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# Functional Response of *Lysiphlebus testaceipes* (Cresson) (Hymenoptera Braconidae) Against *Aphis gossypii* Glover (Homoptera Aphididae) at Two Constant Temperatures (*)

## INTRODUCTION

*Lysiphlebus testaceipes* (Cresson) (Hymenoptera Braconidae) is a beneficial insect mass-reared by some biofactories to be released to control *Aphis gossypii* Glover (Rhynchota Aphididae) in protected crops. Its range of hosts includes most Aphidinae, eg. *Myzus* and *Brachycaudus* spp., *Macrosiphum* and related genera (van Steenis, 1995). The parasitoid was introduced from Cuba (via The Czech Republic) to southern France in 1973/74 to control the exotic aphids *Toxoptera aurantii* (Boyez de Fonscolombe) and *Aphis spiraecola* Patch (= *citricola* van der Goot) (on which it exhibits incomplete parasitism) (Starý *et al.*, 1988). It became established in Mediterranean France whence it spread east, south-east and west; it was found in Italy in 1977 by Tremblay *et al.* (1978). The presence of *L. testaceipes* in Spain since ca. 1982-84 is believed to be due to the spread of this species along the seashore from its release area in France; the parasitoid reached the southern coast of Portugal in 1985 (Costa and Starý, 1988). The rapid and vast dispersal of *L. testaceipes* in the Mediterranean region seemed to be achieved by: i) a genetic diversity which enables the parasitoid to parasitize numerous new host species, becoming an important control agent of indigenous pests; ii) the ability to compete with indigenous parasitoids and the tolerance to various habitats (Starý *et al.*, 1988).

Biological traits of *L. testaceipes* such as longevity, reproduction, development and intrinsic rate of increase are shown by van Steenis (1994).

For many aphid parasitoid species, functional response has been studied to evaluate the effect of the beneficial agent against the target pest (Hågvar and Hofsvang, 1991).

The functional response is the number of hosts successfully attacked per natural enemy (predator or parasitoid) as a function of prey or host density

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(*) Lavoro accettato il 18 gennaio 2000.

(Solomon, 1949). It describes the way a natural enemy responds to the changing abundance of its prey or host by killing or parasitizing more or fewer individuals, and it is a commonly measured attribute of natural enemies of pests.

Holling (1959) considered three types of functional responses. In type I the relationship between prey density and number of prey killed is linear, whereas in type II it is curvilinear and the saturation level is reached in a gradual way; type III is described by a sigmoid relationship and is considered a regulating factor in the population dynamics of the pest and natural enemy (van Lenteren and Bakker, 1976); thus, within certain limits of prey density, the enemies are able to control their prey or hosts without reacting numerically. The failure of natural enemies to keep the density below the economic threshold has been associated with a rapid increase in pest density which overwhelms the enemy's functional and numerical response (Murdoch *et al.*, 1985).

## MATERIALS AND METHODS

Insects *A. gossypii* was reared on *Cucurbita pepo* L. in a rearing cage (60x60x60 cm) inside a greenhouse of Istituto di Entomologia "G. Grandi", University of Bologna.

The *L. testaceipes* strain was provided by Bioplanet biofactory. To obtain experienced females of *L. testaceipes*, the following procedure was carried out: mummies of the parasitoid were put in vials with honey droplets and stored in a climatic chamber (T = 20°C or 28°C in relation to the experiment; 80% RH; photoperiod 16 L:8 D). The emerging females were mated and put for two hours in a plastic box containing a zucchini plant infested by *A. gossypii*. The experienced females were stored for 20 hours in a vial with some honey droplets before the experiment.

Functional response. A petri dish (diameter = 11.5 cm) was used as experimental arena. On the top of the petri dish a zucchini leaf (circa 4 cm diameter) with aphids was fixed by agar. Each experienced female of *L. testaceipes* was exposed to the following host densities: 5, 10, 25, 50 and 100 aphids/leaf (corresponding to 0.4, 0.8, 2, 4 and 8 specimens/cm$^2$). For each density ten females of *L. testaceipes* were individually observed for 30 minutes. For the experiments on functional response only second and third instar nymphs of *Aphis gossypii* Glover were used. After the experiments, the leaves with aphids were put in a petri dish with wet cotton wicks at 20°C for four days. Then all nymphs of *A. gossypii* were dissected with a micro bistoury to detect the larvae of *L. testaceipes*. This procedure was carried out for the experiments at 20 and 28°C.

Statistical analysis. To study the relationships between the number of hosts parasitized and the prey density, the models proposed by Hassell (1978) were used. Type II is described by the classical random parasite equation of Rogers (1972):

$$\mathrm{Na} = \mathrm{N} * \{1\text{-}\exp[(-a' * \mathrm{T})/(1+a' * Th * \mathrm{N})]\} \quad [1]$$

where Na is the number of hosts parasitized, N is the number of hosts exposed for the period T (day), a' the intrinsic rate of attack, *Th* the 'handling time' associated with each host parasitized; a' and Th are estimated by the regression. Type III is a sigmoid response that is described by the equation:

$$Na = a + c/\{1 + \exp[- b * (N - m)]\} \quad [2]$$

where $a$, $b$, $c$ and $m$ are parameters of the function; $a + c$ is the maximum number of aphids that can be parasitized at a given searching time and $m$ is the point of inflection.

To fit equations [1] and [2] the non-linear estimation procedure of the software STATISTICA for Windows (StatSoft™) was used.

Mann-Whitney U test was used to compare the hosts parasitized and the number of larvae observed at 20°C *vs.* 28°C. To compare ratios of females landing on leaves, the percentage of females contacting the host and percentage of females ovipositing at 20 *vs* 28°C, chi-square test in a 2x2 contingency table was used (Zar, 1984).

## RESULTS AND DISCUSSION

The ratio of females landing on leaves, the percentage of females contacting the host and the percentage of females ovipositing were significantly higher at 28°C *vs.* 20°C ($\chi^2$test, $P < 0.05$) at a density of 5 hosts/leaf (tab. 1). A higher value at 28°C *vs.* 20°C, very close to the significance level, was registered for the

Tab. 1. Ratios of *L. testaceipes* females landing on the leaf, contacting the host and parasitizing at different combinations of host densities and temperatures during half an hour.

| Behaviors | Host density | 20°C | 28°C | p |
|---|---|---|---|---|
| Females landing on the leaf | 5 | 0.4 | 0.9 | <0.05 |
| | 10 | 0.7 | 1 | >0.05 |
| | 25 | 0.8 | 0.9 | >0.05 |
| | 50 | 0.8 | 1 | >0.05 |
| | 100 | 0.9 | 0.9 | >0.05 |
| Females contacting host | 5 | 0.3 | 0.8 | <0.05 |
| | 10 | 0.6 | 0.8 | >0.05 |
| | 25 | 0.6 | 0.9 | >0.05 |
| | 50 | 0.7 | 1 | >0.05 |
| | 100 | 0.8 | 0.9 | >0.05 |
| Females parasitizing | 5 | 0.2 | 0.8 | <0.01 |
| | 10 | 0.5 | 0.8 | >0.05 |
| | 25 | 0.6 | 0.9 | >0.05 |
| | 50 | 0.5 | 0.9 | >0.05 |
| | 100 | 0.8 | 0.8 | >0.05 |

ratios of females landing on the leaves at density of 10 hosts/leaf, for the percentage of females contacting the host at a density of 50 hosts/leaf and for the percentage of females ovipositing at 50 hosts/leaf.

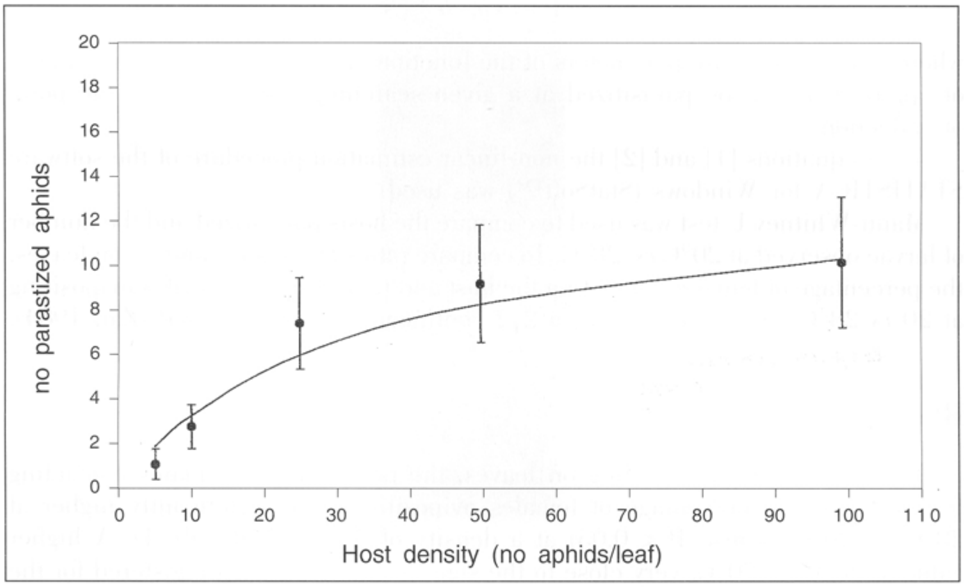

Fig. I. - Functional response of *L. testaceipes* at 20°C. Equation: $Na = N^* \{1-exp[(- 27.24*0.02)/(1+ 27.24*0.0015*N)]\}$; $R = 0.43$. Symbols are explained in the text. Bars represent standard errors.

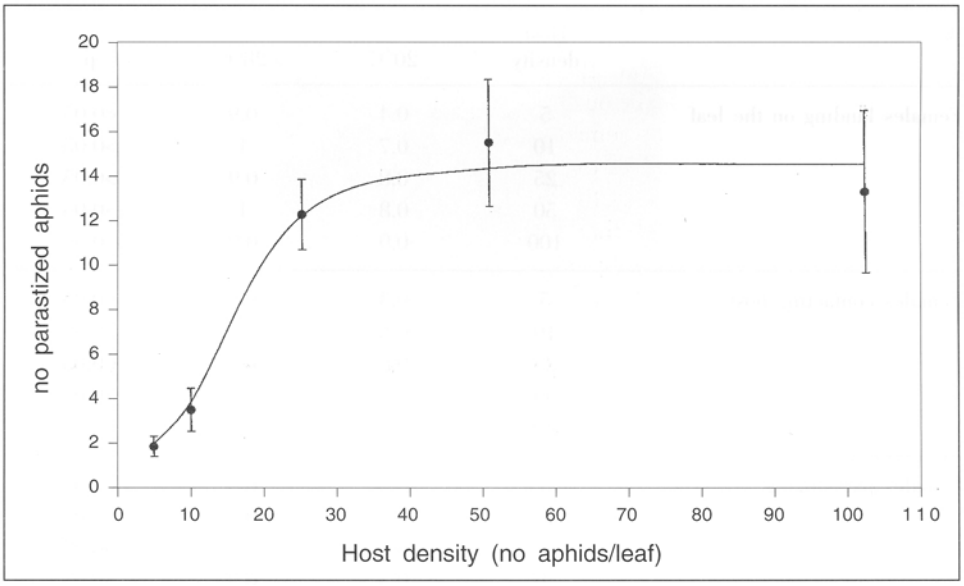

Fig. II - Functional response of *L. testaceipes* at 28°C. Equation: $Na = 0.30 + 13.95/\{1 + exp[- 0.19*(N - 15.84)]\}$; $R = 0.62$. Symbols are explained in the text. Bars represent standard errors.

Tab. 2. - Mean number of aphids parasitized by *L. testaceipes* and mean number of larvae of parasitoid (± se) at various host densities (n=10). Means followed by different letters (in horizontal level) indicate significant differences (Mann-Whitney U test, $P < 0.05$).

| Host densities/leaf | No. hosts parasitized | | No. parasitoid larvae | |
|---|---|---|---|---|
| | 20 °C | 28 °C | 20 °C | 28°C |
| 5 | 1.00 ± 0.70 a | 1.77 ± 0.46 a | 2.00 ± 0.14 a | 1.77 ± 1.46 a |
| 10 | 2.71 ± 1.01 a | 3.40 ± 0.96 a | 3.14 ± 1.18 a | 4.20 ± 1.13 a |
| 25 | 7.12 ± 2.13 a | 12.22 ± 1.60 b | 7.37 ± 1.85 a | 12.88 ± 1.79 b |
| 50 | 7.25 ± 2.70 a | 15.40 ± 2.99 b | 9.12 ± 2.37 a | 15.50 ± 3.04 a |
| 100 | 10.00 ± 2.99 a | 13.00 ± 3.70 a | 10.44 ± 3.28 a | 13.66 ± 4.19 a |

In general, increasing host density, *L. testaceipes* showed an increase in number of hosts parasitized (Tab. 2 and Figs. I-II) at both temperatures. Comparing the two temperature tested, *L. testaceipes* parasitized significantly more aphids at 28 °C than at 20°C for the densities of 25 and 50 hosts/leaf (Mann-Whitney U test, $P < 0.05$), no differences were found for the other densities tested. Also the mean number of larvae of the parasitoid was higher at 28°C *vs.* 20°C for densities of 25 and 50 aphids/leaf (Tab. 2). Some cases of superparasitism are recorded. This is probably also due to the experimental design: females are not allowed to disperse away from the host patch for 30 minutes.

At 20°C *L. testaceipes* showed a II type functional response, while at 28°C a type III (Figs I-II, during the observation period of 30 minutes. The equations showed R values of 0.62 for 28°C and 0.44 for 20°C. The maximum level (calculated by the equation models) of the number of parasitized aphids at 20 and 28°C were 10 and 14 respectively; the point of inflexion of the sigmoid model of parasitization (type III) at 28°C was 15.8 aphids/leaf (Fig. II).

Our results underline that the type of functional response can be strongly influenced by temperature. In our experiments the parasitism, the oviposition and in general the activity of the females were higher at 28°C in comparison to 20 °C for some densities tested. At the density of 100 aphids/leaf the behaviour of parasitism seems similar at both temperatures (not significant difference).

Messenger (1968) found that oviposition of the parasitoid *Praon exoletum* (Nees) was limited to mean temperatures between 8°C and 29°C; near these limits the maximum number of eggs laid and the maximum number of hosts attacked were low.

For many aphid parasitoid species a functional response was determined. In many cases, a type II response was found (Dransfield, 1979; Mackauer, 1983; Cloutier, 1984; Shu-Sheng, 1985) when the duration of exposure of the parasitoid to aphids was large compared to the number of aphids available (van Steenis, 1995): in this case, the parasitism level at lower densities are probably overestimated because of repeated visits to the leaf with hosts (van Lenteren and Bakker, 1976; Hassell *et al.*, 1977).

In other cases, a type III was found (Pandey *et al.*, 1982; 1984; Shirota *et al.*, 1983; Abidi *et al.*, 1987; Bhatt and Singh, 1991; Mishra and Singh, 1993; van

Steenis, 1995), mainly in petri dishes tests with short exposure times or with longer exposures times on a larger area (van Steenis, 1995).

Hofsvang and Hågvar (1983) studied the functional response of *Ephedrus cerasicola* Starý (Hymenoptera Braconidae) at three different time periods. The response during one hour was curvilinear and described by a type II; after 6 hours the response was linear (although it showed a slight sigmoid trend) and after 24 hr curvilinear of type II.

Shu-Sheng (1985) found changes in the functional response throughout the parasite's adult life and Bhatt and Singh (1991) studied *Trioxys indicus* Subba and Sharma (Hymenoptera Braconidae) *vs. A. gossypii* on three cucurbit food plants. Because the functional response is affected by the experimental conditions (time of exposure, age of parasitoids, shape and dimension of arena, species of plant used during the exposure, and temperature as demonstrated here), it appears difficult to compare the different results.

During the first visit *Aphidius colemani* Viereck (Hymenoptera Braconidae), a species commonly used in biological control, showed a type III functional response against *A. gossypii* (van Steenis, 1995), with a point of inflection at a density of 11.9 aphids, compared with 15.84 in our experiment; the maximum number of aphids attacked during the first visit in the experiment of van Steenis (1995) was 55.5: the author, considering a 80% of the attacks being successful (van Tol and van Steenis, 1994), estimated the maximum mean number of parasitized aphids as 45.5. In our experiment the number of aphids parasitized at high density was lower compared to the number of attacks registered by van Steenis (1995) for *A. colemani*, but we took to account aphids successful parasitized by dissecting the specimens of hosts.

The better performance of our *L. testaceipes* strain at 28 rather than 20°C seems to show that this strain is well adapted to high temperatures. In some cases the failure of *A. colemani* to control *A. gossypii* during the summer, might at least be partly caused by the higher temperatures (van Steenis, 1995) and/or hyperparasitoids (Burgio *et al.*, 1997). Field experiments of IPM Project of Emilia-Romagna Region show that the performance of *L. testaceipes* seems to be improved in protected tunnels during the summer, with an increase in mean temperatures (not published data).

The logistic type of functional response (type III) is theoretically considered to be a regulating factor in the population dynamics (Hassell, 1978) but other factors must be considered, such as numerical response and searching efficiency. For example, at a higher density, a strong numerical response may occur by aggregation at places with high densities (Hågvar and Hofsvang, 1987): consequently aphid colonies will be visited repeatedly and parasitism levels can become much higher than during one visit (more parasitoids result in heavier parasitation) (van Steenis, 1995). If the sigmoid response is considered a positive trait for a candidate in biological control, *L. testaceipes* showing a higher parasitism at 28°C in comparison with 20°C, seems more suitable as biological agent in protected crops during summer.

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## SUMMARY

In laboratory experiments we compared the functional response of *Lysiphlebus testaceipes* (Cresson) (Hymenoptera Braconidae) at two constant temperatures (20 *vs* 28°C) using *Aphis gossypii* Glover (Rhynchota Aphididae) as host. Ten experienced females were individually exposed for 30 minutes to 5, 10, 25, 50 and 100 aphid nymphs per leaf (corresponding to 0.4, 0.8, 2, 4, 8 specimens/cm$^2$) in a petridish. Four days after the aphid exposure all nymphs of *A. gossypii* were dissected to assess the parasitism by detecting larvae of the parasitoid. At 20°C *L. testaceipes* showed a II type functional response, but at 28°C a type III. The equations showed R values of 0.44 for 20°C and 0.62 for 28°C. The curve showed a plateau at 20 and 28°C for 10 and 14 aphids parasitized respectively; the point of inflexion of the sigmoid model of parasitism (type III) at 28°C was about 16 aphids/leaf. The max number of aphids parasitized was at the density of 100 aphids/leaf at 20°C and 50 aphids/leaf at 28°C. Comparing the two temperature tested, *L. testaceipes* parasitized significantly more aphids at 28°C than at 20°C for the densities of 25 and 50 hosts/leaf (Mann-Whitney test, $P < 0.05$). The percentage of females landing on leaves, the percentage of females contacting the host and the percentage of females ovipositing were significantly higher at 28°C *vs.* 20°C at a density of 5 hosts per leaf ($\chi^2$ test, $P < 0.05$).

KEY WORDS: *Lysiphlebus testaceipes*, *Aphis gossypii*, functional response, biological control.

## Risposta funzionale di *Lysiphlebus testaceipes* (Cresson) (Hymenoptera Braconidae) contro *Aphis gossypii* Glover (Homoptera Aphididae) a due temperature costanti

## RIASSUNTO

È stata confrontata la risposta funzionale di *Lysiphlebus testaceipes* (Cresson) (Hymenoptera Braconidae) a due temperature costanti (20 e 28°C) usando *Aphis gossypii* Glover (Rhynchota Aphididae) come ospite. Dieci femmine esperte del parassitoide sono state esposte individualmente per 30 minuti a 5, 10, 25, 50 e 100 neanidi di afide per foglia (corrispondenti a densità di 0,4; 0,8; 2; 4; 8 individui/cm$^2$ rispettivamente) in una capsula petri. La parassitizzazione veniva valutata tramite dissezione delle neanidi di *A. gossypii* quattro giorni dopo l'esposizione al parassitoide. A 20°C *L. testaceipes* ha mostrato una risposta funzionale di tipo II e a 28°C di tipo III. Le equazioni hanno registrato un valore del coefficiente di correlazione (R) di 0,44 a 20°C e 0,62 a 28°C. Le curve hanno rilevato un valore massimo a 20 e 28°C di 10 e 14 afidi parassitizzati rispettivamente, il punto di inflessione per il modello sigmoide (tipo III) a 28°C è stato di circa 16 afidi/foglia. Il numero massimo di afidi parassitizzati è stato rilevato alla densità di 100 afidi/foglia a 20°C e a quella di 50 afidi/foglia a 28°C. *L. testaceipes* ha parassitizzato un numero significativamente superiore di afidi a 28°C rispetto a 20°C alle densità di 25 e 50 afidi/ foglia (Mann-Whitney test, $P<0.05$). Non sono state rilevate differenze significative alle altre densità saggiate. La percentuale di femmine atterrate sulla foglia, di femmine che hanno avuto contatto con l'ospite e di femmine ovideponenti è risultata superiore a 28°C che a 20°C per la densità di 5 afidi/foglia ($\chi^2$ test, $P < 0.05$).

PAROLE CHIAVE: *Lysiphlebus testaceipes*, *Aphis gossypii*, risposta funzionale, lotta biologica.

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