Influence of Genetically Manipulated Brassica Genotypes on Parasitism Capacity of Diadegma semiclausum Parasitizing Plutella xylostella

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ABSTRACT

Plant quality in herbivores’ diet may affect the performance of both herbivore and its parasitoids. In the present research, parasitism capacity of Diadegma semiclausum (Hellen) on Plutella xylostella (L.) reared on different genetically manipulated Brassica plants including the canola’s progenitor (Brassica rapa L.), two cultivated canola cultivars (Cultivar-Opera and Cultivar-RGS003), one hybrid (Hybrid-Hyula401), one gamma mutated (Mutant-RGS003), and one transgenic (Transgenic-PF) genotype was determined. All experiments were carried out in a growth chamber at 25±1˚C, 65±5% RH, and a photoperiod of 16:8 (L: D) hour. The value of the net parasitism rate (C₀) of D. semiclausum was 14.94, 20.12, 14.95, 12.20, 13.94, and 12.55 hosts on B. rapa, Cultivar-Opera, Cultivar-RGS003, Hybrid-Hyula401, Mutant-RGS003, and Transgenic-PF, respectively. The transformation rate from host population to parasitoid offspring (Q₀) on all genotypes was close to 1 (C₀≅ R₀). Moreover, the value of the finite parasitism rate (α) was 0.271, 0.285, 0.277, 0.202, 0.202, and 0.202 host parasitoid⁻¹ day⁻¹ on the above-mentioned genotypes, respectively. The finite parasitism rate considers the finite rate of increase, the stable age-stage distribution, and the age-stage specific parasitism rate; therefore, this parameter could be used to assess the efficiency of a parasitoid. In conclusion, D. semiclausum had higher parasitism capacity on canola’s progenitor and the cultivated genotypes which were more suitable for parasitoid’s host based on secondary metabolites concentration.

Keywords: Diamondback moth, Finite parasitism rate, Manipulated canola, Plant chemistry, Two-sex parasitism.

INTRODUCTION

The Diamondback Moth (DBM), Plutella xylostella (L.) (Lepidoptera: Plutellidae) is considered as one of the most important global pests of crucifer crops for several decades. In the recent decade, P. xylostella has caused major problems on Brassica crops in different parts of the world (Golizadeh et al., 2009), mostly due to the increase in levels of resistance to different classes of insecticides even to Bacillus thuringiensis formulations (Shelton et al., 1993, Soufbaf et al., 2010a, b). Therefore, it is crucial to apply a new management tool for its control. Previous studies showed that biological control agents play a major role in regulating the population of this pest (Golizadeh, et al., 2008; Sarfraz et al., 2009; Soufbaf et al., 2012).

Diadegma semiclausum (Hellen) (Hymenoptera: Ichneumonidae), which is a solitary and host-specific larval endoparasitoid, is known as an important biological control agent of P. xylostella (Sarfraz et al., 2005). This parasitoid shows
an excellent search capacity and has been studied as a potential biological control agent in integrated management programs for the diamondback moth (Harcourt, 1960).

Life table parameters are powerful tools for analyzing and understanding the impact of an external factor (e.g., host plant quality and temperature) on the growth, survival rate, reproduction, and increasing rate of a biocontrol agent population (Taghizadeh et al., 2008; Pakyari et al., 2011). Our previous studies revealed profound effect of genetically manipulated canola genotypes on life table parameters of *P. xylostella* (Nikooei et al., 2015) and its parasitoid *D. semiclausum* (unpublished data). Some life table parameters such as the intrinsic rate of increase (*r*) are suitable indices for describing and comparing growth potentials of populations. Since the intrinsic rate of increase cannot alone interpret the parasitism/predation potentials of parasitoids/predators, a standard parameter is needed for description of the capacity of a parasitoid/predator in biological control programs (Chi et al., 2011).

Accordingly, Chi et al. (2011) defined the finite parasitism/predation rate (ω) as a standard parameter linking the finite rate of increase (λ), the stable age-stage distribution (a_{xj}), and the age-stage specific parasitism/predation rate (c_{xj}). The finite parasitism rate considered both the increase rate of parasitoid and the age-stage specific parasitism rate and then it would be capable to describe and compare the parasitism potential of the natural enemies. To compare parasitism/predation capacity among different parasitoids/predators under the same condition or capacity of a parasitoid/predator under different conditions, the finite parasitism/predation rate can be an efficient tool (Yu et al., 2013).

It has been demonstrated that plant quality in herbivores’ diet may not only affect the performance of the herbivore but also that of its natural enemies (Ode, 2006; Harvey et al., 2007; Gols et al., 2007; 2008a, b). Here, we selected *Brassica rapa* L. as a canola’s progenitor, two cultivated canola cultivars (Cultivar-Opera and Cultivar-RGS03), one hybrid (Hyula401), one genetically mutated with gamma radiation (Mutant-RGS03), and one transgenic (Transgenic-PF) genotype which differ dramatically in their degree of manipulation. We previously showed that these genotypes had significant effects on life table parameters and fitness of *D. semiclausum* (unpublished data). Since this parasitoid is an effective biological control agent of *P. xylostella*, the present study was carried out to reveal the effect of these genotypes on parasitism capacity of this parasitoid parasitizing *P. xylostella*.

**MATERIALS AND METHODS**

**Plants**

One of the progenitors of canola (*B. rapa*) as a wild genotype and five genotypes of canola including two cultivars (Opera and RGS03), one hybrid (Hyula401), one gamma mutated (Mutant-RGS03), and one transgenic (Transgenic-PF) were used in the experiments. The seeds were planted in plastic boxes (90×50×30 cm) containing sandy loam soil, peat, and perlite (1:1:1). Twenty seeds were sown in each box. All plants were grown in a climate-controlled growth chamber at 25±1˚C, 65±5% RH, and a photoperiod of 16:8 (L: D) hour without any fertilizer and pesticides.

**Insects**

*Plutella xylostella* moths were originally collected from *Brassica* fields in Tehran province, Iran, during May 2012. Separated colonies of *P. xylostella* were kept on each host plant in ventilated cages (90x80x70 cm). The stock culture was maintained for about two months in the greenhouse. Sub-colonies were established on six plant genotypes separately and maintained in a constant environment at 25±1˚C on the respective host plants for more than three
generations before the trials. At least 130 pairs of moths were used to initiate the colony and 20 wild adult males and females, collected from the field, were added to each colony (stock culture and sub-colonies) weekly.

A potted canola plant with one cohort of 200 early-third instar larvae of P. xylostella was placed in a Perspex cage (30×30×30 cm) with a muslin sleeve on one side of the cage. Ten pairs of 2-day-old mated D. semiclausum were introduced into the cage. The wasps were provided with a diet of 10% honey solution. After 24 hours, the exposed larvae were removed and placed in ventilated plastic containers (20×15×7 cm). Also, fresh canola leaves were added until pupation. Plutella xylostella pupa could not form in case of parasitized larva, so the parasitoid pupae were harvested and put in clean plastic containers for adult emergence. The D. semiclausum culture was established and maintained in a constant environment at 25±1°C, and adults of the second generation were used in the experiments.

Parasitism Capacity of D. semiclausum

All experiments were carried out in a growth chamber set at 25±1°C, 60±5% RH, and a photoperiod of 16:8 (L: D) hour. To evaluate parasitism capacity, when the parasitoid adults emerged, they were sexed, paired, and kept in individual containers (6 cm in diameter, 5 cm in height) with a fresh leaf from the respective host plants and 20 third-instar larvae of the diamondback moth. A piece of cotton wool soaked with 20% honey solution was supplied as food to the adults. After 24 hours, the parasitoids were transferred to a new container with another 20 third-instar larvae of the diamondback moth. The daily observations continued until the death of the last individual.

Parasitism Capacity Analysis

The data obtained from daily parasitism of total cohort were used to calculate the age-stage specific parasitism rate ($c_{ij}$). The age-stage specific parasitism rate $c_{ij}$ is defined as the number of $P$. xylostella larvae parasitized by a parasitoid of age $x$ and stage $j$. Because only females can oviposit in their hosts, there is only a single curve of $c_{ij}$ The age-specific parasitism rate ($k_x$) is the mean number of $P$. xylostella larvae parasitized by $D$. semiclausum at age $x$ and was calculated by the following formula proposed by Chi and Yang (2003):

$$k_x = \frac{\sum_{j=1}^{\infty} s_{xj} c_{xj}}{\sum_{j=1}^{\infty} s_{xj}}$$

Where, $\beta$ is the number representing the life stage and $s_{xj}$ is the age-stage specific survival rate (where $x=$ Age in days and $j=$ Stage). In addition, the age-specific net parasitism rate ($q_x$) was calculated as follows:

$$q_x = l_x k_x$$

According to Chi and Yang (2003), the net parasitism rate ($C_0$) gives the mean number of host parasitized by an average individual parasitoid during its entire life span, and is calculated as:

$$C_0 = \sum_{x=0}^{\infty} \sum_{j=1}^{\infty} s_{xj} c_{xj}$$

According to these equations, the total number of parasitized hosts by a cohort of size $N$ is calculated as $NC_0$. $Q_p$ is the transformation rate from host population to parasitoid offspring. In other words, $Q_p$ is the proportion of the net parasitism rate to the net reproductive rate, and is calculated as:

$$Q_p = \frac{C_0}{R_0}$$

The stable parasitism rate ($\psi$) is the total parasitism capacity of a stable population, of which the total size is one (Chi et al., 2011), and is calculated as follows:

$$\psi = \sum_{x=0}^{\infty} \sum_{j=1}^{\infty} a_{xj} c_{xj}$$

Where, $a_{xj}$ is the proportion of individuals belonging to age $x$ and stage $j$ in a stable age-stage distribution.

Because the parasitoid population will increase at the finite rate $\lambda$, the total number of parasitized hosts will be increased at the rate of $\lambda \psi$. The finite parasitism rate ($\lambda \psi = \omega$) describes the parasitism potential of a
parasitoid population by combining its growth rate ($\lambda$), age-stage parasitism rate ($c_{ij}$), and stable age-stage structure ($a_{ij}$) (Chi et al., 2011), and is calculated as follows:

$$\omega = \lambda \psi = \lambda \sum_{x=0}^{\infty} \sum_{j=1}^{\beta} a_{xj} c_{xj}$$

(6)

Considering this, the intrinsic parasitism rate is calculated as $\ln(\omega)$. In other words, the parasitism capacity will increase exponentially ($\omega = e^{\text{intrinsic parasitism rate}}$) (Khanamani et al., 2015).

Parasitism rate data were analyzed using the computer program CONSUME-MSChart as designed by Chi (2013). The means, variances, and standard errors of parasitism parameters were estimated with the bootstrap resampling method and compared using Tukey-Kramer procedure. In the bootstrap procedure, we randomly took a sample of $n$ individuals from the cohort with replacement and calculated the $C_{0_i,\text{boot}}$ for this bootstrap sample as:

$$C_{0_i,\text{boot}} = \sum_{x=0}^{\infty} \sum_{j=1}^{\beta} s_{xj} c_{xj}$$

(7)

Where, the subscript $i$-boot represents the $i$th bootstrap and $s_{xj}$ and $c_{xj}$ are calculated from the $n$ individuals selected randomly with replacement. Generally, the data on the same individual are repeatedly selected. We repeated this procedure $m$ times ($m=10,000$) and computed the mean of these $m$ bootstraps as:

$$C_{0,B} = \frac{\sum_{i=1}^{m} C_{0_i,\text{boot}}}{m}$$

(8)

The variance (VAR $C_{0,B}$) and standard error (SE $C_{0,B}$) of these $m$ bootstraps were calculated as:

$$\text{VAR } C_{0,B} = \frac{\sum_{i=1}^{m} (C_{0_i,\text{boot}} - C_{0,B})^2}{m-1}$$

(9)

$$\text{SE } C_{0,B} = \sqrt{\text{VAR } C_{0,B}}$$

(10)

All graphs were drawn using Microsoft Excel 2010.

**RESULTS**

**Age-Stage Specific Parasitism Rate**

Age-stage specific parasitism rate ($c_{ij}$) of *D. semiclausum* on *P. xylostella* reared on different genotypes of canola is plotted in Figure 1. Because only females can oviposit in their hosts, there is only a single curve of $c_{ij}$ in Figure 1. Therefore, for the non-parasitic stages (e.g., egg, larva, pupa and male) their parasitism rates ($c_{Eggs}$, $c_{Larva}$, etc.) were zero. First, age-stage specific parasitism rate ($c_{ij}$) on all of the plant genotypes increased with increasing age, and then decreased.

**Net Parasitism Rate and Transformation Rate**

Net parasitism rate ($C_{0}$) and transformation rate from host population to parasitoid offspring ($Q_{fp}$) of *D. semiclausum* on the different genotypes of canola are shown in Table 1. There was a significant difference among the values of net parasitism rate on all plant genotypes. The value of the net parasitism rate ($C_{0}$) of *D. semiclausum* ranged from 12.20 to 20.12 hosts on all studied genotypes. The $Q_{fp}$ gives a demographic estimation for the relationship between the parasitism rate and reproductive rate of a parasitoid. The transformation rate from host population to parasitoid offspring of all genotypes was close to $1$ ($C_{0} \equiv R_{0}$).

**Age-specific Parasitism Rate and Age-specific Net Parasitism Rate**

Age-specific parasitism rate ($k_{x}$) and age-specific net parasitism rate ($q_{x}$) of *D. semiclausum* on the different genotypes of canola are shown in Figure 2. The age-specific parasitism rate is the mean number of *P. xylostella* larvae parasitized by *D. semiclausum* at age $x$. By considering the survivorship, the age-specific net parasitism rate ($q_{x} = l_{x} k_{x}$) is defined as the weighted number of hosts parasitized by a parasitoid of age $x$. 
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Figure 1. Age-stage parasitism rate \( (c_{ij}) \) of \textit{Diadegma semiclausum} on \textit{Plutella xylostella} reared on different \textit{Brassica} genotypes.

Table 1. Parasitism parameters of \textit{Diadegma semiclausum} reared on \textit{Plutella xylostella} on different \textit{Brassica} genotypes under laboratory conditions.\(^a\)

<table>
<thead>
<tr>
<th>Plant genotypes</th>
<th>( C_0 )</th>
<th>( Q_0 )</th>
<th>( \psi ) (hosts parasitoid(^{-1}))</th>
<th>( \omega ) (hosts parasitoid(^{-1}) day(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Brassica rapa}</td>
<td>14.945±3.05(^{ab})</td>
<td>( \cong ) 1</td>
<td>0.227±0.05(^b)</td>
<td>0.271±0.05(^b)</td>
</tr>
<tr>
<td>Cultivar-Opera</td>
<td>20.128±3.54(^a)</td>
<td>( \cong ) 1</td>
<td>0.236±0.03(^a)</td>
<td>0.285±0.04(^a)</td>
</tr>
<tr>
<td>Cultivar-RGS(_003)</td>
<td>14.951±2.93(^{ab})</td>
<td>( \cong ) 1</td>
<td>0.231±0.03(^{ab})</td>
<td>0.277±0.05(^{ab})</td>
</tr>
<tr>
<td>Hybrid-Hyula(_{001})</td>
<td>12.202±2.61(^b)</td>
<td>( \cong ) 1</td>
<td>0.175±0.03(^c)</td>
<td>0.202±0.03(^c)</td>
</tr>
<tr>
<td>Mutant-RGS(_003)</td>
<td>13.943±2.81(^{ab})</td>
<td>( \cong ) 1</td>
<td>0.177±0.03(^{c})</td>
<td>0.205±0.03(^{c})</td>
</tr>
<tr>
<td>Transgenic-PF</td>
<td>12.556±2.52(^{b})</td>
<td>( \cong ) 1</td>
<td>0.175±0.03(^{c})</td>
<td>0.202±0.03(^{c})</td>
</tr>
</tbody>
</table>

\(^a\) Means with the same letters are not significantly different (\( \alpha = 0.05 \)) using Tukey-Kramer procedure.

\[\text{\( 1747 \)}\]
Stable and Finite Parasitism Rates

Stable parasitism rate ($\psi$) and finite parasitism rate ($\omega$) of *Diadegma semiclausum* on *Plutella xylostella* reared on different *Brassica* genotypes are shown in Table 1. The value of the stable parasitism rate ($\psi$) ranged from 0.175 (on Hybrid-Hyula$_{401}$) to 0.236 (on Cultivar-Opera) host/parasitoid. Also, the highest and lowest values of the finite parasitism rate ($\omega$) were observed on Cultivar-Opera and Transgenic-PF, respectively. These results indicated that, if the parasitoid population is stable and the parasitoid number is one, the parasitism capacity of this parasitoid ranged from 0.202 to 0.285 hosts per day.

Figure 2. Age-specific parasitism rate ($k_x$) and age-specific net parasitism rate ($q_x$) of *Diadegma semiclausum* on *Plutella xylostella* reared on different *Brassica* genotypes.
DISCUSSION

Plant quality in herbivores’ diet may affect the parasitism capacity of their parasitoids. The net parasitism rate ($C_0$) gives the mean number of hosts parasitized by an average individual during its entire life span. Since only female parasitoids are capable of laying eggs in their host larvae and all $c_{ij}$ of immature stages are zero, we obtained low value of $C_0$ (ranged from 12.21 to 20.12 hosts) compared with predators (Chi et al., 2011; Farhadi et al., 2011; Khanamani et al., 2014, 2015). The parasitism rate of the parasitoid will be equal to its fecundity when the parasitoid lays only one egg per host and all eggs can develop to the adult stage. If some offspring of a parasitoid kill their hosts but fail to emerge from the larvae, the parasitism rate will differ from the fecundity rate (Ebrahimi et al., 2013).

Chi and Yang (2003) used $Q_p$ (the ratio of the net predate rate to the net reproductive rate, $Q_p = C_0/R_0$) to describe the transformation rate from prey population to predator offspring. For a predator, the age-specific predation rate can be defined as the number of prey killed by an individual predator of a specific age, whereas the age-specific fecundity is the offspring produced by an individual female predator of that age. Therefore, the predation rate of a predator usually differs from its fecundity (Chi and Yang, 2003), in other words, $R_0< C_0$. Whereas, most parasitoids successfully emerged from their hosts, thus, their parasitism rate is almost identical to their fecundity ($C_0= R_0$). On the other hand, for most parasitoids, especially those who lay only one egg in their host, $Q_p$ ($C_0/R_0$) will be 1 or close to 1. The $Q_p$ values of D. semiclausum on all tested genotypes were calculated to be 1.0. A similar result ($Q_p= 1.0$) was reported for Aphidius gifuensis (Ashmead) reared on Myzus persicae (Sulzer) (Chi and Su, 2006). However, when a female parasitoid lays more than one egg in its host, the $Q_p$ value will be <1.0. Indeed, a demographic estimation for the relationship between the reproductive rate and parasitism rate of a parasitoid could be obtained by $Q_p$.

The intrinsic rate of increase ($r$) is a key demographic parameter to depict the population growth potential of an insect (Safuraie-Parizi et al., 2014; Goodarzi et al., 2015). In order to precisely evaluate the effect of parasitism/predation in a biological control program, we should both assess the growth potential of the parasitoid/predator and its parasitism/predation potential. The finite parasitism rate ($\omega$) is a standard parameter which combines the finite rate of increase ($\lambda$), the age-stage structure, and the age-stage parasitism rate ($c_{ij}$) of a parasitoid population, and is then qualified to explain and contrast the parasitism potential of natural enemies used in biological control programs (Chi et al., 2011; Farhadi et al., 2011).

In the present study, the finite parasitism rate ($\omega$) was affected by different genotypes of canola and ranged from 0.202 to 0.285 host parasitoid$^{-1}$ day$^{-1}$. The low $c_{ij}$ and very low proportion of parasitoid individuals in SASD resulted in a low value of the finite parasitism rate. On the basis of parasitism parameters, the results indicated that D. semiclausum had a similar response on Hybrid-Hyula001, Mutant-RGS003, and Transgenic-PF genotypes followed by canola’s progenitor (B. rapa) and the cultivated genotypes (Cultivar-Opera and Cultivar-RGS003). Our results showed that $\omega$, $\psi$ and $C_0$ values were higher on B. rapa, Cultivar-Opera, and Cultivar-RGS003 than the others. Generally, parasitism of D. semiclausum was better on canola’s progenitor (B. rapa) and the cultivated genotypes (Cultivar-Opera and Cultivar-RGS003). A possible explanation for differences in the parasitism parameters may be due to plant chemistry. It is hypothesized that the changes in chemistry of the plants will affect the life history and performance of insects associated with them. Several studies have reported that secondary metabolites in host diets affect the survival rate, growth, development, and body weight
of their parasitoids (Campbell and Duffey, 1979; Barbosa et al., 1991; Szajder and Harvey, 2003; Harvey et al., 2007). We previously measured the levels of secondary metabolites (glucosinolates) in these genotypes (Nikooei et al., 2015). Glucosinolates (GS) concentration was higher in canola's progenitor (B. rapa) and the cultivated genotypes (Cultivar-Opera and Cultivar-RGS003) and lower on Hybrid-Hyula001, Mutant-RGS003, and Transgenic-PF. In addition, the results of our previous study (unpublished data) showed that the life table parameters of D. semiclausum was significantly affected by these genotypes. A better performance of the parasitoid was observed on the genotypes which contained higher level of GS.

Diadegma semiclausum parasitizes P. xylostella specially feeding on Brassicaceae plants containing GS. As a specialist herbivore, P. xylostella uses GS as feeding stimulant and indicator of host plant suitability (Bartlet et al., 1999; Li et al., 2000). Therefore, the performance of host could be better on genotypes having higher level of GS (Nikooei et al., 2015). Since the performance of a parasitoid is followed by its host, we also found that D. semiclausum had higher efficiency on genotypes having higher level of GS (unpublished data). In conclusion, before applying a precise biological control program, it is important to study the interactions among different host plants and biological control agents, considering both growth population potential and parasitism rate of a parasitoid.

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جند کلزا B. rapa رقم زراعی Opera رگس003 و تاریخ20/12/2014 ترتیب تری برابر به 10 میزان بود. نرخ تبدیل از جمعیت میزان به نتایج پارازیتوئید (Qp)، روی تمایل زنوتیپ ها نزدیک به یک بود (R0). همچنین، نرخ متناهی پارازیتوئید (α) روی زنوتیپ های ذکر شده در بالا به ترتیب برابر با 271.99/14/12/20 و 0.95/14/20/12 بود. نرخ متناهی پارازیتوئید پاتامزی است که نرخ متناهی رشد جمعیت، توزیع سنتی - مرحله ای پایدار به نرخ پارازیتوئید ویژه سنتی - مرحله ای را در یک گروه با نیاز آن پارامتر می تواند برای ارزیابی کارآمدی یک پارازیتوئید مورد استفاده قرار گیرد. در پایان، زنیز پارازیتوئید D. semiclausum بالاتری روی جند کلزا و ارقام زراعی بود که بر اساس میزان متابولیت های ثانویه میزان های گیاهی مناسب تری برای بید کلم بودند.