

Functional Response of *Trichogramma evanescens* Parasitizing Tomato Leaf Miner, *Tuta absoluta* on Three Tomato Varieties

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ABSTRACT

The egg parasitoid, *Trichogramma evanescens* Westwood, is used for inundative releases in biological control programs against the tomato leaf miner, *Tuta absoluta* Meyrick, in agro-ecosystems. One of the most important host-parasitoid interactions is functional response that its type and parameters are affected by different factors including host plant characteristics. In this research, functional response of *T. evanescens* to different egg densities (2, 4, 8, 16, 32, and 64) of *T. absoluta* was investigated in a growth chamber (26±2°C, 50±10% RH, and 16:8 hours L:D photoperiod), on three varieties of tomato (Mobil, Riogrande, and Early Urbana-703). Type of functional response was determined by logistic regression and its parameters, i.e. searching efficiency and handling time, were calculated by nonlinear regression model using SAS software. Functional response of this parasitoid was type III on Mobil and Riogrande and type II on Early Urbana-703. Searching efficiency (a) for each host density was 0.0368, 0.0736, 0.1472, 0.2944, 0.5888, and 1.1776 on Mobil, and 0.0374, 0.0748, 0.1469, 0.2992, 0.5984, and 1.1968 h⁻¹ on Riogrande varieties, respectively. Also, on Early Urbana-703, the attack rate was 0.1045 h⁻¹. The handling time was 1.0276, 1.1017, and 1.3289 hours with maximum parasitism of 23.3, 21.7, and 18.06 eggs on these varieties, respectively. It is suggested that physical structure of Early Urbana-703 variety may interfere with the parasitoid's performance, resulting in an impaired searching efficiency and parasitism ability. Some applied aspects of the findings are discussed.

Keywords: Biological control, Host density, Search rate, Tomato moth, Trichogrammatids.

INTRODUCTION

The tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) that has been reported as quarantine pest in Iran since 2010, has become one of the most devastating pests of tomato crops both in the field and under protected conditions (Salek Ebrahimi and Gharekhani, 2014; Ghorbani *et al.*, 2015). It originates from South America and has subsequently spread throughout the Mediterranean Basin and

Europe (Erdogan and Babaroglu, 2014). *T. absoluta* is an oligophagous pest, which feeds on Solanaceous plant species and rapidly develops in favorable environmental conditions, with overlapping life cycles (Guenauoui *et al.*, 2010).

Appearance of *T. absoluta* has led to widespread insecticide use by tomato growers, potentially causing a multitude of undesired side effects on non-target organisms (Desneux *et al.*, 2010; Arno and Gabarra, 2011). To address these issues, natural enemies of the tomato leaf miner are

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increasingly being used in biological control programs designed to protect greenhouse crops (Desneux *et al.*, 2011; Amer *et al.*, 2012; Cagnotti *et al.*, 2012; Cocco *et al.*, 2013).

Egg parasitoids of the genus *Trichogramma* are the most widely used natural enemies in biological control programs, notably through inundative releases, against a range of agricultural pests mainly Lepidopterans (Faria *et al.*, 2008). Species of *Trichogramma* have some unique advantages that make them well-suited for use in the high visibility/low economic threshold systems characteristic of products (Guo, 1986; Chailleux *et al.*, 2013). These include: causing mortality to the egg stage prior to the damaging larval stage, commercial availability, and perhaps most importantly, tiny body size (< 0.5 mm in length), making them unlikely to be noticed by consumers. Among them, an egg parasitoid, *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae) has been used successfully and commercially in the retail trade and the food processing industry in Central Europe to control stored-product moths, mainly *Plodia interpunctella* (Hübner), *Ephestia kuehniella* (Zeller) and *E. elutella* (Hübner) (Gingras and Boivin, 2002; Adarkwah *et al.*, 2015).

Biological control agents are often first tested under laboratory conditions to evaluate their potential for success (Kalyebi *et al.*, 2005). An essential element of dynamics of host-parasitoid association and an important determinant of the stability of the system is the functional response. Functional response tests show the potential of parasitoid ability to suppress the different density of host (Moezipour *et al.*, 2008). Holling (1959) considered three types of functional responses which are determined by the number of killed host raised linearly (type I), curvilinearly (type II), or sigmoidally (type III). Type I (Mills and Lacan, 2004) and II functional responses (Smith, 1996) and even type III have also been reported for *Trichogramma* species

(Wang and Ferro, 1998; Moezipour *et al.*, 2008; Chamani, 2015; Nikbin, *et al.*, 2014). Chamani (2015) reported type III of functional responses for *T. evanescens* at different density of *E. kuehniella*.

The type and rate of a functional response are affected not only by the interaction between parasitoid and host but are also mediated by substrate characteristics. In general, both morphological and biochemical aspects of host plants directly and/or indirectly influence the third trophic level. These effects may be physical (such as trichomes, tissue roughness) or chemical (such as toxins and digestibility reducers) (Price *et al.*, 1980). Host plant surface properties such as trichomes density and shapes may affect the search patterns and foraging behavior of parasitoids and, consequently, changing the searching efficiency may be effective on the functional response of the parasitoids. These effects have been addressed by some authors (Messina and Hanks, 1998; Krips *et al.*, 1999; DeClercq *et al.*, 2000; Karami Jamur and Shishehbor, 2012). Despite many literatures dealing with the effect of plant physical traits on searching efficiency of parasitoids, the effect of leaf surface physical traits on parasitism of *T. evanescens* has not been addressed so far. Therefore, in this study, we aimed to find and compare the effect of three varieties of tomato (Mobil, Rio Grande and Early Urbana-703) on the ability and performance of *T. evanescens* in parasitizing the host eggs, in order to clarify parasitoid–host–plant interactions.

MATERIALS AND METHODS

Host and Parasitoid Cultures

The colony of *Tota absoluta* was established from insects collected in a tomato (*Lycopersicon esculentum* Mill) commercial plantation in Shoshtar (Iran). Adults were kept in mesh netting cages (40×40×40 cm) and fed with a 10% honey solution. Tomato

plants were provided for egg laying of *T. absoluta*.

Eggs of *T. absoluta* parasitized by *T. evanescens* were obtained from Biological Control Research Department (BCRD) of the Iranian Research Institute of Plant Protection (IRIPP). After the emergence of adults, they were reared on eggs of a substitute host, *E. kuehniella*. Rearing was carried out in glass tubes (4.5 cm in length and 0.7 cm in diameter), the parasitoids were fed on honey droplets. *E. kuehniella* eggs were glued on a small strip of paper cards (1.2×6 cm) with 20% diluted honey solution. Parasitoids were maintained for at least one generation in an incubator at 26±2°C, 50±10% RH, and 16:8 hours L:D photoperiod on *E. kuehniella* eggs before starting the experiments. Females and males used in the experiments were 24 hours old.

Functional Response

To study the functional responses of *T. evanescens*, different egg densities (2, 4, 8, 16, 32, and 64) of *T. absoluta* were used. Each host density was replicated 10 times. For each host density, eggs of *T. absoluta* (< 24 hours) were placed on a small leaf of tomato and inserted into glass vials (12×100 mm). One-day-old mated female wasps (fed on drop of 20% honey/water) were exposed to different density levels of *T. absoluta* eggs. After 24 hours, the female parasitoids were removed and host egg were maintained under 26±2 °C, 50±10% RH, and 16:8 hours L: D photoperiod condition. To determine the number of parasitized egg, the number of black eggs was counted. These experiments were conducted on three varieties of tomato, separately. Three varieties (Mobil, Rio Grande and Early Urbana-703) were tested.

Statistical Analysis

A logistic regression proportion of parasitized host versus the initial number of hosts offered, (N_a / N_0) is usually

recommended (Juliano, 2001). Therefore, a polynomial function was fitted as follows:

$$\frac{N_a}{N_0} = \frac{\exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)}{1 + \exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)} \quad (1)$$

Where, N_a is the number of parasitized hosts and N_0 is initial host density. P_0 , P_1 , P_2 and P_3 are the intercept, linear, quadratic, and cubic coefficients, respectively. These parameters were estimated using the CATMOD procedure in SAS software (Juliano, 2001, SAS Institute, Inc. 2004). The sign of P_1 and P_2 was used to distinguish the shape of the curves. A positive linear parameter (P_1) indicates that the functional response is type III, whereas the functional response is type II when linear parameter is negative.

As the host densities were depleted during the experiment, non-linear least square regression was used for estimating the parameters of the functional response, (NLIN procedure, SAS Institute Inc. 2004) based on Rogers (1972) random parasitoid equation (Equation 2):

$$N_a = N_0 \{1 - \exp[a(T_h N_0 - T)]\} \quad (2)$$

Where, N_a is the Number of host attacked, N_0 the initial Number of hosts, T is the Time of exposure (24 hours), a the rate of successful attack, and T_h the handling Time.

For a type III response, a is assumed to increase with host density according to the Equation (3):

$$a = (d + bN_0) / (1 + cN_0) \quad (3)$$

Where, b , c , and d are constants (Hassell, 1982). In cases where both d and c are not significantly different from 0, reduced type III equations were used (Equation 4):

$$N_a = N_0 \{1 - \exp[(d + bN_0)(T_h N_a - T)(1 - cN_0)]\} \quad (4)$$

Thus, for each host density, the attack coefficient (a) could be found as $a = bN_0$.

Pairwise comparisons of functional response's parameters for all possible pairs of treatments were performed by using the indicator variable method (Juliano, 2001) as follows:



$$N_a = \frac{\exp[b+D_b(j)]N_0^2T}{1+\exp[b+D_b(j)]N_0^2[T_h+D_{T_h}(j)]} \quad (5)$$

Where, j is an indicator variable which takes value 0 for the first treatment and 1 is for the second treatment. The parameters D_b and D_{T_h} indicate the Difference between b and T_h parameters in different treatments, respectively (Juliano, 2001; Allahyari et al., 2004). To find a difference between two handling times, it must be proved that D_{T_h} is a significant number, and it is not equal to zero. If D_{T_h} is not significantly different from zero, the difference between T_h and $T_h+D_{T_h}$ is not significant and the two handling times are not statistically different (Juliano, 2001). The coefficient of determination was calculated as: $r^2 = 1 - \text{Residual sum of squares} / \text{Corrected total sum of squares}$ (Messina and Hanks, 1998).

RESULTS

The outcome of logistic regression analysis of the proportion of hosts parasitized by female adults of *T. evanescens* on different varieties is presented in Table 1. It shows that the functional response of a female parasitoid on Mobil and Rio Grande varieties is type III, as the sign of the linear term is positive in both cases, and on Early Urbana-703 is type II, as the sign of the linear term is negative (Table 1). Functional response curves of female adults and

percentage of parasitism to various densities of host eggs on different varieties are shown in Figure 1.

Table 2 shows the estimated parameters of functional response. Results of nonlinear least square regression indicated that parameters c and d were not significantly different from zero; therefore, we eliminated them from the model and the searching efficiency (a) reduced model was used. Estimated b value, on Mobil and Rio Grande were 0.0184 ± 0.0031 and 0.0187 ± 0.0029 , respectively, and the estimated handling Times (T_h) in these two varieties were 1.0276 ± 0.0246 and 1.1017 ± 0.0263 , respectively (Table 2). Thus, for each host density (2, 4, 8, 16, 32 and 64), the searching efficiency (a) estimated by the equation ($a = bN_0$) was 0.0368, 0.0736, 0.1472, 0.2944, 0.5888 and 1.1776 on Mobil and 0.0374, 0.0748, 0.1469, 0.2992, 0.5984 and 1.1968 h^{-1} on Rio Grande variety, respectively. Estimated confidence interval for D_b and D_{T_h} in different data sets can be used to compare parameters b and T_h . Data analyses using Equation (5) indicated that D_b included 0 and there was no significant difference between b values on these two varieties, but that of D_{T_h} was greater than 0, showing that handling times for Rio Grande were significantly longer than those for Mobil variety (Table 3). Also, on Early Urbana-703, the attack rate (a) ($0.1045 \pm 0.0150 h^{-1}$) and the handling Time

Table1. Results of logistic regression analysis of the proportion of parasitized eggs by *Trichogramma evanescens*, against initial number of eggs on three varieties of tomato.

| Varieties | Coefficient | Estimate | SE | X ² value | P value |
|------------------|-------------|----------|---------|----------------------|---------|
| Mobil | Constant | 1.22 | 0.63 | 3.76 | 0.0524 |
| | Linear | 0.31 | 0.108 | 8.54 | 0.0035 |
| | Quadratic | -0.015 | 0.004 | 12.94 | 0.0003 |
| | Cubic | 0.00015 | 0.00004 | 13.38 | 0.0003 |
| Rio Grande | Constant | 0.96 | 0.56 | 2.93 | 0.0868 |
| | Linear | 0.30 | 0.095 | 9.86 | 0.0017 |
| | Quadratic | -0.014 | 0.003 | 14.64 | 0.0001 |
| | Cubic | 0.00014 | 0.00003 | 14.96 | 0.0001 |
| Early Urbana-703 | Constant | 3.09 | 0.5926 | 27.24 | <0.0001 |
| | Linear | -0.23 | 0.0794 | 8.78 | 0.003 |
| | Quadratic | 0.005 | 0.0028 | 3.84 | 0.05 |
| | Cubic | -0.00004 | 0.00002 | 2.76 | 0.0967 |

Table 2. Estimated parameters for the random parasitoid equation, functional response of *Trichogramma evanescens* to densities of *Tuta absoluta* eggs on three varieties of tomato.

| Varieties/ Parameters | Estimate | Asymptotic SE | 95% (Lower) | CI ^a | 95% (Upper) | CI ^a | T/T _h | r ² |
|--------------------------|----------|---------------|----------------|-----------------|----------------|-----------------|------------------|----------------|
| Mobil | | | | | | | 23.3 | 0.984 |
| <i>b</i> | 0.0184 | 0.0031 | 0.0121 | | 0.0247 | | | |
| <i>T_h</i> | 1.0276 | 0.0246 | 0.9783 | | 1.0769 | | | |
| Rio Grande | | | | | | | 21.7 | 0.983 |
| <i>b</i> | 0.0187 | 0.0029 | 0.0128 | | 0.0246 | | | |
| <i>T_h</i> | 1.1017 | 0.0263 | 1.0491 | | 1.1542 | | | |
| Early Urbana-703 | | | | | | | 18.06 | 0.976 |
| <i>a</i> | 0.1045 | 0.0150 | 0.0744 | | 0.1346 | | | |
| <i>T_h</i> | 1.3289 | 0.0576 | 1.2137 | | 1.4442 | | | |

^a CI: Confidence Interval.

(*T_h*) (1.3289±0.0576 h) were estimated from the Roger's random parasitoid equation using a nonlinear least square regression (Table 2). The estimated maximum rate of parasitism (T/*T_h*) on Mobil, Rio Grande, and Early Urbana-703 determined as 23.3, 21.7, and 18.06 eggs, respectively (Table 2). The coefficient of determination (*r*²) indicated that the random parasitoid equation adequately describes the functional response of *T. evanescens*. The *r*² values for Mobil, Rio Grande, and Early Urbana-703 were 0.98, 0.98, and 0.97, respectively (Table 2).

DISCUSSION

Functional response is useful in evaluating potential biocontrol candidates and, among the behavioral activities, it is widely used to evaluate the host-parasitoid interactions

(Savino, *et al.*, 2012). In laboratory studies, the type of functional response of parasitoid/predator may change from one type to another as environmental conditions change (Wang and Ferro, 1998). Female parasitoids need to find suitable hosts to reproduce and, because of the direct link between successful host finding and offspring production, the parasitoid foraging behavior is expected to be strongly influenced by natural selection (Gingras and Boivin, 2002).

The type of functional response of *T. evanescens* and its parameters such as searching efficiency and handling time was influenced by varieties of *L. esculentum*. The type III functional response, corresponding to a sigmoid type curve, was observed for *T. evanescens* on Mobil and Rio Grande varieties, and type II functional

Table 3. Parameters estimated by an equation with indicator variable for comparing functional response parameters of *Trichogramma evanescens* between Mobil and Rio Grande.

| Parameters ^a | Estimate | Asymptotic SE | 95% CI ^b (Lower) | 95% CI ^b (Upper) |
|----------------------------------|----------|---------------|-----------------------------|-----------------------------|
| <i>D_b</i> | 0.00028 | 0.00431 | - 0.0082 | 0.0088 |
| <i>D_{T_h}</i> | 0.0741 | 0.0361 | 0.0026 | 0.1455 |

^a *D_b*: indicator variable estimates the differences between the treatments in the value of the parameter *b*, *D_{T_h}*: indicator variable estimates the differences between the treatments in the value of the parameter *T_h*.

^b CI: Confidence Interval. Significant difference of parameters shown in bold face.

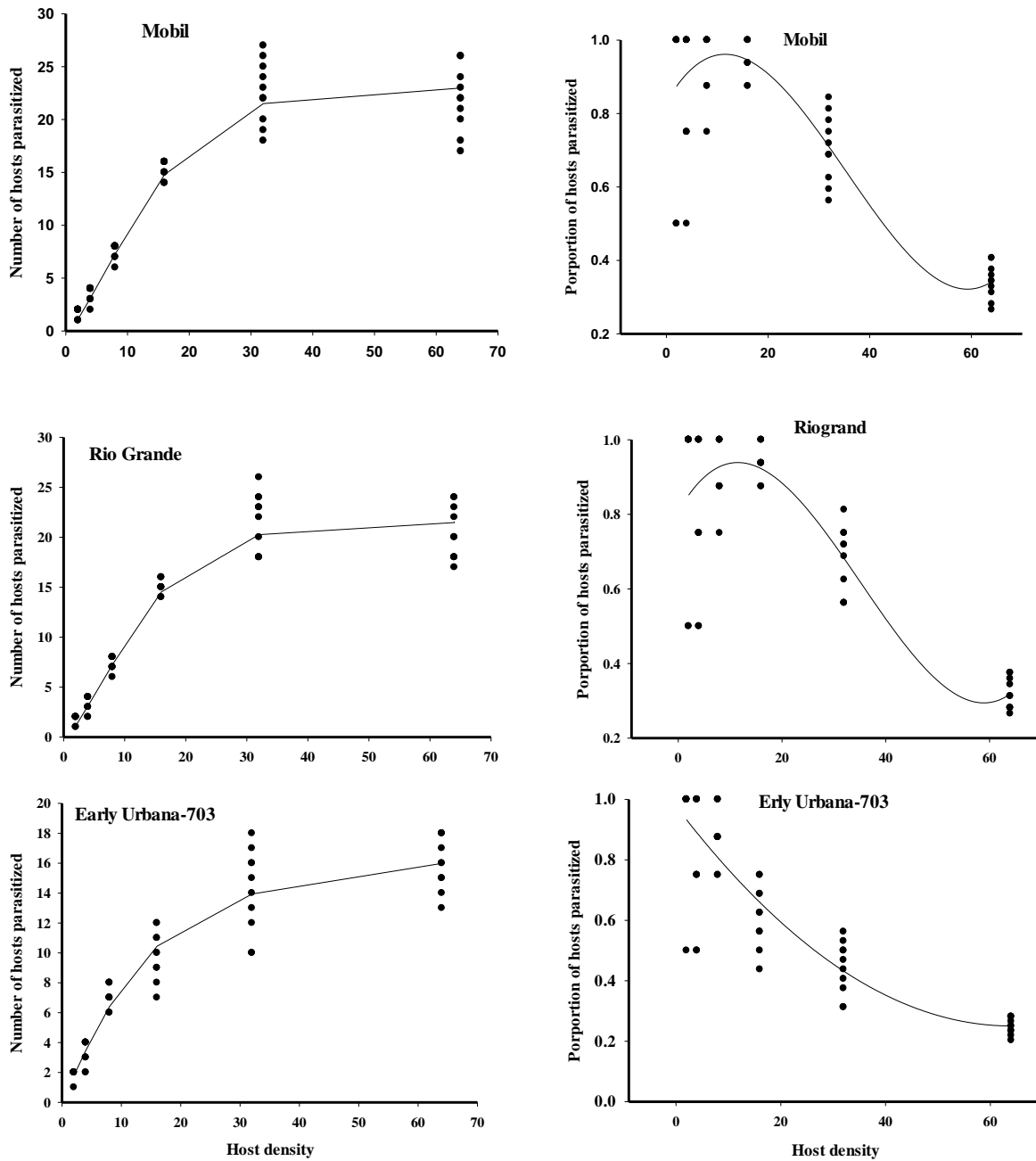


Figure 1. Functional responses of *Trichogramma evanescens* in different densities of *Tuta absoluta* eggs on three varieties of tomato. Left: Number of hosts parasitized (Symbols are observed data and lines are predicted by the model), and Right: Proportion of hosts parasitized.

response was on Early Urbana-703. Species of *Trichogramma*, under different laboratory conditions, exhibit either the type III (ArbabTafti *et al.*, 2004; Reay Jones *et al.*, 2006; Moezipour *et al.*, 2008; Nikbin *et al.*, 2014; Chamani *et al.*, 2015), or a type II functional response (Reay-Jones *et al.*, 2006; Moezipour *et al.*, 2008; Farrokhi *et al.*, 2010; Nikbin *et al.*, 2014). This difference among studies may be attributed partly to the range of host densities or to the searching area available to the parasitoid.

Foraging success of *Trichogramma* spp. can be influenced by other plant parts or plant characteristics such as plant surface area and plant height (Monteith, 1960, Thorpe, 1985). Physical characteristics of plants can thus mediate ecological interactions and influence parasitoid-host population dynamics (Gingras and Boivin, 2002). Some studies on the role of different trichome style in the resistance of tomato varieties to *T. absoluta* indicated that the highest preference was observed on Mobil and the lowest on Early Urbana (Javadi Khedri *et al.*, 2014). Moreover, trichome type and density of the assayed varieties appeared to be related to population density of tomato leaf miner. The most infested genotype (Mobil) displayed the lowest IV and VI trichomes style ranks of leaf blade, vein and domatia (glandular trichomes). Thus, significant negative relations were found between larvae and adult density with IV and V style trichome (Javadi Khedri *et al.*, 2014; Fathi *et al.*, 2015). According to the results of Ghorbani (2015), the Mobil and then Rio Grande had the highest while Early Urbana-703 had the lowest nutrition utility to the tomato leaf miner, respectively. Thus, the Mobil variety was the sensitive host to the increase of population of this pest and also, its parasitoid, *T. evanescens*, had higher performance on Mobil and Rio Grande.

In this study, the highest searching efficiency and the lowest handling time was observed on Rio Grande. In other words, this parasitoid spent more time handling the host on Early Urbana-703. Unsuccessfully

foraging efficiency of *T. evanescens* on Early Urbana-703 may be related to the physical and chemical properties of the host plant. Glandular trichomes on this variety may have acted as impediment factors for successful search and parasitism and acted as shelter for protecting the hosts. In addition, plant allelochemicals may also have influenced the parasitoid's performance (Gingras and Boivin, 2002). Gingras and Boivin (2002) reported that plant structure affects rate of egg discovery of *T. evanescens*, which is higher on simple plants and decreases as plant increases in complexity. Furthermore, level of parasitism increased when host egg density increased, which can be seen as an effect of functional response (Gingras and Boivin, 2002).

It is assumed that parasitoids showing a type III functional response have better opportunities to regulate their host populations than type II parasitoids (Nikbin *et al.*, 2014). Holling (1965) postulated that stable equilibrium densities and damping of population oscillations would be more likely with type III rather than type II functional responses. Other investigators have similarly postulated that a parasitoid with a type III functional response could contribute more to regulation of host density than a parasitoid with a type II functional response (Murdoch, 1969). The genus of *Trichogramma* possibly possess a learning ability to discriminate between parasitized and unparasitized eggs through experience (Wang and Ferro, 1998) which may result in type III response. Bjorksten and Hoffmann (1998) suggested that oviposition experience had a stronger effect on host preference than pre-adult experience (learning through development in rearing host). Another postulated mechanism producing type III functional responses is concentration of host parasitizing effort in high-density patches (Faria *et al.*, 2008).

Our results address the dearth of information about host plant-parasitoid interactions, which have not been studied extensively (Gingras and Boivin, 2002). The obtained information in this study may be useful for evaluation of *T. evanescens* as a



candidate for the biological control of tomato leaf miner, especially on Mobile and Rio Grande varieties with the highest density of *T. absoluta*. Therefore, to achieve an efficient control, according to the highest value of a (searching ability of parasitoid) and the lowest value of T_h , it is recommended that the Rio Grande be used as relatively resistant variety against tomato leaf miner and suitable for performance of *T. evanescens* in greenhouses (Faria et al., 2008).

Other characteristics such as a short handling time and a tendency to aggregate in areas where hosts are common, a high intrinsic rate of natural increase, female parasitoid's age as well as host traits, biotic and abiotic factors, may influence the behavioral aspects and efficiency of natural enemies (Davoodi Dehkordi and Sahragard, 2013). Routinely, functional response experiments, like the present study, are carried out in controlled conditions. However, it is obvious that, in the field conditions, natural enemies encounter unstable and highly variable conditions. On the other hand, it is difficult to predict the natural enemies' reactions to these changes. Therefore, it would be necessary to measure functional response of parasitoid in a condition closer to that of fields. Our results suggest additional work to confirm whether effects are representative of field conditions.

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واکنش تابعی *Trichogramma evanescens* پارازیتوئید تخم مینوز گوجه‌فرنگی
Tuta absoluta روی سه وارپته گوجه‌فرنگی

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چکیده

زنبور پارازیتوئید تخم، *Trichogramma evanescens* Westwood در برنامه‌های کنترل بیولوژیکی علیه مینوز گوجه‌فرنگی، *Tuta absoluta* Meyrick در اکوسیستم‌های کشاورزی مورد استفاده قرار می‌گیرد. یکی از مهم‌ترین روابط میزبان-پارازیتوئید، واکنش تابعی می‌باشد که نوع و پارامترهای آن تحت تاثیر عوامل مختلفی از جمله ویژگی‌های گیاه میزبان قرار می‌گیرد. واکنش تابعی زنبور *T. evanescens* در تراکم‌های مختلف (۲، ۴، ۸، ۱۶، ۳۲ و ۶۴) تخم *T. absoluta* روی سه وارپته گوجه‌فرنگی (مبیل، ریوگراند و ارلی یوربانا-۷۰۳) در شرایط 26 ± 2 درجه سلسیوس، رطوبت نسبی 50 ± 10 درصد و نسبت روشنایی به تاریکی ۱۶:۸ مطالعه شد. نوع واکنش تابعی از طریق رگرسیون لجستیک و پارامترهای آن (قدرت جستجوگری و زمان دستیابی) از طریق مدل رگرسیون غیر خطی و با استفاده از نرم افزار SAS محاسبه گردید. واکنش تابعی زنبور روی وارپته‌های مبیل و ریوگراند از نوع سوم و روی ارلی یوربانا-۷۰۳ از نوع دوم بود. قدرت جستجوگری (a) برای هر تراکم میزبان با استفاده از معادله ($a = bNo$) به ترتیب ۰/۰۳۶۸، ۰/۰۷۳۶، ۰/۱۴۷۲، ۰/۲۹۴۴، ۰/۵۸۸۸ و ۱/۱۷۷۶ روی رقم مبیل و ۰/۰۳۷۴، ۰/۰۷۴۸، ۰/۱۴۶۹، ۰/۲۹۹۲، ۰/۵۹۸۴ و ۱/۱۹۶۸ بر ساعت بر روی رقم ریوگراند به دست آمد. همچنین نرخ حمله روی رقم ارلی یوربانا-۷۰۳، ۰/۱۰۴۵ بر ساعت بود. زمان دستیابی روی این وارپته‌ها به ترتیب ۱/۰۲۷۶، ۱/۱۰۱۷ و ۱/۳۲۸۹ ساعت با حداکثر نرخ پارازیتیسیم ۲۳/۳، ۲۱/۷ و ۱۸/۰۶ تخم می‌باشد. پیشنهاد می‌شود که ساختار فیزیکی رقم ارلی یوربانا-۷۰۳ ممکن است در عملکرد پارازیتوئید تداخل ایجاد کند و منجر به اختلال در قدرت جستجوگری و توانایی پارازیتیسیم آن شود. برخی جنبه‌های کاربردی این نتایج مورد بحث است.