

Host population density influence on host acceptance in *Trichogramma*

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Abstract

In 7 *Trichogramma* species (Hymenoptera, Trichogrammatidae) the percentage of females, parasitizing at least one host, directly depends on the number of host eggs. *Trichogramma* females that refused *Sitotroga* eggs will still oviposit in natural hosts. These refusing females were yet active, moved in test tubes and sometimes contacted the host. It seems that the sequence of behavioural reactions resulting in parasitization is interrupted at the stage of arrestment and host recognition. Host density within a habitat proved to be an important factor in host acceptance. Possible mechanisms that formed the basis of the observed effect (behavioural response to host kairomones, the learning ability, endocrine processes) are discussed.

Introduction

Host acceptance by insect parasitoids is usually considered as a species-specific characteristic. Several species-specific factors have been found to play a role in host acceptance by *Trichogramma*: host eggs shape, size and colour (Laing, 1937, 1938; van Dijken *et al.*, 1986; Pak & de Jong, 1987), volatile and nonvolatile chemicals-kairomones (Lewis *et al.*, 1972, 1975, 1976; Noldus & van Lenteren, 1983; Nordlund *et al.*, 1977; Gross *et al.*, 1981, 1984; Gardner & van Lenteren, 1986; Zaborski *et al.*, 1987). Host acceptance can also be influenced at subspecies level. Various strains of *Trichogramma* were found to differ in their acceptance of two lepidopterous hosts (Curl & Burbutis, 1978; Pak & de Jong, 1987). Host acceptance is furthermore influenced by learning (de Jong & Pak, 1984; Kaiser *et al.*, 1989; Wajnberg, 1989). Host age

also plays a role in host acceptance (Pak *et al.*, 1986).

For a number of years we have been studying the phenomenon of 'refusal to parasitize' which is manifested by the complete absence of parasitization of freely accessible grain moth, *Sitotroga cerealella* Oliv. (Lepidoptera, Gelechiidae), eggs by females of various species of *Trichogramma*, while practically all the females parasitize natural hosts (Reznik & Umarova, 1988). It was found that if a few hosts are presented they may be insufficient to start parasitization (Reznik & Umarova, 1989).

Here we report the results of our studies of the influence of host population density on the percentage of females, parasitizing at least one host. We want to answer three questions: 1. Does the host population density influence the host location and acceptance of *Trichogramma*?, 2. Which steps in the parasitoid's behaviour are influenced

by the host population density?, and 3. How long is the duration of this influence?

Materials and methods

We used laboratory lines of *Trichogramma embryophagum* Htg., *T. evanescens* Westw., *T. principium* Sug. & Sor., *T. semblidis* Aurivil, *T. pintoi* Voegele, *T. dendrolimi* Matsumura, and *T. sp.* (a species collected in Turkmenia and similar to *T. evanescens*), cultivated for many generations on the eggs of the grain moth. In all our studies females were placed individually into test tubes (40 × 5 mm) containing a strip of paper with a certain number of grain moth eggs pasted onto it. In cases with 5, 10 and 15 eggs their number was exact, while in cases with 35, 60 and 90 eggs the number of eggs was estimated by the area of the strip covered. Eggs were pasted densely, so that the distance between the neighbouring eggs did not exceed the length of an egg.

All experiments were conducted at 20 °C under a light regime of L : D = 17 : 7. Females were not fed during the experiment. After 1–2 days the host eggs were taken out of test tubes and maintained under the same conditions. The number of females parasitizing at least one host was calculated in each experimental run. The number of females parasitizing at least one host was then divided by the total number of females in the run, which resulted in deducing the proportion of ovipositing females.

For statistical comparison the 'chi-square' test and the analysis of variance were used.

All species were subjected to two series of experiments, each including four variants. In the first series females were offered 5, 10, 15 or 60 grain moth eggs for parasitization, in the second series – 5, 15, 35 or 90 eggs. Each variant was repeated 2–3 times. Each trial involved at least 50 females (at least 100 females for a variant in total). Because of the high variability of the percentage of egg-laying females, which is normally observed in the generation sequences of *Trichogramma* cultured on grain moth eggs (Zaslavsky & May, 1982; Reznik & Umarova, 1988), the results of the two series are presented separately.

To observe the behaviour of *Trichogramma* directly, 20–40 females in similar test tubes were observed at 15–20 min intervals for the 12-h duration of the experiment. Thus, every female was watched about 50 times. For each observations the behaviour of the female was recorded.

Four behaviour categories were used:

- 1) Sitting on the glass or on the paper,
- 2) Moving along the glass or the paper,
- 3) Contacting the host, but manifesting no behaviour associated with parasitization (just sitting on an egg, moving along it),
- 4) Parasitizing the host, inspecting it with antennae, ovipositor, etc.

To analyze the results the females were divided into two groups on the basis of whether or not she parasitized at least one egg, after which the data for the two groups were analyzed separately. The time spent on various forms of activity was used for the final index.

The third experiment was conducted with *T. embryophagum*. Females were offered different strips of paper with grain moth eggs on the first and on the second day of this experiment. In the first variant of this experiment females were offered 60 eggs on the first day and 60 new eggs on the second day, in the second variant – 10 and 10, in the third – 10 and 60, in the fourth – 60 and 10. This experiment was repeated five times, each replicate of each variant involved 50 females (250 females/variant in all).

Results and discussion

For all species studied the percentage of females parasitizing at least one egg is dependent on the host population density (Table 1). The analysis of variance shows, that species of *Trichogramma* (multiple $R = 0.64$, $F = 12.9$, $P < 0.001$) and the host population density ($R = 0.61$, $F = 13.1$, $P < 0.001$) influence the percentage of ovipositing females. The influence of the serial number of the replicate is not significant ($P = 0.066$).

Refusal to parasitize, therefore, can be compared with a functional response to host population density: as the number of eggs in the test tube

Table 1. The influence of host population density on the percent of ovipositing females of *Trichogramma*¹

The number of host eggs		5	10	15	35	60	90
The species of <i>Trichogramma</i>	No of series	Percentage of ovipositing females					
<i>T. embryophagum</i>	1	27a	41b	56c	-	68d	-
	2	16a	-	20a	56b	-	70c
<i>T. pintoi</i>	1	5a	8ab	14b	-	47c	-
	2	6a	-	10a	26b	-	54c
<i>T. sp.</i>	1	38a	50ab	57b	-	75c	-
	2	17a	-	27a	40b	-	56c
<i>T. evanescens</i>	1	58a	52a	62a	-	89b	-
	2	57a	-	81b	87b	-	88b
<i>T. semblidis</i>	1	40a	57b	61b	-	79c	-
	2	30a	-	43ab	56b	-	75c
<i>T. dendrolimi</i>	1	5a	11ab	17b	-	64c	-
	2	20a	-	29a	65b	-	76b
<i>T. principium</i>	1	10a	18a	16a	-	41b	-
	2	9a	-	19b	22bc	-	34c

¹ Values followed by the same letter within row are not significantly different at 0.05 level (ANOVA test).

increase the percentage of females that parasitize at least one host increase. The functional response of parasitoids to host population density (Holling, 1959) has recently become the object of intensive study. Two surveys concerning the matter have appeared recently (Stiling, 1987; Walde & Murdoch, 1988).

The results of the direct observation of *Trichogramma* behaviour may help to explain the mechanisms of 'refusal to parasitize' in more detail (Table 2). It is seen that the females which 'refused' were active and moved in the test tube no less (if not more) than those which parasitized. They also contacted the hosts. These contacts might have been incidental and inevitable in a small test tube. However, the elements of behaviour specific for parasitization either were not manifested by 'refused' females or were extremely rare.

It seems that the sequence of behavioural reactions resulting in parasitization (Vinson, 1985) is interrupted at the stage of discovering the host. Perhaps the concentrations of kairomone which arrest the parasitoid and starts parasitization is not sufficient to cause parasitization by all females. As the number of eggs increase, the

Table 2. The time (in %) spent by ovipositing and 'refused' females of *Trichogramma* on various forms of activity¹

Females: The number of host eggs:	Ovipositing		Refused	
	15	60	15	60
Forms of behaviour and species of <i>Trichogramma</i>				
Percentage of time spent on:				
Moving along the glass or the paper				
<i>T. embryophagum</i>	43.3a	11.7b	40.5a	36.0a
<i>T. principium</i>	8.0a	11.8a	11.3a	14.7a
<i>T. semblidis</i>	15.5a	8.0a	16.8a	18.2a
Contacting the host				
<i>T. embryophagum</i>	11.3a	9.2a	4.1a	5.5a
<i>T. principium</i>	3.1a	11.8b	0.4c	0.7c
<i>T. semblidis</i>	9.8a	5.5a	1.0b	1.0b
Parasitizing or manifesting associated forms of behaviour				
<i>T. embryophagum</i>	7.3a	59.2b	0.1c	0.5c
<i>T. principium</i>	6.2a	16.4b	0.0c	0.0c
<i>T. semblidis</i>	10.2a	15.7a	0.0b	0.0b

¹ Values followed by the same letter within row are not significantly different at 0.05 level ('chi-square' test).

concentration of kairomone increases and the percentage of females which parasitized increase. The functional response of parasitoids to host population density is also based on the reaction to host kairomones (Morrison, 1986).

Kairomone-mediated finding *Sitotroga* eggs has been demonstrated by Laing (1937), but Zaborski *et al.* (1987) failed to reproduce this result. However Laing (1937) used the smell of living moths and Zaborski *et al.* (1987) used hexane extract of scales.

The highest number of females refusing to parasitize was observed in the tubes with 5 host eggs (Table 1). It should be mentioned that the absence of even one darkened egg in many test tubes resulted from lack of parasitization, but not from superparasitization or inadequacy of grain moth eggs for the development of *Trichogramma* larvae. The majority of the eggs which were not parasitized produced caterpillars. In cases when females in the 5-egg test tubes started parasitization, they usually parasitize the majority of hosts (about 70%: from 40% in *T. pintoi* to 79% in *T. sp.*). However, these results make us think

Table 3. Influence of host population density on the various types of oviposition behaviour of *Trichogramma* sp. females¹

Variant number	Number of eggs		Groups of females (%)			
	First day	Second day	Continuing	Finishing	Starting	Refusing
1	10	10	30.4a	6.4b	21.6c	41.6b
2	60	60	50.8b	9.6b	11.6b	28.0a
3	10	60	36.8a	1.6a	31.6d	30.0a
4	60	10	47.2b	10.0b	6.4a	36.4b

¹ Values followed by the same letter within column are not significantly different at 0.05 level ('chi-square' test)

that some grain moth eggs used in the study were unacceptable for parasitization for some unknown reason, e.g. due to damage from handling.

Females, used in the third experiment, were divided into four groups on the basis of whether or not they parasitized on the first and on the second day of the experiment. Females, called 'continuing', oviposited both on the first and on the second day, 'finishing' females – only on the first day, 'starting' females – only on the second day, 'refusing' females – neither on the first nor on the second day (Table 3).

Trichogramma appeared to maintain a relatively stable 'parasitization state' during the experiment: in the first and in the second variants the majority of females are 'continuing' and 'refusing', not 'starting' or 'finishing'. The tendency of females to behave in the same manner both on the first day and on the second day of the experiment proved that dividing into parasitizing and refusing is determined by the differences between females, not by the difference between their portions of the grain moth eggs.

The stability of the parasitization state suggests that it can be based not on behavioural, but rather on endocrine mechanisms. Refusal to oviposit therefore can be considered as a specific form of imaginal diapause: oviposition inhibition in unfavourable conditions without resorption of eggs.

Another possible mechanism of this stability is learning. Learning has been found to play a role in host searching, acceptance, parasitization in numerous parasitic wasps (see review: Vet, 1988; Godfray & Waage, 1988) as well as in *Trichogramma* (de Jong & Pak, 1984; Kaiser *et al.*,

1989; Wajnberg, 1989). Ovipositional preferences can be modified by an oviposition experience: the affinity for a given host is higher after an oviposition on the same species (Kaiser *et al.*, 1989).

The percentage of 'starting' females was significantly higher in the first variant than in the second variant (the percentage of 'continuing' was lower). Possibly, 60 eggs caused the 'parasitization state' more quickly, than 10 eggs did and that is why in the second variant 84% of the ovipositing females began oviposition on the first day of the experiment, while in the first variant it was 63% (the difference is significant: $P < 0.01$).

The percentage of 'finishing' females was practically the same in the second and in the fourth variants, while in the third variant it was significantly lower than in the first variant (the percentage of 'continuing' females is higher). The number of eggs on the second day of the experiment influenced the prolongation of the parasitization only in case the number of eggs on the first day was little. Possibly, the state of parasitization, induced by 60 eggs was more stable than that induced by 10 eggs.

The percentage of the 'starting' females in the second variant was significantly higher than in the fourth variant, and that in the third variant was higher than in the first variant. Obviously, on the second day 60 eggs induced parasitization more intensely than 10 eggs, independently of the number of eggs offered on the first day of the experiment.

Finishing the analysis of results we conclude, that the probability of induction of the 'parasitizing state', the stability of parasitizing state

and probability of its prolongation in *Trichogramma* females depend on host population density.

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Résumé

La densité de population de l'hôte influence l'acceptation de l'hôte chez Trichogramma

En utilisant l'hôte de laboratoire (*Sitotroga cerealella* Oliv.) nous avons démontré qu'une partie des femelles refuse la parasitisation. L'analyse de la succession des actes constitutifs de la parasitisation montre que celle-ci est interrompue au moment de l'acceptation. Le pourcentage des femelles parasitantes est en dépendance directe du nombre des œufs de l'hôte. Les résultats de cette étude suggèrent que l'acceptation de l'hôte par le parasitoïde est fonction de la densité de population de celui-ci. Les mécanismes possibles de cette dépendance (la réaction aux kairomones, l'apprentissage, les modifications endocrines) sont discutés.

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