Tracking switching behavior of *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) over 45 generations

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Abstract: The effects of long-term rearing of *Trichogramma brassicae* (Bezdenko) on host preference and switching behavior was examined when parasitizing two common factitious hosts, including Mediterranean flour moth (MFM), *Ephestia kuehniella* Zeller and Angoumois grain moth (AGM)*, Sitotroga cerealella* (Olivier) over 45 generations. Both host species eggs were offered to the parasitoid over 24 h. Significantly more MFM eggs were parasitized in all generations, but a distinct negative switching behavior was found in all experiments. The parasitoid negatively switched from MFM when they became abundant compared to AGM and vice versa. The preference coefficient (*c*) estimated using Murdoch's index ranged from 2.056 (in G5) to 1.734 (in G45) for MFM and 0.486 (in G5) to 0.577 (in G45) for AGM. The linear regression indicated that despite preferring MFM, the intensity of the preference decreased over 45 generations. Negative switching behavior in *T. brassicae* may be advantageous for this species in controlling pests in given situations.

Keywords: biological control, foraging behavior, host preference, mass rearing, negative switching, *Trichogramma brassicae*

Introduction

Parasitoids that attack the egg stage of their hosts are an important part of the pest control strategy to reduce chemical applications and still produce crops. *Trichogramma brassicae* (Bezdenko) is an important biological control agent used widely against many lepidopteran pests. Annually, a large number of *T. brassicae* wasps are produced locally or by pioneer companies on factitious hosts, the Mediterranean flour moth (MFM), *Ephestia kuehniella* Zeller, and the Angoumois grain moth (AGM)*, Sitotroga cerealella* (Olivier) and used against different insect species across the world (Smith 1996, Knutson 1998, Cônsoli et al. 2010). In most cases, successive generations of *T. brassicae* are maintained for a long time.

In parasitoid-host systems, switching plays an important role in increasing the stability of the system in the long term (Van Baalen et al.

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2001, Heidarian et al. 2012). Switching behavior is the ability of a general natural enemy to become entrained to the most abundant prey/host species such that the number of attacks on that prey/host is disproportionately greater than would be expected by chance encounters. If another prey/host becomes dominant, the predator/parasitoid "switches" to and concentrates its attacks disproportionately upon the new dominant prey/host. The natural enemy thus exhibits a flexible search image influenced by learning (Cornell 1976).

Switching occurs when natural enemies change to an alternative host, and the density of the preferred host decreases (Murdoch 1969, Fathipour and Maleknia 2016). Briefly, a natural enemy might distribute its attacks among several prey/host species in response to the species' relative frequencies in which the attacks concentrate disproportionately upon the more abundant prey/host (Murdoch et al. 1975). Switching can be tested by offering natural enemies mixtures of different host species (or host stages) in single-patch experiments (Fathipour and Maleknia 2016). To determine the stabilizing characteristics of switching in a particular parasitoid-host system, assumptions about the heterogeneity of host distribution, the dynamic features of parasitoid and host, and how the parasitoid and host are coupled (e. g., functional response) must be specified (Cornell and Pimentel 1978). Parasitoid searching capacity, host preference, and dispersion are some traits that have been also evaluated as criteria for strain selection (Pakyari et al. 2008, Hegazi et al. 2019).

The concept of negative switching (anti-switching) (Chesson 1978a) describes a situation where the ratio of two prey/host species in the diet of a natural enemy increases slower than expected from their ratio in the environment. Variation in preference for the prey/host, from one natural enemy individual to another, leads to a partial decoupling of the per capita predation/parasitism rates (Chesson 1984, Abrams 2010). In other words, negative switching is said to occur where

disproportionately more of the rarer type of prey/host is accepted (Chesson 1978b, Visser 1981, Jervis 2007). A predator whose preference for a prey decreases as a function of the abundance of this prey will maintain its diet composition virtually constant irrespective of changes in prey density and may destabilize trophic interactions by increasing the predation mortality of a prey species whose abundance declines (Rindorf et al. 2006).

Many natural enemies are mass-reared and applied for inundative biological control (Cônsoli et al. 2010). We could find no information on the changes which may occur in the switching ability of insects under such long-term breeding programs, even though changes here could have dramatic effects on the success of such programs. Previous studies showed decreased *T. brassicae* efficiency in long-term mass rearing (Ghaemmaghami et al. 2021 a, b). The present study investigated the possible changes in host preference and switching behavior of *T. brassicae* over 45 generations of rearing on two factitious hosts, the Mediterranean flour moth, *Ephestia kuehniella,* and the Angoumois grain moth, *Sitotroga cerealella*. Our study contributes to quality control of the parasitoid wasp *T. brassicae* during long-term mass rearing in laboratories and insectaries.

Materials and Methods

Insect rearing
A colony of Angoumois grain moth was established on barley grains under ambient temperature (25 ± 1 ºC) in a biocontrol laboratory of the Technical and Vocational Training Center of Estahban, Fars, Iran. After emerging adult insects were transferred to funnels (20 cm) covered with a fine mesh net over the mouth. After mating, eggs were collected on papers placed under a mesh net and transferred daily to containers (20 × 50 cm diameter) with two kg of barley. Mediterranean flour moth (MFM) was established on an artificial medium consisting of one kilogram of flour (prepared from Omid wheat cultivar), 200 grams of wheat bran, and 5 grams

of bakery yeast and maintained in the same condition as AGM.

To initiate the colony of *T. brassicae*, *S. cerealella* egg traps were placed on tomato farms in Karaj, Iran, in May 2017, and the traps were collected after 48 h. The parasitized eggs were incubated in a growth chamber at 25 ± 1 °C, 65 ± 5% RH, and a 16:8 h (L: D) photoperiod until the emergence of *T. brassicae* adults (G0). The adult *T. brassicae* were reared in rectangular plastic containers (35 × 20 × 15 cm) on *S. cerealella* eggs under the abovementioned conditions.

Host preference experiments
Females of *T. brassicae* (< 24 h old) were individually placed in glass vials (100 × 16 mm). Eighty (40 + 40) fresh eggs of MFM and AGM (50:50 ratio) were simultaneously introduced to every wasp. The parasitized eggs were counted 24 h after starting the experiment. No host was replaced during the experiment. The experiment had twenty replicates. These procedures were repeated for generations 5, 10, 15, 20, 25, 26, 27, 28, 29, 30, 31, 35, 40, and 45. Host preference data were analyzed using independent–sample–tests at P = 0.05 using the SPSS (SPSS-Inc 2009) program to determine any significant differences in each generation regarding the mean number of parasitized eggs of each host species. The percentage of each host egg parasitized (after the necessary data transformation) was compared using ANOVA (Tukey multiple comparisons) among different generations. The graphs were plotted using Excel 2013.

Switching experiments
Female *T. brassicae* wasps (maximum 24 h old) were provided with fresh eggs of MFM and AGM in a glass vial (100 × 16 mm) with both host eggs at one of the seven ratios: 70: 10, 60: 20, 50: 30, 40: 40, 30: 50, 20: 60 and 10:70 of MFM: AGM. Each ratio was carried out with twenty replicates. After 24 h, the wasps were removed, and the number of parasitized eggs was counted after 48 h.

The null case is a simple parasitism model that describes results from experiments in which *T. brassicae* exhibited a preference between two host species. The null or no-switch model (Murdoch et al. 1975) for a system consisting of two host species is:

$$P_1 = (cF_1)/(1 - F_1 + (cF_1)) \qquad (1)$$

Where F_1 is the proportion of $host_1$ in the environment, P_1 is the proportion of $host_1$ among all parasitized hosts, and c is a parameter described in Eq. (2):

$$E_1/E_2 = c(N_1/N_2) \qquad (2)$$
If $N_1 = N_2$ therefore $c = E_1/E_2$

Where E_1/E_2 is the ratio of two host species parasitized, N_1/N_2 is the ratio of the number of two host species available in the environment, and c is constant; c measures preference and can be defined as the ratio of parasitized $host_1$ to $host_2$ when the two species of hosts are equally abundant. When $c = 1$, there is no preference. When c > 1, there is a preference for $host_1$. When $c < 1$, there is a preference for $host_2$ (Murdoch 1969).

Finally, to test the hypothesis of the host switch, we compared the observed ratio with the expected ratio for the corresponding treatment. When switching occurs, the observed ratio E_1/E_2 is higher than the expected ratio at high values of N_1/N_2 (Murdoch and Marks 1973).

Results

Host preference
Although the female wasps parasitized both species, the most parasitized eggs belonged to MFM in all generations (Fig. 1). T–tests among the mean number of each host species showed that the wasps parasitized more *E. kuehniella* eggs rather than *S. cerealella* in an equal ratio of hosts along 45 generations (Fig. 1). The highest (14.80 ± 0.85 eggs) and lowest (12.05 ± 0.46 eggs) MFM eggs were parasitized in G5 and G45, respectively. For AGM eggs were in G30 and G45, respectively (7.70 ± 0.35 and 6.95 ± 0.34 eggs).
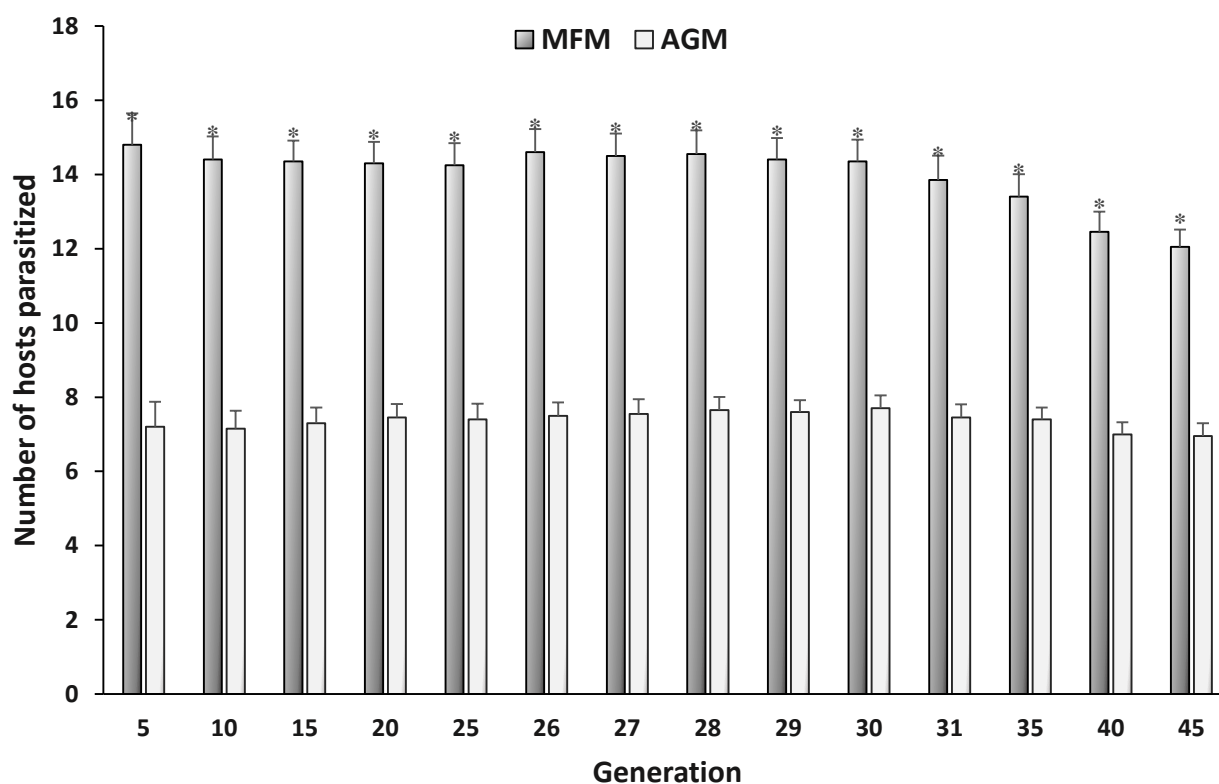
Figure 1 Host preference of *Trichogramma brassicae* on Mediterranean flour moth (MFM), *Ephestia kuehniella* and Angoumois grain moth (AGM), *Sitotroga cerealella* over 45 generations. Data with an asterisk differed significantly according to t–test at P < 0.05.

Switching

A distinct negative switching behavior was found in *T. brassicae* in all experiments. The parasitoid switched from MFM when they became abundant to AGM, which was fewer in number and vice versa. In these experiments, the higher parasitism of MFM occurred in its lower proportion. This trend decreased as its offering increased, indicating the occurrence of negative switching behavior (Fig. 2). Similarly, the parasitoids switched from AGM to MFM when the proportion of AGM was more than MFM (Fig. 3). The curves of switching tests based on Murdoch's model (Figs. 2 and 3) indicated the preference behavior did not remain constant and varied directly with increasing relative density even though MFM eggs were significantly preferred to AGM by *T. brassicae*. The preference value

(*c*) estimated using Murdoch's index (Eq. 2) (Table 1) was used to plot the Murdoch's no-switch line. The highest and lowest *c* values for MFM were observed in G5 (2.056) and G45 (1.734), respectively. Also, G45 (0.577) and G5 (0.486) had the highest and lowest *c* values for AGM. In all generations, this index favored MFM, confirming this host species was more preferred by *T. brassicae* (Table 1).

The linear regression between preference values (*c*) for MFM and the generation number of the parasitoid indicated that although *T. brassicae* preferred MFM to AGM eggs, the intensity of the preference decreased slightly over generations (Fig. 4). The merit of the model was supported by the high value of the coefficient of determination (R^2) (F = 179.787; df = 1, 12; P < 0.0001; R^2 = 0.937).
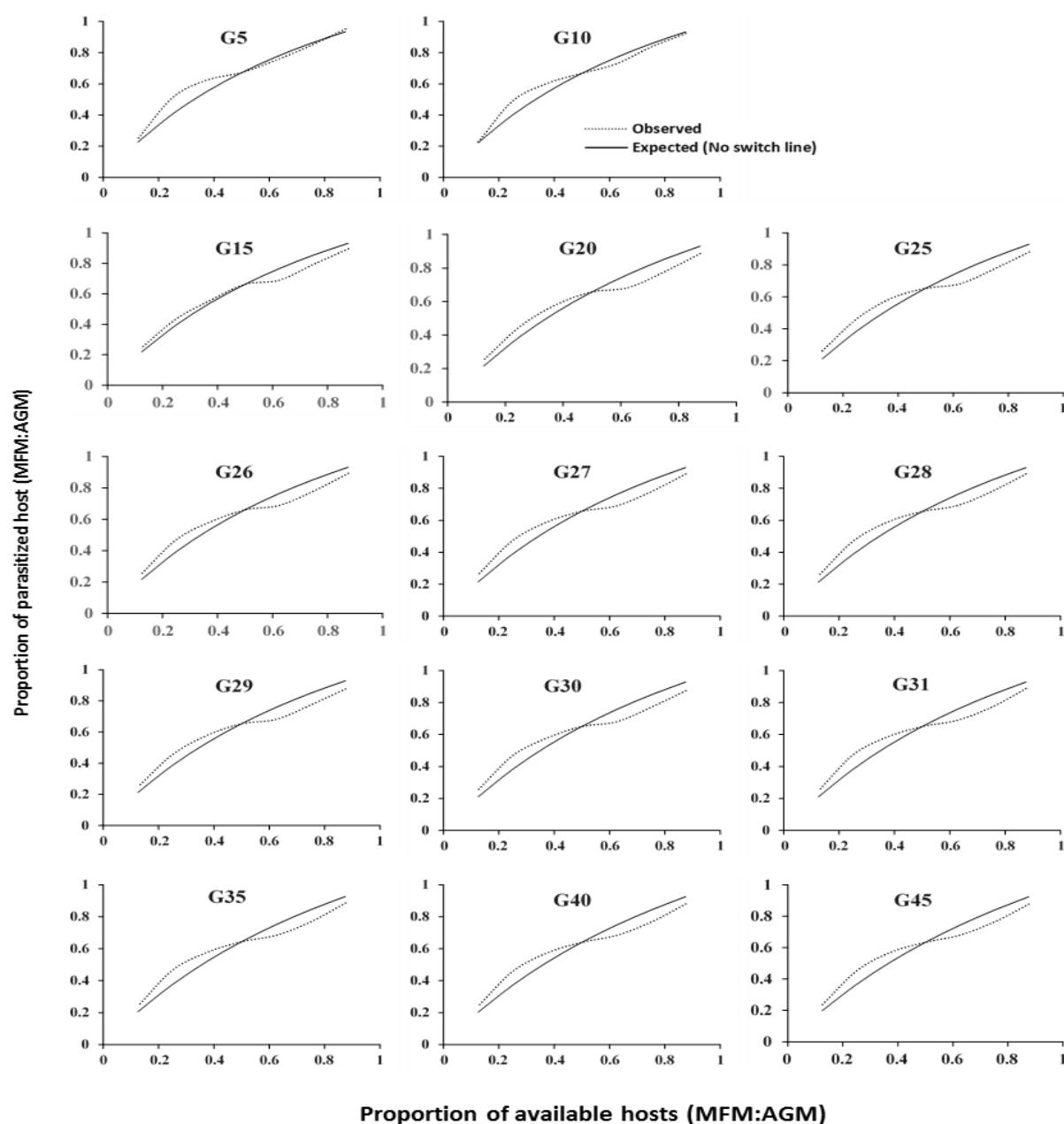
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Figure 2 Proportion Mediterranean flour moth, *Ephestia kuehniella* eggs parasitized by *Trichogramma brassicae* as a function of their relative abundance in the environment over 45 generations (G).

Analysis of variance indicated that the number of generations and host ratios had significant effects on the total parasitism of *T. brassicae* and the rate of MFM and AGM parasitism when offered at different ratios (Table 2). The mean comparison revealed that except in 87.5% available MFM, the MFM parasitism rate [(MFM/(MFM + AGM)] × 100

did not differ over generations (Table 3). Accordingly, the highest and lowest MFM parasitism rates were observed in G5 and G45, respectively. Also, the AGM parasitism rate [(AGM/(MFM + AGM)] × 100 was only significant at 12.5% of available AGMs in which G45 and G5 had the highest and lowest AGM parasitism rates (Table 4).

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Except at 12.5 and 25% available MFM, for the other ratios of available hosts, the total parasitism was significant (Table 5). G5 had the highest total parasitism for 87.5%, 75%, 62.5%, 50%, and 37.5% available MFM eggs. The lowest total parasitism was observed in G45 for 87.5%, 75%, 62.5%, 50%, and 37.5% available MFM eggs (Table 5).
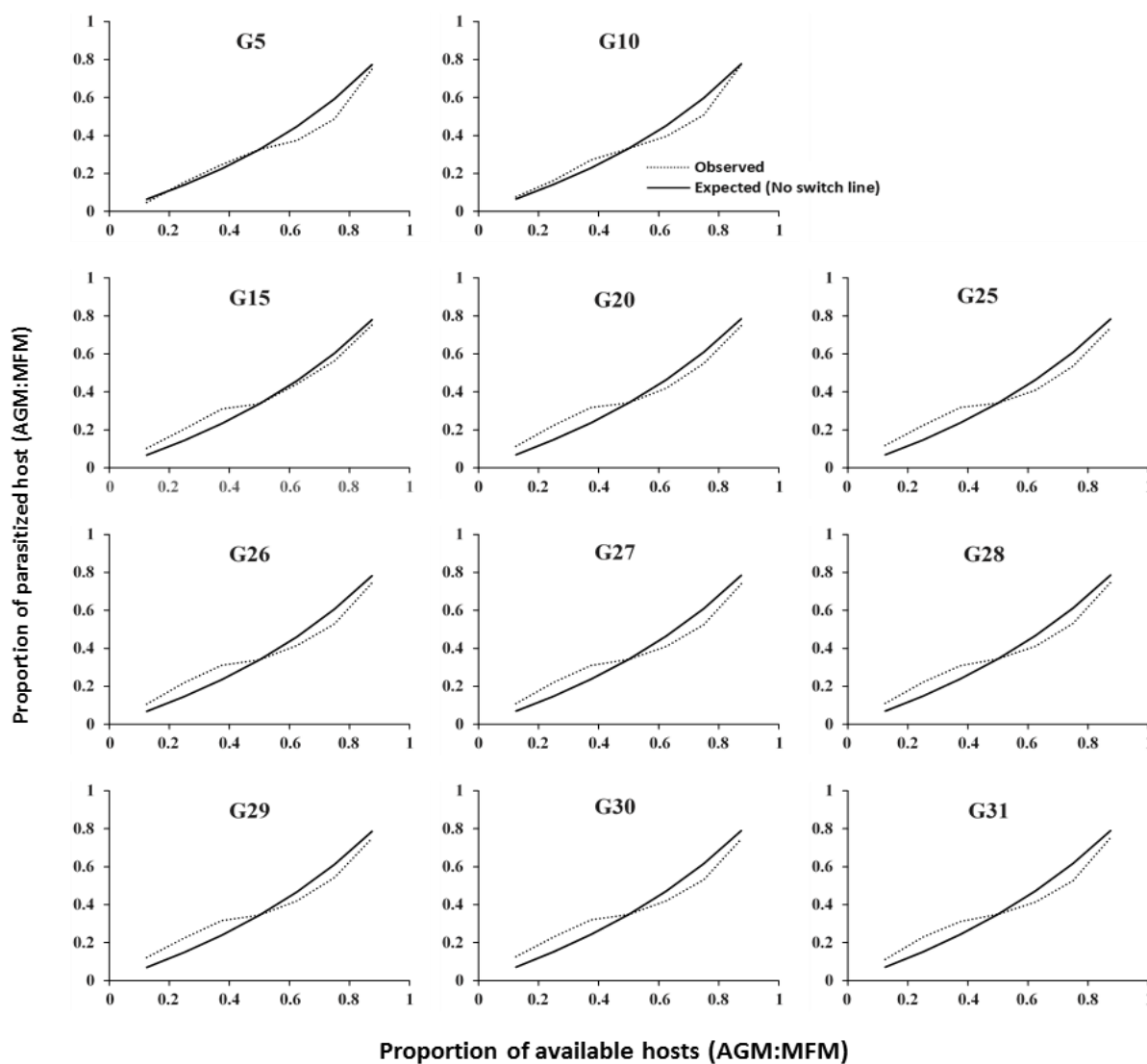

Figure 3 Proportion of Angoumois grain moth, *Sitotroga cerealella* eggs parasitized by *Trichogramma brassicae* as a function of their relative abundance in the environment over 45 generations (G).

Table 1 The preference values (c) estimated using Murdoch's index, for two host eggs, Mediterranean flour moth (MFM) *Ephestia kuehniella* and Angoumois grain moth (AGM), *Sitotroga cerealella*, parasitized by *Trichogramma brassicae* over generations.

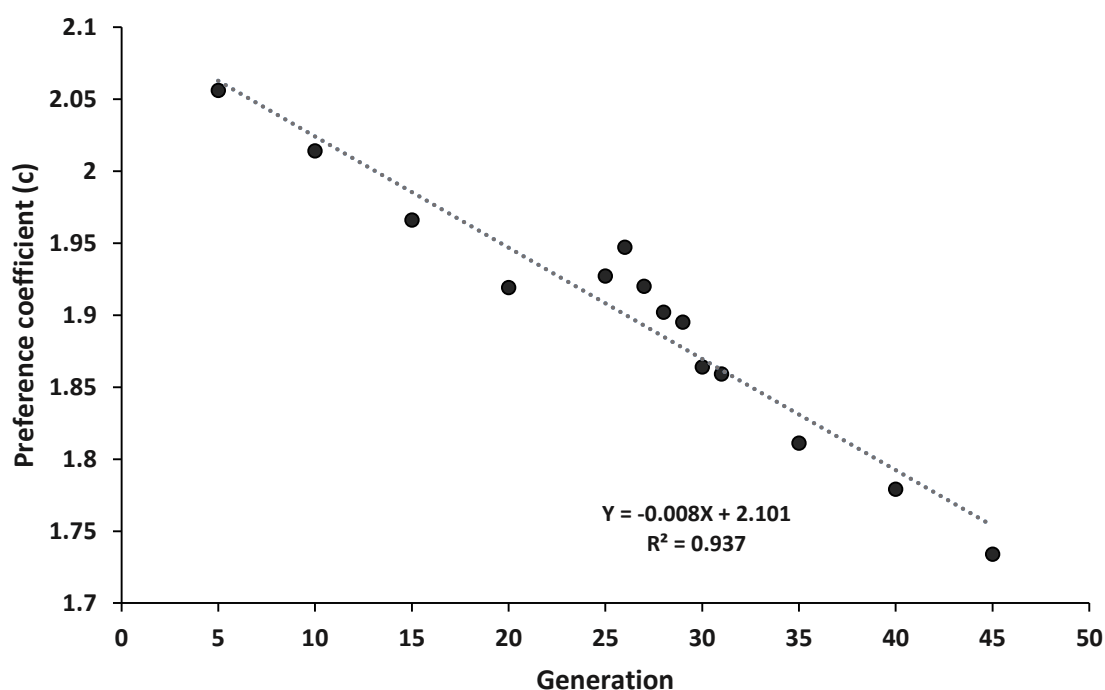| Generation	MFM	AGM
5	2.056	0.486
10	2.014	0.496
15	1.966	0.509
20	1.919	0.521
25	1.927	0.520
26	1.947	0.514
27	1.920	0.521
28	1.902	0.526
29	1.895	0.528
30	1.864	0.537
31	1.859	0.538
35	1.811	0.552
40	1.779	0.562
45	1.734	0.577

Figure 4 Regression curve of the preference coefficient (*c*) for Mediterranean flour moth (MFM), *Ephestia kuehniella* eggs with generations of *Trichogramma brassicae*. The *c* is the ratio of parasitized MFM to Angoumois grain moth (AGM), *Sitotroga cerealella* when the two species of hosts are equally abundant.

Table 2 Univariate factorial ANOVAs for the effect of host ratio and parasitoid generations on total parasitism and number of parasitized hosts of *Trichogramma brassicae*.

Source	Total parasitism				Parasitized MFM eggs				Parasitized AGM eggs			
	SS	df	F	*P* value	SS	df	F	*P* value	SS	df	F	*P* value
Generation	1661.31	13	13.28	< 0.001	1594.19	13	15.42	< 0.001	187.76	13	3.25	< 0.001
Host ratio	3618.96	6	62.65	< 0.001	48875.57	6	1024.47	< 0.001	27494.60	6	1031.80	< 0.001
Generation × Host ratio	838.10	78	1.12	0.232	822.96	78	1.33	0.032	117.72	78	34	1.000
Error	17925.05	1862			14805.40	1862			8269.55	1862		
Total	963247.00	1960			438092.00	1960			165103.00	1960		

MFM, Mediterranean flour moth, *Ephestia kuehniella*; AGM, Angoumois grain moth, *Sitotroga cerealella*.

Table 3 Mean comparison for percentage of *Ephestia kuehniella* (MFM) eggs (MFM/(MFM + AGM) × 100) parasitized by sequential generations of *Trichogramma brassicae*.

Generation	Percentage of available MFM eggs (MFM/(MFM + AGM) ×100)						
	87.5	75	62.5	50	37.5	25	12.5
5	94.69 ± 1.12 [a]	84.01 ± 1.98 [a]	74.95 ± 2.15 [a]	66.89 ± 3.23 [a]	62.33 ± 2.61 [a]	51.10 ± 3.16 [a]	24.65 ± 2.44 [a]
10	92.41 ± 1.24 [ab]	83.46 ± 1.85 [a]	73.51 ± 2.53 [a]	66.75 ± 2.15 [a]	59.62 ± 2.83 [a]	48.33 ± 2.94 [a]	22.15 ± 2.79 [a]
15	89.51 ± 1.43 [ab]	79.66 ± 1.72 [a]	69.12 ± 1.80 [a]	66.44 ± 1.68 [a]	55.93 ± 2.52 [a]	43.40 ± 1.78 [a]	23.74 ± 2.43 [a]
20	88.64 ± 1.39 [b]	77.38 ± 2.03 [a]	68.13 ± 1.60 [a]	65.68 ± 1.41 [a]	57.93 ± 2.19 [a]	45.12 ± 2.56 [a]	24.34 ± 2.28 [a]
25	88.06 ± 1.28 [b]	77.75 ± 1.89 [a]	68.17 ± 1.69 [a]	65.87 ± 1.56 [a]	58.88 ± 2.14 [a]	46.30 ± 2.71 [a]	25.91 ± 1.84 [a]
26	89.25 ± 1.23 [ab]	78.15 ± 1.67 [a]	68.71 ± 1.78 [a]	65.95 ± 1.39 [a]	58.40 ± 2.14 [a]	46.92 ± 2.49 [a]	25.22 ± 1.87 [a]
27	88.94 ± 1.23 [ab]	77.96 ± 1.49 [a]	69.00 ± 1.62 [a]	65.51 ± 1.86 [a]	59.20 ± 1.78 [a]	47.34 ± 2.54 [a]	25.64 ± 1.50 [a]
28	88.98 ± 1.10 [ab]	78.03 ± 1.34 [a]	69.15 ± 1.48 [a]	65.28 ± 1.61 [a]	58.88 ± 2.26 [a]	46.73 ± 2.41 [a]	25.03 ± 1.66 [a]
29	87.78 ± 1.19 [b]	77.74 ± 1.78 [a]	68.17 ± 1.45 [a]	65.30 ± 1.18 [a]	57.94 ± 1.88 [a]	45.67 ± 2.25 [a]	24.79 ± 1.96 [a]
30	87.29 ± 0.84 [b]	76.96 ± 1.64 [a]	67.76 ± 1.37 [a]	64.78 ± 1.74 [a]	57.99 ± 2.34 [a]	46.82 ± 2.16 [a]	24.82 ± 1.32 [a]
31	88.81 ± 1.07 [b]	76.93 ± 1.61 [a]	68.62 ± 1.60 [a]	64.72 ± 1.68 [a]	58.72 ± 1.88 [a]	47.14 ± 2.01 [a]	24.05 ± 1.98 [a]
35	88.31 ± 1.27 [b]	76.80 ± 1.45 [a]	68.44 ± 1.60 [a]	64.16 ± 1.49 [a]	57.82 ± 1.96 [a]	46.37 ± 2.06 [a]	23.21 ± 1.71 [a]
40	87.91 ± 1.21 [b]	76.26 ± 1.89 [a]	68.28 ± 2.19 [a]	63.79 ± 1.59 [a]	57.48 ± 2.05 [a]	45.83 ± 2.12 [a]	23.71 ± 1.58 [a]
45	87.60 ± 1.32 [b]	76.37 ± 1.34 [a]	68.44 ± 1.64 [a]	63.42 ± 1.51 [a]	57.28 ± 1.60 [a]	45.26 ± 1.89 [a]	23.49 ± 1.62 [a]
F	2.755	2.023	1.445	0.353	0.440	0.537	0.269
dfT,dfE	13,266	13,266	13,266	13,266	13,266	13,266	13,266
P	0.001	0.019	0.139	0.982	0.954	0.900	0.995

The means followed by the same letter in each column are not significantly different (P < 0.05, Tukey's test). *T*: Treatment; *E*: Error.

Table 4 Mean comparison for percentage of *Sitotroga cerealella* (AGM) eggs (AGM/(MFM + AGM) × 100) parasitized by sequential generations of *Trichogramma brassicae.*

Generation	Percentage of available AGM eggs (AGM/(MFM + AGM) ×100)						
	12.5	25	37.5	50	62.5	75	87.5
5	5.31 ± 1.12 [b]	15.99 ± 1.98 [a]	25.05 ± 2.15 [a]	33.11 ± 3.23 [a]	37.67 ± 2.61 [a]	48.90 ± 3.16 [a]	75.35 ± 2.44 [a]
10	7.59 ± 1.24 [ab]	16.54 ± 1.85 [a]	26.49 ± 2.53 [a]	33.25 ± 2.15 [a]	40.38 ± 2.83 [a]	51.67 ± 2.94 [a]	77.85 ± 2.79 [a]
15	10.49 ± 1.43 [ab]	20.34 ± 1.72 [a]	30.88 ± 1.80 [a]	33.56 ± 1.68 [a]	44.07 ± 2.52 [a]	56.60 ± 1.78 [a]	76.26 ± 2.43 [a]
20	11.36 ± 1.39 [a]	22.62 ± 2.03 [a]	31.87 ± 1.60 [a]	34.32 ± 1.41 [a]	42.07 ± 2.19 [a]	54.88 ± 2.56 [a]	75.66 ± 2.28 [a]
25	11.94 ± 1.28 [a]	22.25 ± 1.89 [a]	31.83 ± 1.69 [a]	34.13 ± 1.56 [a]	41.12 ± 2.14 [a]	53.70 ± 2.71 [a]	74.09 ± 1.84 [a]
26	10.75 ± 1.23 [ab]	21.85 ± 1.67 [a]	31.29 ± 1.78 [a]	34.05 ± 1.39 [a]	41.60 ± 2.14 [a]	53.08 ± 2.49 [a]	74.78 ± 1.87 [a]
27	11.06 ± 1.23 [ab]	22.04 ± 1.49 [a]	31.00 ± 1.62 [a]	34.49 ± 1.86 [a]	40.80 ± 1.78 [a]	52.66 ± 2.54 [a]	74.36 ± 1.50 [a]
28	11.02 ± 1.10 [ab]	21.97 ± 1.34 [a]	30.85 ± 1.48 [a]	34.72 ± 1.61 [a]	41.12 ± 2.26 [a]	53.27 ± 2.41 [a]	74.97 ± 1.66 [a]
29	12.22 ± 1.19 [a]	22.26 ± 1.78 [a]	31.83 ± 1.45 [a]	34.70 ± 1.18 [a]	42.06 ± 1.88 [a]	54.33 ± 2.25 [a]	75.21 ± 1.96 [a]
30	12.71 ± 0.84 [a]	23.04 ± 1.64 [a]	32.24 ± 1.37 [a]	35.22 ± 1.74 [a]	42.01 ± 2.34 [a]	53.18 ± 2.16 [a]	75.18 ± 1.32 [a]
31	11.19 ± 1.07 [a]	23.07 ± 1.61 [a]	31.38 ± 1.60 [a]	35.28 ± 1.68 [a]	41.28 ± 1.88 [a]	52.86 ± 2.01 [a]	75.95 ± 1.98 [a]
35	11.69 ± 1.27 [a]	23.20 ± 1.45 [a]	31.56 ± 1.60 [a]	35.84 ± 1.49 [a]	42.18 ± 1.96 [a]	53.63 ± 2.06 [a]	76.79 ± 1.71 [a]
40	12.09 ± 1.21 [a]	23.74 ± 1.89 [a]	31.72 ± 2.19 [a]	36.21 ± 1.59 [a]	42.52 ± 2.05 [a]	54.17 ± 2.12 [a]	76.29 ± 1.58 [a]
45	12.40 ± 1.32 [a]	23.63 ± 1.34 [a]	31.56 ± 1.64 [a]	36.58 ± 1.51 [a]	42.72 ± 1.60 [a]	54.74 ± 1.89 [a]	76.51 ± 1.62 [a]
F	2.755	2.023	1.445	0.353	0.440	0.537	0.269
dfT,dfE	13,266	13,266	13,266	13,266	13,266	13,266	13,266
P	0.001	0.019	0.139	0.982	0.954	0.900	0.995

The means followed by the same letter(s) in each column are not significantly different (P < 0.05, Tukey's test). *T*: Treatment; *E*: Error.

Table 5 Mean comparison for total parasitism of sequential generations of *Trichogramma brassicae* on eggs of *Ephestia kuehniella* (MFM) and *Sitotroga cerealella* (AGM).

Generation	Percentage of available MFM eggs (MFM/(MFM + AGM) × 100)						
	87.5	75	62.5	50	37.5	25	12.5
5	26.10 ± 0.98 [a]	25.65 ± 0.86 [a]	24.70 ± 0.75 [a]	22.00 ± 0.48 [ab]	23.10 ± 0.83 [a]	22.45 ± 0.60 [a]	18.45 ± 0.44 [a]
10	25.30 ± 0.76 [a]	23.60 ± 0.67 [abc]	21.20 ± 0.73 [bcd]	21.55 ± 0.57 [ab]	20.70 ± 0.77 [ab]	22.40 ± 0.87 [a]	19.25 ± 0.53 [a]
15	24.65 ± 0.83 [a]	24.20 ± 0.93 [abc]	22.40 ± 0.74 [abcd]	21.65 ± 0.70 [ab]	21.95 ± 0.69 [ab]	20.20 ± 1.09 [a]	19.45 ± 0.73 [a]
20	24.15 ± 0.73 [ab]	24.60 ± 0.60 [abc]	22.50 ± 0.60 [abcd]	21.75 ± 0.71 [ab]	21.10 ± 0.76 [ab]	20.75 ± 0.73 [a]	19.55 ± 0.68 [a]
25	24.00 ± 0.61 [ab]	24.60 ± 0.81 [abc]	22.50 ± 0.64 [abcd]	21.65 ± 0.78 [ab]	21.65 ± 0.85 [ab]	21.25 ± 0.69 [a]	19.85 ± 0.60 [a]
26	23.85 ± 0.56 [ab]	25.10 ± 0.62 [ab]	23.00 ± 0.46 [abc]	22.10 ± 0.74 [a]	21.20 ± 0.72 [ab]	21.55 ± 0.90 [a]	20.20 ± 0.60 [a]
27	23.75 ± 0.59 [ab]	25.15 ± 0.65 [ab]	23.10 ± 0.56 [ab]	22.05 ± 0.52 [a]	21.50 ± 0.84 [ab]	21.70 ± 0.81 [a]	20.15 ± 0.69 [a]
28	23.60 ± 0.62 [ab]	25.10 ± 0.70 [ab]	23.15 ± 0.65 [ab]	22.20 ± 0.64 [a]	21.55 ± 0.51 [ab]	21.85 ± 0.85 [a]	20.05 ± 0.54 [a]
29	23.65 ± 0.58 [ab]	24.50 ± 0.50 [abc]	23.00 ± 0.62 [abc]	22.00 ± 0.72 [ab]	21.30 ± 0.83 [ab]	21.55 ± 0.90 [a]	20.05 ± 0.49 [a]
30	23.35 ± 0.55 [ab]	24.20 ± 0.56 [abc]	22.60 ± 0.76 [abcd]	22.05 ± 0.51 [a]	21.50 ± 0.55 [ab]	21.90 ± 1.00 [a]	20.70 ± 0.69 [a]
31	23.10 ± 0.59 [ab]	24.10 ± 0.59 [abc]	22.75 ± 0.66 [abcd]	21.30 ± 0.66 [ab]	21.35 ± 0.67 [ab]	21.25 ± 0.90 [a]	19.90 ± 0.66 [a]
35	21.10 ± 0.54 [bc]	23.30 ± 0.68 [abc]	21.85 ± 0.54 [abcd]	20.80 ± 0.67 [ab]	20.45 ± 0.53 [ab]	20.50 ± 0.88 [a]	19.75 ± 0.76 [a]
40	19.90 ± 0.66 [c]	22.20 ± 0.69 [bc]	19.85 ± 0.55 [cd]	19.45 ± 0.57 [ab]	19.95 ± 0.51 [ab]	19.85 ± 0.74 [a]	19.45 ± 0.57 [a]
45	19.45 ± 0.59 [c]	21.30 ± 0.80 [c]	19.65 ± 0.90 [d]	19.00 ± 0.57 [b]	19.60 ± 0.65 [b]	19.65 ± 0.80 [a]	19.35 ± 0.49 [a]
F	8.085	2.909	4.042	2.445	1.497	1.105	0.774
dfT,dfE	13,266	13,266	13,266	13,266	13,266	13,266	13,266
P	< 0.001	0.001	< 0.001	0.004	0.118	0.355	0.687

The means followed by the same letter (s) in each column are not significantly different (P < 0.05, Tukey's test). *T*: Treatment; *E*: Error.

Discussion

Switching behavior usually occurs when a natural enemy has a weak preference for one of the host species and switches to the abundantly available host or when total prey density is constant. Mortality in each prey is density-dependent (Murdoch 1969). In the present study, *T. brassicae* preferred MFM to AGM, which is consistent with the findings of Cornell and Pimentel (1978). Our results did not support the positive switching behavior across the experiments and the existence of a sigmoid-shaped curve in which the relationship between the numbers of prey eaten versus the numbers given will be sigmoid according to Murdoch (1969).

Switching behavior may positively or negatively affect some life history traits, such as fecundity. When *Trichogramma minutum* Rile switched from *Choristoneura fumiferana* (Clemens) as a natural host to MFM as a factitious host, their fecundities decreased in generations. Conversely, when *T. minutum* reared on MFM switched to the natural host, fecundities increased after five generations (Bourchier et al. 1994). Here, we found that *T. brassicae* preferred MFM to AGM, which may reflect MFM eggs' size. Bigger host size and longer larval development time may allow natural enemies to take up more nutrients and produce larger insects with higher fecundity (Bigler et al. 1987, Nordlund et al. 1997). It is well-known that different *Trichogrumma* species vary in size based on their colonizing host eggs (Cônsoli et al. 2010). Such wasps may be much better in quality (e. g. larger and greater in several female individuals) than those reared on small host eggs (Smith 1996). The host density influences the acceptance and allocation of host eggs by Trichogrumma, and parasitoid fecundity or clutch size is adjusted according to host availability relative to abundance, host egg size, and spacing between eggs (Smith 1996, Cônsoli et al. 2010). Bigler et al. (1987) found that *Trichogramma* wasps emerging from either *Ephestia* or *Sitotroga* were equivalent in parasitism under field conditions. Still, better performance in the laboratory conditions was achieved by rearing on *Ephestia*. The protein concentration of host eggs may play a vital role in the preference and acceptance of a host by its parasitoid (Lü et al. 2015). Different life stages of an insect host may not provide a natural enemy in the same way. Pakyari et al. (2008) found that female *Scolothrips longicornis* Priesner preferred to feed more on the adults of *T. urticae* than the juvenile stages. Also, Heidarian et al. (2012) showed that, compared with the other life stages, the larvae and protonymphs of *Schizotetranychus smirnovi* Wainstein were more attacked by *S. longicornis* throughout the experiment.

Natural enemy species may behave differently when faced with two host/prey items. Therefore, in the diet of the natural enemy population, the ratio of the two prey/host species may vary inversely with their ratio in the environment, i. e., negative switching occurs at the population level even though individual predators show no switching behavior.

This was the case with *T. brassicae,* except that negative switching behavior occurred for both hosts regardless of host preference. In other words, when an abundance of MFM was higher, *T. brassicae* switched to AGM and reciprocally switched from AGM to MFM when AGM eggs were more available. Chesson (1984) argued that population-negative switching might occur when preference is highly variable, food is sufficiently abundant, functional responses are near saturation, and individuals have no switching behavior. The explanation for negative switching is not fully clear, but suggestions include the effect of prey actively attempting to avoid the predator when the risk of predation is high (Abrams and Matsuda 1993); it is an artifact of the estimation of mean preference from samples consisting of several individuals with different preferences (Chesson 1984), the confusion of the search image of the predator due to the more abundant prey (Rindorf et al. 2006), and the predator maintaining a balanced supply of nutrients (Visser 1981). Switching behavior is not usual among natural enemies (Murdoch 1969). As there are many

examples of switching behavior in insects (Cornell and Pimentel 1978, Bourchier et al. 1994, van Baalen et al. 2001, Heidarian et al. 2012, Fathipour et al. 2020), many natural enemies have been identified with no switching behavior (Murdoch 1969, Ashley et al. 1974).

Since the culture was founded, switching shapes have changed with generation times. In earlier (G5 and G10), the wasps parasitized hosts more than expected (no switch line), but in the later generations (G15 onwards), negative switching was observed. Population-level switching behavior has implications for population dynamics. Indeed, a general tendency toward population negative switching might occur when prey is abundant, possibly destabilizing the prey populations (Chesson 1984). MFM's preference value (c) decreased, and AGM increased over generations. Furthermore, this index may be varied due to relative host density. Chesson (1978a) stated, "if *c* is a decreasing function of relative density, we say that negative switching occurs".

Despite preferring MFM, the preference decreased slightly over 45 generations in *T. brassicae*. It may be because the long-term mass-rearing process of the parasitoid on AGM may lead to adaptation to the factitious host. The parasitoid was predicted to switch to AGM in later generations, but this did not happen. This result is consistent with the findings of Bourchier et al. (1994).

Switching behavior in nature rarely occurs, and natural enemies generally do not stabilize prey/host populations by this mechanism (Murdoch 1969). Still, host switching is a fundamental characteristic of populations of *T. brassicae* during the mass production process in insectaries. Lab-cultured parasitoids often experience inadvertent selection. The rearing procedure may select a preference for large, fast-laying individuals that are effective at attacking a relatively homogeneous substrate (Hopper et al. 1993). The ability to respond to host egg size, shape, or other qualities may be lost in these rearing procedures. Parasitoids originating from different hosts may be better equipped to deal with variable natural hosts. Selection for

switching ability or variation in response to host eggs may result in higher realized fecundity in the field (Bourchier et al. 1994).

In general, our goal in biological control may define the tactics used. Increased insight into alternative foods' effects may help us devise new tactics to make the natural enemy more useful. Natural enemies with switching behavior may increase diversity, and those without this behavior could increase prey mainly if they prefer prey/host, which tends to win in the competition. However, negative switching behavior in *T. brassicae* may benefit this species in controlling the pests it parasitizes.

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Statements and Declarations

The authors have no competing interests to declare relevant to this article's content.

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پایش رفتار سوئیچینگ زنبور پارازیتوئید
Trichogramma brassicae (Hymenoptera: Trichogrammatidae) طی ۴۵ نسل

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چکیده: ترجیح میزبانی و رفتار سوئیچینگ زنبور پارازیتوئید (Bezdenko) *Trichogramma brassicae* روی دو گونه میزبان آزمایشگاهی بید آرد، Zeller *Ephestia kuehniella* و بید غلات، *Sitotroga cerealella* (Olivier) طی ۴۵ نسل مورد بررسی قرار گرفت. تخم‌های هر دو میزبان به‌مدت ۲۴ ساعت در اختیار زنبور پارازیتوئید قرار داده شد. در همه نسل‌های مورد آزمایش، میزان پارازیتیسم روی تخم‌های بید آرد به‌صورت معنی‌داری بیشتر از بید غلات بود ولی در همه آزمایش‌ها، رفتار سوئیچینگ منفی مشاهده شد. ترجیح زنبور پارازیتوئید از میزبان با فراوانی بیشتر به میزبان با فراوانی کمتر تغییر یافت (سوئیچینگ منفی). ضریب ترجیح (*c*) در تراکم مساوی میزبان‌ها، از ۲/۰۵۶ (در نسل ۵) تا ۱/۷۳۴ (در نسل ۴۵) برای بید آرد و از ۰/۴۸۶ (در نسل ۵) تا ۰/۵۷۷ (در نسل ۴۵) برای بید غلات به‌دست آمد. تجزیه رگرسیون خطی نشان داد که با وجود ترجیح بید آرد توسط زنبور پارازیتوئید، ضریب ترجیح این میزبان با گذشت نسل به‌صورت معنی‌داری کاهش یافت. رفتار سوئیچینگ منفی در زنبور پارازیتوئید *T. brassicae* ممکن است برای کنترل آفات در شرایط ویژه، مزیت نسبی محسوب شود.

واژگان کلیدی: کنترل بیولوژیک، رفتار کاوشگری، ترجیح میزبانی، پرورش انبوه، سوئیچینگ منفی