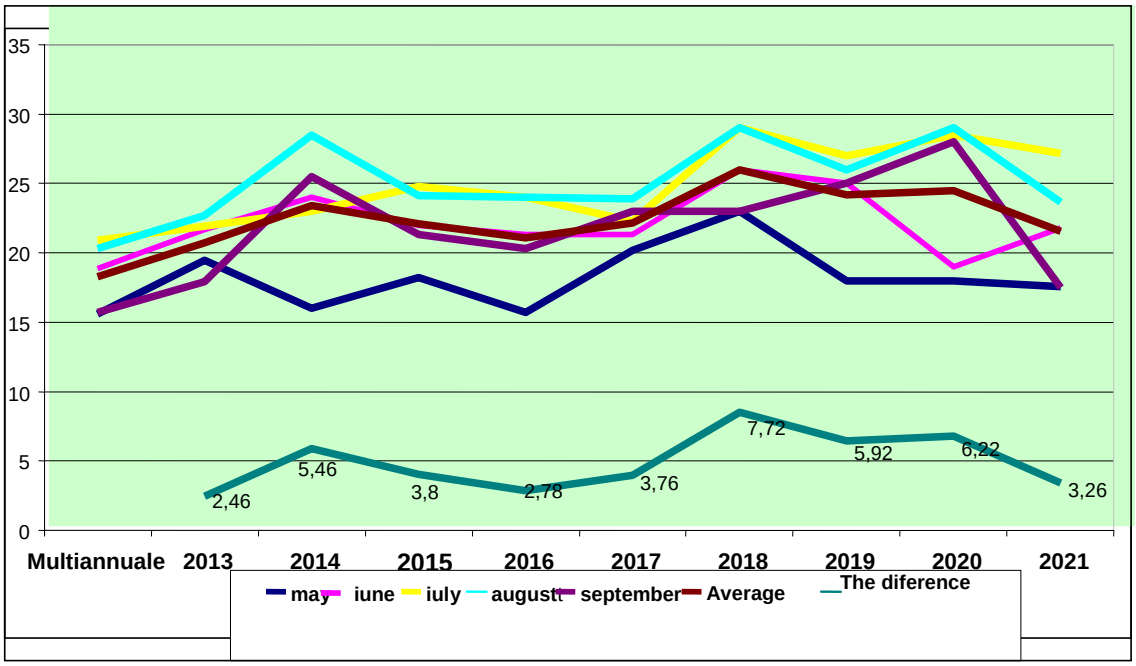

Fig. 1. Average monthly temperatures for the years 2013-2021 and the average multiannual monthly temperature

CONCLUSIONS

1. The research given can be similar to the situation created in nature, after a heavy dew, after rain, after irrigation, for rice plants, which grow in water, etc. when the leaves of the plants are wet and the pest's eggs are in the water.
2. Our experience has shown that under water (water) conditions the *Trichogramma* can move on water, find the pest's eggs and parasitize them, where the percentage of parasitism was 71%.
3. The lowest average monthly temperature (may-september) was in 2013, 2016).the difference of the average monthly temperature for the years 2013-2021 compared to the multi annual average monthly temperature (10 years) varied from 2.46-7.72^{o}C.
4. Moth eggs of cereals aged 24-26 hours were treated at a temperature of 40-70℃, the time of treatment with hot water was: 20 and 40 seconds each variant, where the hatching of the larvae continued until the temperature of 60℃.

ABSTRACT

The research given may be similar to the situation created in nature, after heavy dew, after rain, after irrigation, for rice plants, which grow in water, etc., when the leaves of the plants are wet and the pest's eggs are in the water. Our experience has shown that in watery conditions *Trichogramma evanesces* can move on water, find the pest's eggs and parasitize them, where the percentage of parasitism was 71%. In extreme conditions the moth eggs of cereals aged 24-26 hours were treated at a temperature of 40-70℃, the time of treatment with hot water was: 20 and 40 seconds each variant, where the hatching of the larvae continued until the temperature of 60℃.

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AUTHOR’S ADDRESS

GAVRILITA LIDIA, Institute of Genetics, Physiology and Plant Protection of Republic Moldova, Chişinău, e-mail: lidia_gavrilita@yahoo.com