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Article

Performance of *Trichogramma pintoi* when parasitizing eggs of the oriental fruit moth *Grapholita molesta*

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With 5 figures

Abstract: In recent years, *Grapholita molesta* Busck has become a serious pest to fruit trees, especially to peach trees in China. In the present study, the parasitism of the pest by the parasitoid *Trichogramma pintoi* Voegele was studied, with assessment of the impact of UV-light, host egg age, host and parasitoid density, as well as different inoculation time. The UV-light treatment significantly decreased parasitism rate of the parasitoid compared to non-UV light treatment. *T. pintoi* preferred to parasitize newly laid *G. molesta* eggs, and parasitism rate decreased with increasing age of host eggs. Moreover, the parasitism rate on *G. molesta* increased with *Trichogramma* density although the rate of parasitism per parasitoid female reduced overall. The parasitism rate also increased when the releasing duration extended to 48 h. The results provided key information for developing the use of *T. pintoi* as biocontrol agent for management of *G. molesta*. Further studies will be needed to document potential method for field development of *T. pintoi*–based biological control of *G. molesta* in peach orchards.

Keywords: peach orchard; parasitism rate; emergence rate; egg age; biological control

1 Introduction

Grapholita molesta Busck (Lepidoptera: Tortricidae), also known as the oriental fruit moth, is a worldwide pest infesting fruit trees such as pears, peaches and apples (Knight & Larsen, 2004). The larvae of *G. molesta* feed on fruit tree shoots and fruits, causing extensive damages and yield loses (Zhang et al., 2018). In recent years, *G. molesta* showed major outbreaks in peach and pear orchards (as well as other fruit orchards) in China (Yang et al., 2003). Notably, it also showed overlapping of generations, which makes it hard for control and management (Li et al., 2016). Moreover, the outbreak period is affected by temperature, humidity and other environmental factors, causing difficulty in implementing control methods in a preventive way.

Comprehensive prevention measures and control methods could be usually combined with various other methods for pest management, including artificial clearance of the crops to reduce the source of insects, trapping adults with sweet and sour liquid and light traps, as well as pesticides application (Witzgall et al., 1999). Chemical control has lost its preference (e.g. Il'Ichev et al., 2006) because of potential multiple problems related to the use of insecticides, notably the known increasing resistance in agricultural pests (e.g. Guo et al. 2013; Liu et al. 2017; Kampouraki et al. 2018; Roditakis et al. 2018), and potential side effects on

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beneficial arthropods (Lu et al. 2012; Decourtye et al. 2013; Fogel et al. 2013; Biondi et al. 2015; Cabrera et al. 2018; and see Desneux et al. 2007 for a thorough review) and human health (Weisenburger 1993). Throughout the world, pest management methods based on biocontrol agents are gradually receiving more and more attention from farmers (Heimpel & Mills 2017; Buchanan et al. 2018; Kaser & Heimpel 2018; Michaud 2018; Toivonen et al. 2018; Xu et al. 2018; Jaworski et al. 2019). More specifically, integrated pest management methods have received more attention and notably include monitoring, trapping and killing of *G. molesta* during the emergence period, the release of *Trichogramma* parasitoids during the oviposition period of the pest and the use of mating disruption barriers and sex pheromone (Yang et al., 2003; Il'Ichev et al., 2004; 2006). The use of some *Trichogramma* species (i.e. *T. japonicum, T. dendrolimi* etc.) was proved to be effective against many Lepidopterous pests worldwide and got acceptance by farmers gradually but would need further development in most cases (Smith 1996; Boivin 2010; Desneux et al. 2010; Tabone et al. 2010; El-Arnaouty et al. 2014).

As egg parasitic wasps, *Trichogramma* parasitoids utilize their host to develop and reproduce, thus the host pests are controlled (Gardner et al., 2011; Wang et al., 2014; Altoe et al., 2012). *Trichogramma* has been widely used in the biological control and integrated control of various lepidopteran pests in agriculture and forestry (van Lenteren & Gurr, 2000; Dahlan & Gordh 1998; Jarjees & Merritt 2000), including *G. molesta* (Busck) (Li et al., 2016). For example, in the 1980s, *Trichogramma dendrolimi* Matsumura was used to control forest pests in China and effective management was achieved (Huang et al., 2018). Moreover, there has been increasing attention on ways to simultaneously improve the efficiency and reduce the cost of inundative releases of *Trichogramma*; in this context, studying the biology (e.g. Andrade et al. 2011; Coelho et al. 2017; Huang et al. 2017; Souza et al. 2018; Wu et al. 2018) of economically important parasitoid species is important to promote economically-sound development in use of *Trichogramma* parasitoids as biocontrol agents (Smith 1996; Parreira et al. 2018; Wang et al. 2018).

Trichogramma pintoi Voegele is mainly distributed in northwest China, and has been widely studied by Chinese scientists since late 1990s (Wang et al., 2014). However, the mass rearing of this species is not fully developed and need to be studied. Although *Trichogramma* species can parasitize *G. molesta*, the parasitism rate and potential control efficacy may be modulated by various factors known to affect such egg parasitoids (Chailleux et al. 2012; 2013b; Yang et al., 2016; Zhang et al., 2017; Du et al., 2018). In practice, UV-light exposure on host eggs is a common procedure for *Trichogramma* mass-rearing, but it could affect parasitism of various hosts by *Trichogramma* parasitoids (e.g. Edwin et al., 2016). The mass rearing methods of *T. pintoi* are still under development and assessing how UV-light exposure may influence the performance of *T. pintoi* would help to optimize rearing conditions. Besides UV-light, both the age of host eggs and of parasitoid females could have a significant effect on parasitism (Zhang et al. 2014; Song et al. 2015). For example, *Lobesia botrana* eggs older than 3–4 days were less parasitized than newly laid eggs by *Trichogramma cacoeciae* Marchal (Pizzol et al., 2012), and five *Trichogramma* species showed preference toward younger eggs of *Mythimna separata* (Walker) (Hou et al., 2018). The development status of host embryo can also influence the choice of parasitoid females when selecting hosts for parasitism, among others factors (e.g. see Chailleux et al. 2013a; Thiéry & Desneux 2018).

In this context, we tested how factors related to mass rearing conditions of *Trichogramma* parasitoids may influence the parasitism and emergence of *T. pintoi*. We studied how UV-light treatment, host egg age, host and parasitoid density, as well as releasing time, could affect parasitism and parasitoid emergence. We aimed at providing information enabling to improve the efficiency of the parasitoid *T. pintoi* when releasing it to control *G. molesta*.

2 Materials and methods

2.1 Biological materials

T. pintoi adults were obtained from Beijing Academy of Agriculture and Forestry Sciences (BAAFS, Haidian district, Beijing, China). The colony was maintained for more than 10 generations using *Corcyra cephalonica* Stainton eggs as hosts in a growth chamber at the Lab of Natural Enemy Research (LNER), Institute of Plant & Environment Protection, BAAFS, at 25 ± 0.5 ºC, 60% R.H., 16: 8 h L: D.

Grapholita molesta (Busck) larvae were collected from peach shoots (1 to 13 cm) in orchards (Pinggu, Beijing). Extra leaves of the shoots were cut with scissors, then 30 to 40 leafless peach branches were put in a glass (15 cm tall, 8 cm in diameter, absorbent cotton was put at the bottom of the glass) immediately after collection, after which 15 mL water was added to each beaker to keep the shoots moisturized. The glass was covered with 2 layers of gauze and put in dark in the same growth chamber as described above. The number of *G. molesta* larvae collected was more than 500. When *G. molesta* pupate in the tip of shoots, they trend to expose half of the body outside of the shoot, so the pupae were easily picked out after about a week per incubation in the glass. All of the pupae and prepupae were collected from the glasses and put in petri dishes. Then the petri dishes were placed in a glass dish with absorbent cotton moisturized with 70 mL distilled water. The glass dishes were covered with a glass lid and a gap was left on the lid. Then the glass dishes were placed in a plexi glass box, and reared in dark conditions at 26 ºC until adult emergence.

2.2 *G. molesta* egg collection

The newly emerged adults of *G. molesta* were put in a plexi glass box. An iron plate was put in the bottom of the box. Some absorbent cotton was placed in the plate, then the plate was covered with gauze and fixed with rubber band. After which about 100 mL distilled water was put into the plate and absorbed by the cotton. This method can not only keep moisture, it also prevents *G. molesta* to be soaked in water. Two holes were made at the top left and top right of the plexiglass box with electric soldering iron respectively, and then a 1.5 mL centrifuge tube was plugged into the hole. Afterwards, a piece of cotton was put in the box from the centrifuge tube. So 10% honey could be added from the cotton as food source. The oviposition site was placed on the top of the box with a filter paper, which was pasted tightly. Eggs of *G. molesta* were then collected from the filter paper.

2.3 Parasitism of *T. pintoi* on *G. molesta*

After obtaining enough *G. molesta* eggs, different treatments were designed to investigate the parasitism of *T. pintoi* on *G. molesta*. The experimental treatments were divided into five groups. 1) The first group was UV-light treatment. Fifty *G. molesta* eggs were UV-light treated (15 W) for 30 min, then one *T. pintoi* was put into the glass cups. The control group was not treated by UV-light. 2) The second group was different egg age treatment. Fifty *G. molesta* eggs aged 1, 2, and 3 d were parasitised by *T. pintoi*. 3) The third group was different egg density treatment. The number of *G. molesta* eggs were 15, 30, 45, 60 and 75. 4) The fourth group was different *T. pintoi* density treatment. Two hundred *G. molesta* eggs were put in the glass cup, then 1, 3, 5, 7, and 9 *T. pintoi* were added for parasitism. 5) The fifth group was different time to add *T. pintoi*. Fifty *G. molesta* eggs were put in the glass cup, and then one *T. pintoi* was put into the cup for 12, 24, 36, 48 and 60 h. In the first three and the fifth treatments, only one *T. pintoi* was put into the glass cup for parasitism. In each group, each treatment was replicated 9 times and the parasitism of *T. pintoi* was recorded daily.

2.4 Data analysis

The data were analyzed with SPSS 17.0. The parasitism rate was the number of parasitized eggs divided by the number of host eggs. The emergence rate was number of newly emerged parasitoids divided by the number of parasitized hosts. Arc sine and logarithmic transformations were made when the data were expressed as percentages. One-way ANOVA (Tukey-Kramer HSD test) was used for comparing the differences among different treatments when homogeneity of variance was met. Independent *t*-test was used for analyzing the effect of UV-treatment on parasitism and emergence rates.

3 Results

3.1 Impact of UV light on the parasitism rate of *T. pintoi* on *G. molesta* eggs

The UV treatment of *G. molesta* eggs negatively influenced *T. pintoi* parasitism (t = –2.550, P = 0.021); decreasing the parasitism rate from 76% to 58.7% (Fig. 1). By contrast, the parasitoid emergence rate was high (all ≥ 71.9%) and was not significantly influenced by the UV-treatment (t =1.033, P = 0.317).

3.2 Parasitism of *T. pintoi* on *G. molesta* eggs in different age

Parasitism rate decreased significantly when the age of *G. molesta* eggs increased ($F_{2,26}$=22.876, P < 0.001; Fig. 2). The 1-day-old eggs of *G. molesta* had a parasitism rate of 76%, while the parasitism rate of 2-day-old eggs was 41.3% and that of 3-day-old eggs was only 24.7%. By contrast, the emergence rate of *T. pintoi* showed no significant difference when the age of *G. molesta* eggs increased ($F_{2,26}$=1.439, P = 0.257).

3.3 Impact of host egg density on parasitism of *T. pintoi*

Both the number of eggs parasitized and the parasitism rate varied significantly depending on the host egg density ($F_{4,44}$=14.922, P < 0.001; $F_{4,44}$=7.228, P < 0.001, respectively; Fig. 3). The number of parasitized eggs increased first, and then stabilized at 31.67 on average when *G. molesta* egg density was 45, 60 and 75, and differed significantly among different treatments. The parasitism rate decreased significantly when the host egg density were 60 and 75, and it was lowest at the highest host egg density tested (75). The results indicated that the lower the density of eggs was, the higher parasitism was obtained.

3.4 Effect of *T. pintoi* density on parasitism

Under the condition of fixed number of *G. molesta* eggs, the parasitism rates of *T. pintoi* increased significantly when the parasitoid density increased ($F_{4,44}$=162.226, P < 0.001; Fig. 4). The parasitism rate was 16% when only one *T. pintoi* was released, which increased to 80.67% when nine *T. pintoi* adults were released. However, in such case the parasitism rate per female decreased significantly ($F_{4,44}$=21.418, P < 0.001), from 16% to 8.96%. The *T. pintoi* density had significant effect on the emergence rate ($F_{4,44}$=5.259, P = 0.020), which was lowest when *Trichogramma* number was set to five parasitoids.

3.5 Effect of *T. pintoi* releasing time on parasitism

The parasitism rate as well as the emergence rate increased with the releasing duration ($F_{4,44}$=27.455, P < 0.001;

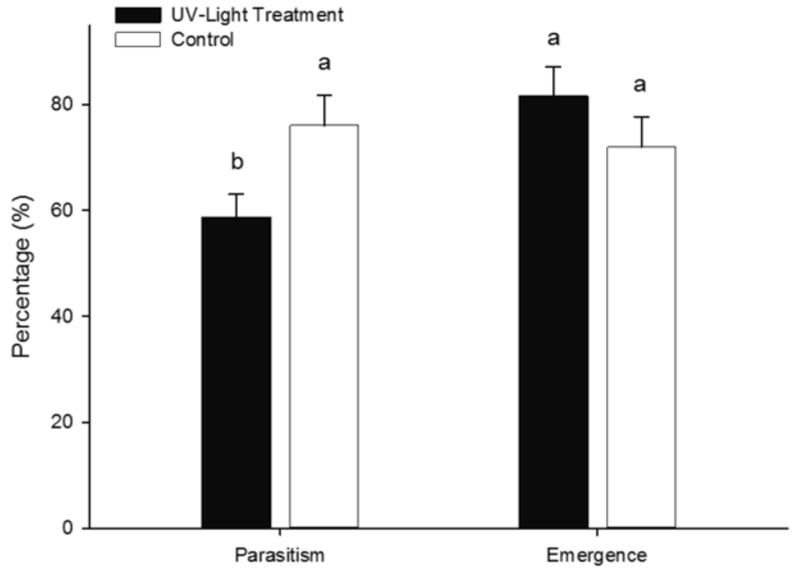

Fig. 1. Mean (± SE) values of parasitism and emergence rate of *T. pintoi* on *G. molesta* eggs after UV-light treatment. Within each group, same letters indicate no significant differences (at the $P < 0.05$ level).

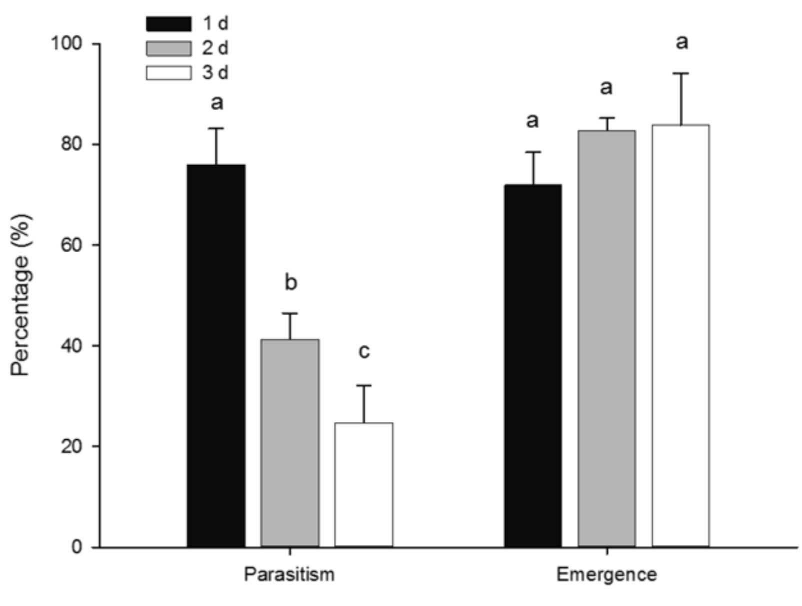

Fig. 2. Impact of the age of *G. molesta* eggs on parasitism and emergence rate of *T. pintoi*. Data in the column are means ± SE. Within each group, same letters indicate no significant difference (at the $P < 0.05$ level).

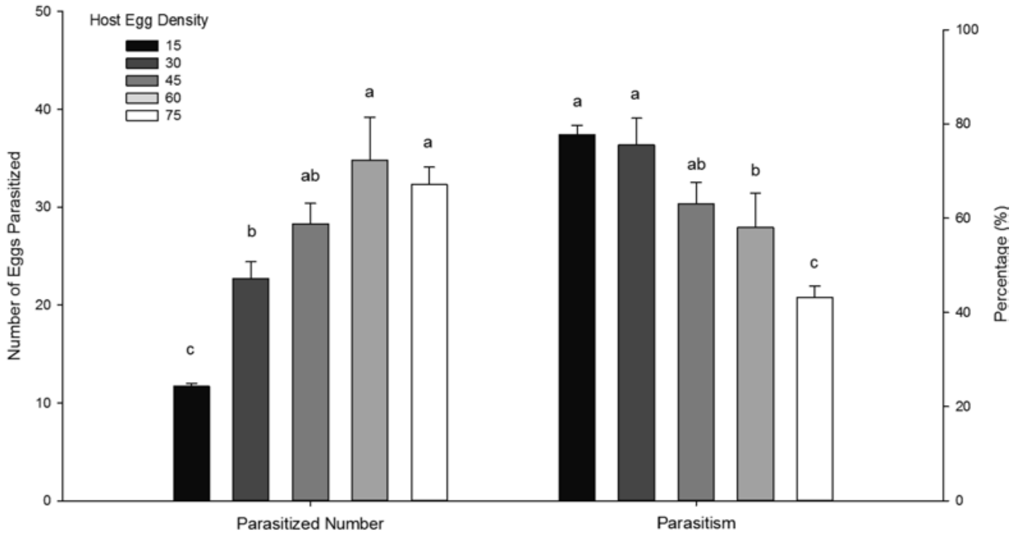

Fig. 3. The number of parasitized eggs and parasitism of *T. pintoi* when treated with different *G. molesta* eggs density. Data in the column are means ± SE. Same letters in the figure indicate no significant difference at the $P < 0.05$ level (Tukey-K ramer HSD test).

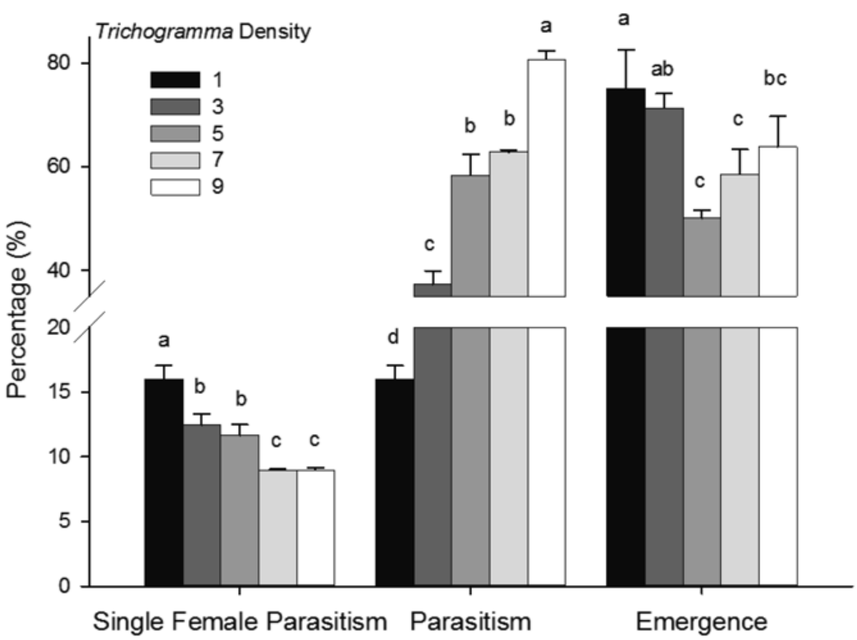

Fig. 4. The effects of *T. pintoi* density on parasitism and emergence rate. Data in the column are means ± SE. Within each group, same letters indicate no significant difference (at the $P < 0.05$ level).

$F_{4,44}$=3.608, P = 0.039, respectively; Fig. 5). The parasitism rate increased from 13.33% after 12 h of parasitism to 64% after 48 h of parasitism and then stabilized. The emergence rate increased from 59.29% after 12 h and then stabilized after 24, 36, 48 and 60 h.

4 Discussion

Host age (Pizzol et al. 2010; 2012), density (Khanh et al. 2012), UV-sterilization on host eggs (Edwin et al. 2016), different host species (Ozder & Kara 2010), competition among parasitoid individuals (Pintureau 1997; Pizzol et al. 2010), releasing duration (Zhang et al. 2014), as well as temperature and humidity (Smith 1996) may influence parasitizing performance and emergence rate of parasitoids. At present, improving the application efficiency of biological control agents is one of the most crucial questions in organic farming (Liu et al. 1998; Wang et al. 2014). To demonstrate how host age, density and UV treatment, as well as parasitoid density and releasing duration influence the application efficiency of *T. pintoi* on *G. molesta*, parasitism and emergence rates were tested. Among these assays, UV-light treatment, host age and density, parasitoid wasp density and inoculation duration influenced the parasitizing performance of *T. pintoi* significantly. Most remarkable, our findings showed that appropriate host egg age, density and parasitoid releasing time and number could enhance the parasitism of *T. pintoi*, which could promote the quality and efficiency of massing rearing and utilization of the parasitoid wasp.

4.1 Effect of UV-sterilization on parasitism and emergence of *T. pintoi*

UV sterilization is widely used to extend the host egg storage in *Trichogramma* mass rearing, and has been reported to be feasible in several cases (Ksentini et al. 2014; Xu et al. 2016). The parasitism rate of *T. pintoi* declined on UV-treated *G. molesta* eggs, which was consistent with the findings by Lü et al (2014) and indicated the parasitism rate of *Trichogramma* decreased further as the intensity of UV treatment increased. Under our testing conditions, although the use of UV light in *Trichogramma* mass rearing may help extending the shelf-life of host eggs, it may also influence the productive efficiency of the parasitoids by reducing the availability of active nutrients in host eggs when physiological changes occur after UV treatment (Hu et al. 1999). Thus UV treatment might have decreased the host acceptance to *T. pintoi*. As for emergence rate, despite the emergence could be enhanced slightly by less competition with host embryo (through being killed by UV light), this did not compensate the effective nutrition lost. The results were also in agreement with findings reported by Moreno et al. (2009) in which UV treatment did not affect the number of progeny produced. Similarly, higher intensity and longer exposure time were suggested by Romeis et al. (1997) in mass rearing of *T. chilonis* to keep host eggs inactive and extend the storage period, but irradiated eggs were also found to be less suitable than untreated eggs. Nevertheless, UV sterilization could still be an effective method for long-term host

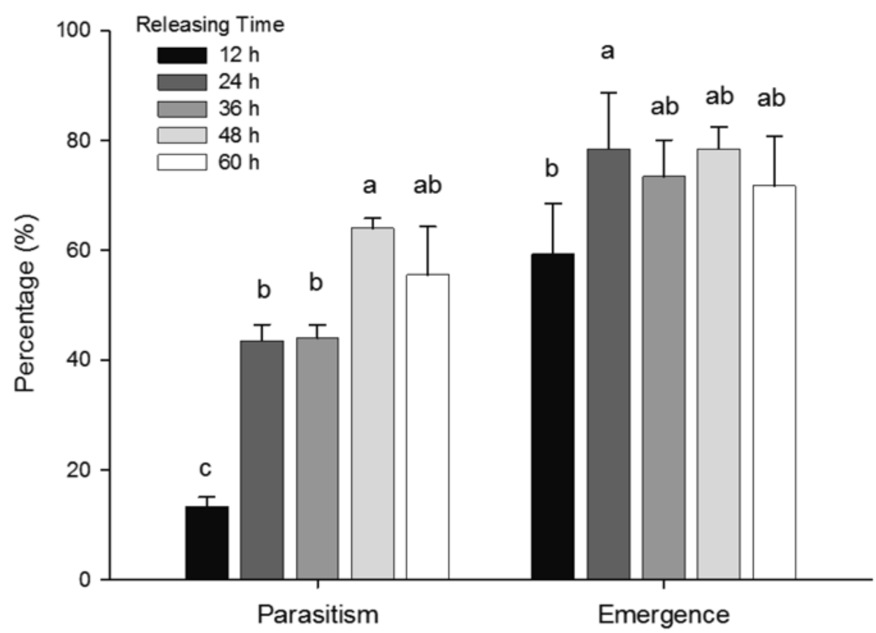

Fig. 5. The effects of *T. pintoi* releasing time on parasitism and emergence rate. Data in the column are means ± SE. Within each group, same letters indicate no significant difference (at the P < 0.05 level).

egg storage to reduce the cost in *Trichogramma* mass rearing with appropriate timeliness, intensity and distance of UV application (Ayvaz et al., 2008).

4.2 Effect of host egg age on the parasitism and emergence of *T. pintoi*

Host egg age is widely considered in *Trichogramma* releasing. Our results showed that releasing *T. pintoi* in the early stage of *G. molesta* eggs was beneficial to the parasitism efficiency. Under present conditions, *T. pintoi* showed higher preference to the young aged eggs of *G. molesta*, which could due to the development of host embryo. Host embryo growth may have an adverse effect on the development of parasitoid eggs (Pizzol et al. 2012). Similarly, most reports demonstrated that *Trichogramma* wasps prefer young and medium aged host eggs to old developed ones (Moreno et al. 2009; Pizzol et al. 2010; 2012; Makee 2005), as the nutrients in old eggs were incorporated and assimilated by the growing embryo (Ruberson & Kring 1993). Also the rotation and cephalic capsule sclerozitation in old aged hosts prevents the development of parasitoid eggs (Moreno et al. 2009).

75% of the host embryonic development was taken as the age boundary that whether *Trichogramma* could develop successfully in most host species (Pak 1986), which proportion reflects the age gap in host eggs. *T. pintoi* in this assay showed great preference for freshly laid eggs, in which the host embryo might have the least ability in nutrition competition. Our results contributed to proof the hypotheses that the older host embryos compete stronger with parasitoid larvae, thus *T. pintoi* grew less efficiently in *G. molesta* eggs aged 2–3 d compared with 1-d old eggs. When practically applied in mass rearing of *Trichogramma*, this phenomenon should be taken into consideration that using younger host eggs to facilitate higher efficiency of artificial rearing. As for orchard pest management, *Trichogramma* should be released at the very early stage in the appearance of pest eggs.

4.3 Effect of host egg density on the parasitism and emergence of *T. pintoi*

Host egg density usually influences the host searching efficiency and parasitism success in the narrow environment (Gingras & Boivin 2002). Pest density is also one of the key factors for deciding parasitoid releasing numbers in agriculture. The density dependent effect of host eggs was demonstrated by some researches (Henderson et al. 1993; Moreno et al. 2009; Lü et al. 2014), which is in consistent with our findings. When different densities of *G. molesta* eggs were provided, the parasitism rate of wasps showed a downward trend despite the amount of parasitized egg increased. We observed the highest parasitism rate when there were 15 to 45 eggs under our test conditions, although the numbers of parasitized eggs were higher in the 45, 60 and 75 eggs treatments. Likewise, *T. dendrolimi* showed similar parasitism rate as 85% when the host density was 15 eggs, but it decreased when the number of pest eggs increased (Davies et al. 2011). Some other *Trichogramma* species were also reported to show type II functional response to host density, such as *T. pretiosum* (Rami 1983).

The trend indicated that there is a certain difference between the parasitic efficiency of different *Trichogramma* species, but what is consistent is that the parasitism rate generally decreases with the continuous increase of host density. This could due to the increased probability of ineffective sting without enough fertilized eggs, while host eggs in low density lead to higher effective sting rates. Thus the parasitism rate could be enhanced. It was suggested that 3–4 pairs of *T. chilonis* should be released with fifty *Sitotroga cerealella* eggs, so the female production could be maximized in laboratory rearing (Muhammad et al. 2004). However, with fixed number of parasitoid, only the gradient of host eggs was considered in this assay. Future studies could be done by conducting the orthogonal experiments of host density and *Trichogramma* density to find best rearing combination of both.

4.4 Effect of *T. pintoi* density and releasing duration on the parasitism and emergence of *T. pintoi*

The release number of *Trichogramma* and duration are also key factors in mass rearing and pest management. The two factors showed a significant impact on parasitism rate in our assay. With the increase of number and time of *T. pintoi* inoculation, the parasitism rate showed an upward trend in our findings. Host locating time of parasitoids restricts the parasitism of *Trichogramma*: 48 h duration of parasitizing facilitates higher parasitism, while prolonged releasing time did not accumulate higher efficiency. This was probably due to the energy for host location and sting of *Trichogramma* individuals was limited (Khanh et al. 2012) and they tend not to trade off the parasitism to ineffective host selection (Pizzol et al. 2010). Therefore increased releasing time for 60 h was not beneficial in this case.

It is noteworthy that higher densities of *Trichogramma* wasps may lead to higher super parasitism (Muhammad et al. 2004). Thus the growth and development of the surviving larvae could be affected, which consequently results in low efficiency in parasitoid releasing. The decreased single female parasitism in our tests also indicated that *Trichogramma* density influenced intra-population competition (Pizzol et al. 2012). The competition among parasitoid female individuals might increase in limited space and with fixed number of hosts. Hyperparasitism rate might be the highest in 5 *Trichogramma* releasing group, because the lowest emergence was tested in this treatment. The phenomenon also verified that *T. pintoi* female individuals might have traded off some energy on host competition and ineffective oviposition (Pizzol et al. 2012). In the meantime, in *Trichogramma* mass rearing, optimal density should be tested and carefully chosen to obtain higher emergence rate.

Our findings provided baseline data for the biological fitness and release of *T. pintoi*. The results also contributed to the control of *G. molesta* in peach orchards as well as other fruit trees in the future. In order to acquire better effectiveness and control efficiency, the investigation and detection of population occurrence of *G. molesta* should be carefully conducted. Then the appropriate releasing time and amount of the parasitoids could be decided. On the other hand, mass rearing of *Trichogramma* already has almost ten decades history, but with the increasing demand of these biology control agents in China with the development of organic farming, we are faced with a vital issue that sufficient *Trichogramma* are in bad need. Even so, we could not just focus on quantity. Quality is still the main issue when it refers to enhancing the releasing efficiency and reducing the rearing and shipping cost. Thus it is important for testing the female progeny and successful offspring development to better understand how UV-light, host age and density, parasitoid density and releasing duration affect the reproduction of *T. pintoi*. Also, to better estimate the releasing efficiency, mortality and emergence of *Trichogramma* parasitoids should be investigated after inundative releases in the field. Moreover, future studies are also required to test the releasing method of *T. pintoi* for the management of *G. molesta* in orchards, e.g. how they could be used in IPM programs without being impaired by pesticides commonly applied in crops (Desneux et al. 2005; 2007).

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