

Parasitism of different *Trichogramma* species and strains on *Plutella xylostella* L. on greenhouse cauliflower

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Abstract The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is the most important pest of cultivated Brassica worldwide, including those grown in greenhouses like cauliflower. In this article, we evaluate the potential of various species (and various strains of some species) of Trichogrammatidae (Hymenoptera: Chalcidoidea) to control this pest on cauliflower in greenhouse in France. We assessed the parasitism levels on *P. xylostella* of 17 Trichogrammatidae strains, belonging to 12 different species (2 indigenous strains from France), under greenhouse conditions. Parasitism levels for each of the Trichogrammatidae species and strains were determined on cauliflower leaves (*Brassica oleracea botrytis* L., Brassicaceae) infested with *P. xylostella* eggs. Nine strains parasitized 60% (or more) of the *P. xylostella* eggs. Compared to previous results in laboratory conditions, climatic conditions of the greenhouse did not influence parasitism levels. The presence of the cauliflower plants may have a positive effect on eight strains, a negative effect on four strains and no effect on five strains. Our study points out the importance of including the host

plant of *P. xylostella* when conducting studies aiming to select the most efficient parasitoid against this pest.

Keywords Egg parasitoids · Strains · Parasitic efficiency · Diamondback moth · Greenhouse · Brassica

Introduction

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is the most important pest of cultivated brassicas worldwide (Talekar and Shelton 1993). *P. xylostella* occurs throughout the year wherever its host plants are grown. The larvae feed on many plants in the crucifer family (notably canola, mustard, cabbage, cauliflower and kohlrabi) and on several greenhouse plants. Because of the widespread use of insecticides to control *P. xylostella*, it has developed resistance to insecticides, and also to several *Bacillus thuringiensis* products (Talekar and Shelton 1993; Tabashnik 1994; Gassmann et al. 2009). In addition, insecticides are harmful for the environment and present multiple potential side effects on beneficial arthropods (Theiling and Croft 1988; Desneux et al. 2007). This hints a new interest in developing alternatives to the usage of insecticides, such as biological control and development of resistant plants (Thomas and Waage 1996).

Wasps of genus *Trichogramma* (Hymenoptera) are the principal egg parasitoids of Lepidoptera (Smith 1996). Notably, these tiny insects (length 1 mm) parasitize the eggs of many types of agricultural pest insects (Mills and Carl 1991; Smith 1996; Vinson 1998; Hazarika et al. 2009; Agamy 2010; Suckling and Brockerhoff 2010), and they are easy to rear (Ayvaz and Karabörklü 2008; Pizzol and Pintureau 2008) and release in fields suffering from pest

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outbreaks. In order to develop biological control against *P. xylostella* in cabbage crops in France, the efficiency of 30 *Trichogramma* species, strains and *Trichogrammatidae* sp. were determined under plant-free laboratory conditions (see Smith 1996; Tabone et al. 1999, 2001). However, given the artificial conditions of this primary screening, parasitoid efficiency waits to be determined under more realistic conditions. Under natural conditions, various factors like crop architecture, plant morphology, prey distribution and climatic conditions could affect the efficiency and behaviour of natural enemies and may affect the efficacy of biological control (Andow and Prokrym 1990; Rutledge et al. 2003; Desneux et al. 2006; Tabone et al. 2006; Carrillo et al. 2008; Desneux and O’Neil 2008; Desneux and Ramirez-Romero 2009). For *Trichogrammatidae*, parasitism levels vary greatly among different habitats, plant species and plant structures, and plant spacing, odour and chemistry all play a role in moulding the efficiency of *Trichogramma* spp. as biological control agents (Gingras et al. 2002; Romeis et al. 2005). For different *Trichogramma* species, parasitism is inversely correlated with plant size (Ables et al. 1980; Thorpe 1985) and architecturally complexity of plants (Andow and Prokrym 1990; Lukianchuk and Smith 1997; Romeis et al. 1998; Gingras et al. 2003). Plant volatiles also influence *Trichogrammatidae* behaviour (Bar et al. 1979; Cabello et al. 1985; Vet and Dicke 1992), with certain plant species being attractive and others repellent (Romeis et al. 1997).

In this article, we compare 17 *Trichogrammatidae* strains, belonging to 12 different species: 2 indigenous strains (Northern France) and 15 strains that did show the highest

parasitic efficiency in previous laboratory assays (Tabone et al. 1999, 2001). The efficacy of various strains of *Trichogrammatidae* in parasitizing *P. xylostella* was assessed on cauliflower plants without taking into account for parasitoids dispersal ability in the greenhouse. In this study, we assess parasitism performance of the different species and strains under greenhouse conditions and determine whether climate (greenhouse) and host plant (plants used as substrate) affect parasitism by *Trichogrammatidae*.

Materials and methods

Plants and insects

Plutella xylostella was reared in plastic boxes (28.5 × 27.5 × 9.5 cm) on cauliflower leaves at 21 ± 1°C, 70 ± 10% R.H. and L16:D8. *Trichogrammatidae* strains were reared on *Ephestia kuehniella* Zeller eggs, in plastic tubes (7.5 × 1.2 cm), at 25 ± 1°C, 75 ± 10% R.H. and L16:D8 (the various strains/species were maintained for 50–80 generations on *E. kuehniella*). Table 1 shows a list of the 17 strains used with their geographical origin. Cauliflowers were grown in pots in a glass insect-proof growth chamber (21 ± 1°C, 60 ± 5% R.H.). Plants used for all experiments were 3 months old.

Experimental protocol

For the experiment, a cauliflower plant was placed into a 75 × 85 × 80 cm cage with twelve, 24-h-old mated

Table 1 Host and country of origin of the *Trichogrammatidae* and *Trichogramma* strains studied

Species	Strain	Host	Origin
<i>Trichogrammatidae bactrae</i> Nagaraja	Bac-1	<i>Plutella xylostella</i> Linnaeus	Thailand
<i>Trichogramma bourarachae</i> Pintureau and Babault	Bou-1	<i>Vanessa cardui</i> Linnaeus	Morocco
<i>Trichogramma bourarachae</i>	Bou-2	<i>Helicoverpa armigera</i> Hubner	Portugal
<i>Trichogramma buesi</i> Voegelé	Bue-1	<i>Ephestia kuehniella</i> Zeller	Canada
<i>Trichogramma cacoeciae</i> Marchal	Cac-1	<i>Ephestia kuehniella</i>	Canada
<i>Trichogramma chilonis</i> Ishii	Chi-1	<i>Plutella xylostella</i>	Japan
<i>Trichogramma chilonis</i>	Chi-2	<i>Chilo sacchariphagus</i> Bojer	Reunion Island
<i>Trichogramma chilonis</i>	Chi-3	<i>Ephestia kuehniella</i>	Taiwan
<i>Trichogramma dendrolimi</i> Matsumara	Den-1	<i>Lobesia botrana</i> Denis and Schiffermüller	Italy
<i>Trichogramma evanescens</i> Westwood	Eva-1	<i>Pectinophora gossypiella</i> Saunders	Egypt
<i>Trichogramma evanescens</i>	Eva-2	<i>Plutella xylostella</i>	France
<i>Trichogramma oleae</i> Voegelé and Pointel	Ole-1	<i>Prays oleae</i> Bernard	France
<i>Trichogramma ostriniae</i> Pang and Chen	Ost-1	<i>Ephestia kuehniella</i>	Japan
<i>Trichogramma ostriniae</i>	Ost-2	<i>Ephestia kuehniella</i>	Moldavia
<i>Trichogramma principium</i> Sugonjaev and Sorokina	Pri-1	<i>Earias insulana</i> Boisduval	Syria
<i>Trichogramma semblidis</i> Aurivillius	Sem-1	<i>Plutella xylostella</i>	France
<i>Trichogramma voegelei</i> Pintureau	Voe-1	<i>Helicoverpa armigera</i>	France

P. xylostella females. On these plants, only two leaves situated at the median level of the plant were kept. After 24 h, the plant was withdrawn from the cage and *P. xylostella* eggs were counted. The aim was to obtain 200 *P. xylostella* eggs per leaf; therefore, additional eggs were removed with a brush. A total of three plants were placed in a glass greenhouse (20 × 4 m). Three greenhouses were used at the same time, with three cauliflower plants placed into each greenhouse (spaced at 5 m intervals).

Parasitized *E. kuehniella* eggs (i.e. black eggs) stuck on a yellow card (placed in a glass tube) were counted just before parasitoid emergence to have 200 females (extra parasitized eggs were removed). The number of females was calculated according to the average sex ratio of each strain (Tabone et al. 2006; E. Tabone, unpublished data). When more than 50% of the parasitoids emerged, the glass tube containing them was opened and placed in a latticed sleeve attached between the two leaves of each cauliflower plant in the greenhouses.

After 5 days, cauliflower leaves were removed and brought to the laboratory where *P. xylostella* eggs were incubated at 25°C and 75% R.H. Empty eggs (non-parasitized eggs) and black eggs (parasitized) were counted. For each trial, nine replicates were undergone with the strains tested randomly assigned to each of the three greenhouses (three replicates of the same strain per greenhouse). A total of 12 replicates were done for each strain, and replicates for which the cauliflower plant started senescing before being brought back to the lab were discarded. Experiments were conducted between May and October at the INRA (French National Institute for Agricultural Research) station of Valbonne (southern France) and temperature and humidity were recorded during each trial (temperature and relative humidity recorded hourly using HOBO data loggers, Onset Computer, Pocasset, MA, USA).

Data analysis

The mean percentages of parasitized eggs (black) were compared among strains with ANOVA on arcsin-transformed data followed by Tukey's post-hoc test. Simple linear regressions were used to determine relationships between parasitism rates and temperature (mean temperature and number of hours per day at temperatures exceeding 35°C or below 16°C) as well as between parasitism rates and humidity (mean relative humidity and number of hours per day at a relative humidity below 40%).

Results

Factors related to the greenhouse as the temperature and humidity did not affect parasitism (mean temperature:

$R^2 = 0.19$, $F = 2.86$, $df = 148$, $P = 0.093$; hours per day with $T > 35^\circ\text{C}$: $R^2 = 0.13$, $F = 1.99$, $df = 148$, $P = 0.160$; hours per day with $T < 16^\circ\text{C}$: $R^2 = 0.24$, $F = 3.61$, $df = 148$, $P = 0.059$; mean R.H.: $R^2 = 0.001$, $F = 0.08$, $df = 148$, $P = 0.777$; hours per day with R.H. <40%: $R^2 = 0.08$, $F = 1.25$, $df = 148$, $P = 0.265$.

Levels of parasitism differed significantly between strains ($F_{(16,131)} = 10.51$, $P < 0.001$) (Fig. 1). Nine of the 17 strains had a parasitism equalling or exceeding 60%. In contrast, two strains parasitized less than 20% of the *P. xylostella* eggs. Levels of parasitism significantly differed (all $P < 0.05$) between the two strains of *Trichogramma bourarachae*, the two strains of *Trichogramma chilonis*, and largely between the two strains of *Trichogramma ostriniae*. In this case, the percentage of parasitism of *P. xylostella* by the Japanese strain of *T. ostriniae* (Ost-1) was nearly four times the parasitism by the Moldavian strain (Ost-2). Compared to laboratory trials undergone previously (Tabone et al. 2001), eight strains (Chi-1, Eva-2, Eva-1, Ole-1, Chi-3, Chi-2, Sem-1 and Bou-2) had significantly higher parasitism levels in the greenhouse than in the laboratory (all $P < 0.05$), whereas four strains had higher parasitism levels in laboratory conditions (Bue-1, Voe-1, Ost-2 and Cac-1) (all $P < 0.05$, Fig. 1).

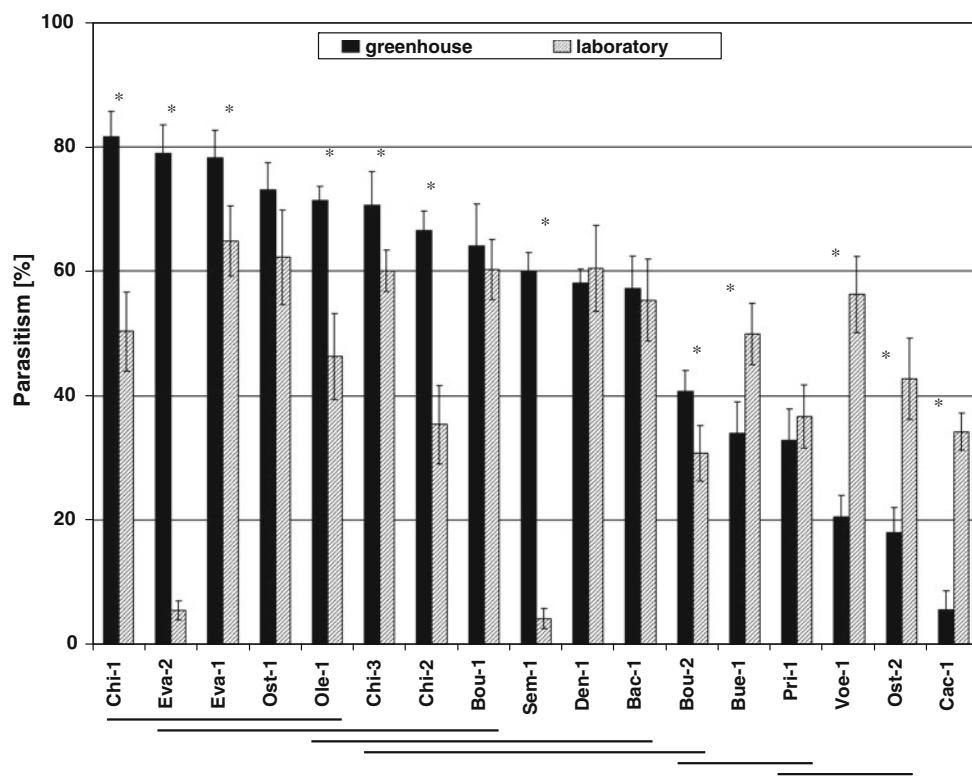
Discussion

A primary step to select suitable *Trichogramma* spp. for biological control of *P. xylostella* is the assessment of inter- and intraspecific variation in tested species (Smith 1996). Of the 17 strains, highest levels of parasitism on *P. xylostella* under greenhouse conditions were recorded for *T. chilonis*-1, *T. evanescens*-1, *T. evanescens*-2, *T. ostriniae*-1, *T. oleae*-1, *T. chilonis*-3, *T. chilonis*-2, *T. bourarachae*-1 and *T. semblidis*-1. These nine strains parasitized 60% or more of the *P. xylostella* eggs.

Levels of parasitism varied among strains of the same species in the case of *T. bourarachae*, *T. ostriniae* and *T. chilonis*. This variation could be ascribed to a local adaptation of the different strains to particular conditions of their respective habitats. For example, an Italian strain of *T. brassicae* Bezdenko is more efficient in controlling *Ostrinia nubilalis* Hübner in Spain than the French strain (Eizaguirre et al. 1998). Klug and Meyhöfer (2009) described similar findings when comparing performance of two strains of *T. brassicae* in locating eggs of the silver Y moth (*Autographa gamma* Linnaeus). However, this is not always the case in host-parasitoid systems (Vos and Vet 2004) and our results showed that strains of *T. evanescens* did not differ in their parasitism level.

Twelve strains obtained different results in the greenhouse experiment when compared to results to laboratory

Fig. 1 Comparison of mean parasitism rates (\pm SD) among *Trichogramma* species and strains on *P. xylostella* on cauliflowers in the greenhouse and in laboratory conditions (glass tubes, data from Tabone et al. 2001). Strains are classified according to greenhouse results, means of parasitism for strains subtended by lines do not differ ($P > 0.05$, ANOVA followed by Tukey's post-hoc test). * Significant difference in parasitism rates between laboratory and greenhouse conditions (at $P < 0.05$)



experiments (eight showed higher parasitism rates in the greenhouse than in the laboratory, whereas it was the opposite for four strains, Tabone et al. 1999, 2001). These changes may be caused by abiotic factors varying largely in the greenhouse but in the laboratory. The thermoperiod that occurred in the greenhouse (in contrast to laboratory conditions, e.g. without thermoperiod) could have impacted the efficiency of the parasitoids as it can influence Trichogrammatidae behaviours (Prasad et al. 1999) and physiological state (Bigler et al. 1997). Direct observations made during the experiments also revealed that the four strains (Bue-1, Voe-1, Ost-2 and Cac-1, Fig. 1) which displayed higher level of parasitism in the laboratory showed a lower walking activity in the greenhouse than during the laboratory trials (Gonsard, personal communication). In addition, the dispersal capacities of *Trichogramma* often vary between strains, which may ultimately impact their effectiveness in attacking hosts (Klug and Meyhöfer 2009) and notably between the strains tested in our study (E. Tabone, unpublished data). Similarly, the horizontal and vertical searching activities of *Trichogramma* may also affect parasitism efficiency (El-Wakeil et al. 2008). Thus variable host searching capacities may be the cause of observed lower effectiveness of some strains in more realistic conditions than in laboratory conditions.

Other factors may also be of concern for efficiency of the strains tested against *P. xylostella* on cauliflower. Plants emit attractive odours (Altieri et al. 1981; Nordlund et al.

1985), repelling odours (Bar et al. 1979, Romeis et al. 1997) or have no effect (Cabello et al. 1985) on Trichogrammatidae. Reddy et al. (2002) observed that *T. chilonis* was attracted by green leaf cabbage volatiles, whereas *T. chilonis*, *T. japonicum* and *T. poliae* were attracted to acetone leaf extracts of cauliflower (Ganesh et al. 2002). Further olfactometer studies are necessary to determine the real impact of plant volatiles on Trichogrammatidae. In addition, physical characteristics of plant leaves (notably epicuticular lipids of Cruciferae plants) can impact insect movement on the plant (Eigenbrode and Espelie 1995). Thus, particular Trichogrammatidae species (and strains) tested may be more adapted to these waxes (as demonstrated for aphid parasitoids, Desneux and Ramirez-Romero 2009).

The chi-1 strain of *T. chilonis* and both strains of *T. evanescens* showed the strongest potential for controlling *P. xylostella* on greenhouse cauliflower. Five other strains showed parasitism rates superior to 60% and thus can be also considered as good candidates for biological control of the pest on cauliflower. However, given preliminary results on dispersal capacity of these various strains in cabbage crops in greenhouses, the potential of these various strains still need to be tested further, using a cauliflower crop infested with natural *P. xylostella*, to determine their impact on this pest under normal cropping conditions. Further studies would also aim to assess the efficacy of the various strains when used in combination

(e.g. the chi-1 strain of *T. chilonis* and both *T. evanescens* strains) for optimizing their efficiency. Finally, if potential risks related to the introduction of exotic species are considered and if there is a urge to consider native species, *T. evanescens* appears to be the best candidate for biological control of *P. xylostella* on cauliflower in France.

Although Trichogrammatidae selection tests conducted on target hosts in laboratory conditions (in glass tubes) have long been widely used (Hassan 1990; Smith 1996), our results show that it is not always justified to exclude species that display poor parasitic efficiencies in the laboratory and that multiple factors should be integrated for selecting the most appropriate parasitoid for biological control of *P. xylostella* on cauliflower in greenhouse.

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