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ORIGINAL ARTICLE

Inoculative releases of *Trichogramma dendrolimi* for suppressing the oriental fruit moth (*Grapholita molesta*) in peach orchard in China

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Abstract – Introduction. Integrated pest management is becoming popular in China. To supply safe peach fruits (*Prunus persica* cv. Okubo) to the population and to preserve the environment, this study aimed to investigate the biological control of *Grapholita molesta* (oriental fruit moth, OFM) using *Trichogramma dendrolimi* and to discuss how to effectively use this wasp for managing OFM. **Materials and methods.** *T. dendrolimi* was released with 1-week frequency at different release distances (5.0, 7.5, 10.0, 12.5 and 15.0 m) in 5 experimental plots, and the wasp density was surveyed after release. The OFM adult number, effective wilting shoot number, and damaged peach number were surveyed twice. **Results and discussion.** *T. dendrolimi* was the dominant natural enemy of OFM in the peach orchard. Wasp density was reduced in a release distance-dependent manner in the treated plots and was much lower than that in the control plots. The OFM adults of the 2nd generation were not obviously suppressed by *T. dendrolimi*. Compared with the control plot, the numbers of effective wilting shoots and damaged peaches were significantly reduced by releasing wasps ($P < 0.05$), especially in the 15 m release plot. However, there was no significant difference in the density of the wasps during the two surveys, the rate of increase of the number of effective wilting shoots, the rate of decrease of the number of damaged peaches or the number of OFM adults. **Conclusion.** The inoculative release of *T. dendrolimi* is an effective and safe approach to inhibiting OFM population. Moreover, releasing the wasps at a distance of 15 m is strongly recommended in practice.

Keywords: China / peach (*Prunus persica*) / oriental fruit moth (*Grapholita molesta*) / *Trichogramma dendrolimi* / biological control / orchard pest management

Résumé – Inoculation par lâcher de *Trichogramma dendrolimi* pour la suppression de la tordeuse orientale du pêcher (*Grapholita molesta*) dans les vergers en Chine. Introduction. La gestion intégrée des ravageurs est devenue populaire en Chine. Pour assurer la sécurité d'approvisionnement des populations en fruits du pêcher (*Prunus persica* cv. Okubo) et pour préserver l'environnement, l'étude du contrôle biologique de la tordeuse orientale (OFM) *Grapholita molesta* a été menée en utilisant *Trichogramma dendrolimi* et en discutant l'efficacité des méthodes de gestion de l'OFM par ces guêpes. **Matériels et méthodes.** Les guêpes *T. dendrolimi* ont été libérées chaque semaine à des distances différentes des arbres (5.0, 7.5, 10.0, 12.5 et 15.0 m) dans 5 parcelles expérimentales, et leur densité a été enregistrée après la libération. Le nombre d'OFM adultes, le nombre effectif de pousses flétries, et le nombre de pêches endommagées ont été enregistrés à deux reprises. **Résultats et discussion.** *T. dendrolimi* s'est révélé être la population dominante d'ennemis naturels des tordeuses orientales dans les vergers de pêchers. La densité de ces guêpes diminuait en fonction de la distance des lâchers dans les parcelles traitées et était beaucoup plus faible que dans la parcelle témoin. Les adultes OFM de 2e génération n'ont évidemment pas été éliminés par *T. dendrolimi*. Par rapport à la parcelle témoin, le nombre de pousses de pêcher atteintes de flétrissement effectif et le nombre de fruits endommagés ont été significativement réduits par l'introduction des guêpes dans les vergers ($P < 0,05$), en particulier dans un rayon de 15 m. Aucune différence significative n'a été trouvée sur les deux enquêtes concernant la densité de population de guêpes, le taux d'augmentation du nombre de pousses effectivement flétries, le taux de diminution du nombre de pêches endommagées ou le nombre d'adultes OFM. **Conclusion.** Les lâchers de parasitoïdes *T. dendrolimi* sont une approche efficace et sûre pour contrôler la population de tordeuse orientale du pêcher. En outre, la libération des guêpes dans un rayon de 15 m est une pratique fortement recommandée.

Mots clés : Chine / pêcher (*Prunus persica*) / tordeuse orientale du pêcher (*Grapholita molesta*) / *Trichogramma dendrolimi* / contrôle biologique / gestion des ravageurs des vergers

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1 Introduction

The oriental fruit moth (OFM), *Grapholita molesta* (Lepidoptera: Tortricidae), is an important worldwide pest of stone and pome fruits [1, 2]. Its larvae damage the fruit directly by boring into them or cause shoot dieback by feeding on twigs. Currently, the management of OFM is largely dependent on the use of broad-spectrum insecticides [3]. However, the chemical control of this pest is difficult because of its concealed and large host distribution characteristics [4]. Moreover, the extensive use of chemical insecticides causes several problems, including resistance to insecticides, toxicity to natural enemies and food residue. Therefore, it is important to develop alternative control technologies.

Currently, several harmless methods, such as the application of sugar and vinegar trapping [5] and sex-pheromone-mediated mating disruption [6, 7], have been researched and developed. However, the former traps both OFM and other non-target insects, including natural enemies, and the latter is costly and time-consuming for the season-long control of OFM, making them unsuitable [8]. The biological control of OFM might solve the problems described above. The introduction of natural enemies has been proved to be a safe and effective method that can significantly reduce the economic losses caused by OFM [9].

Our previous study has demonstrated that the dominant natural enemy of OFM in peach orchards is *Trichogramma dendrolimi* [10]. These wasps lay their eggs inside the eggs of insect pests [11]. Preliminary results indicate that these natural enemies can be used for the management of OFM. The chalcidoid wasps *T. dendrolimi* have been extensively used in the biological control of certain lepidopterous species [12]. In China, rearing of this wasp species *in vitro* has been researched for large-scale production [13]. However, there is no information concerning the biological management of OFM in China. It was important to investigate how to effectively use these chalcidoid wasps for the bio-control of OFM. A series of release densities of *T. dendrolimi* in peach orchards were established in the present study to check the control efficiency of OFM at different *T. dendrolimi* release densities.

2 Materials and methods

2.1 Experimental fields design

All the experiments were performed between May 2013 and June 2013, at a large peach orchard zone (112°29′ E, 37°18′ N) of Xishandi village of Houcheng town, Taigu county, Shanxi province, China. The peach trees (*Prunus persica* cv. Okubo) used for field experiments were 6 years old with average tree height of 2.5 m. The trees were spaced 3.5 m × 5.0 m apart and were arranged in single rows. The humid continental climate here with annual mean temperature of 9.8 °C and mean precipitation of 442 mm was very suitable for the growth of peach trees.

In this large peach orchard zone, 6 plots (parcel area of each is 667 m^2) were randomly selected for the experiments. Five were chosen as treatment plots and the last one as a control plot.

2.2 Survey of dominant natural enemy population

Before the natural enemy control experiment, yellow water traps filled with water were used to trap natural enemies of OFM. A dilute detergent was added to the water to break the surface tension and thus keep the insects in the water [14]. The traps were placed in a five-sampling pattern [15,16] throughout the whole peach orchard. A total of 4 surveys were conducted to identify the dominant natural enemy population throughout the peach orchard. The number of each trapped natural enemy, especially the parasitic wasps, was recorded. Different species of parasitic wasps were identified by referring to the literature [17] or consulting an expert.

To identify the parasitic natural enemies, the eggs of OFM, which can be parasitized by these natural enemies, were collected using the five-point sampling method and brought back with the moth to our laboratory for raising them. The eggs were raised in a manual climatic box with a temperature of 26 ± 1 °C, a humidity of 70% to 80%, a photoperiod (light: darkness) of 15:9 h, and light intensity of 60 μmol m^{-2} s^{-1}. Once the wasps hatched, their species were identified.

2.2.1 Release of *T. dendrolimi*

T. dendrolimi was released at 8 points in each plot at distances of 5, 7.5, 10, 12.5 and 15 m. The prospective density of *T. dendrolimi* at each release distance was 8×10^4 wasps ha^{-1}, 3.6×10^4 ha^{-1}, 2×10^4 wasps ha^{-1}, 1.3×10^4 wasps ha^{-1}, and 0.9×10^4 wasps ha^{-1}, respectively. Release points were more than 10 m from the plot margins. The egg cards were hung with a pin on the leaves or under the branches. The release was performed at 10 am or 4 pm on a sunny day between 5 June and 24 July 2013 every 7 days, 7 times. The control plot was not treated.

2.2.2 Survey of *T. dendrolimi*

To survey the density of these parasitic wasps after release, yellow water traps with water and detergent were placed at 10 m intervals in the 6 plots at 8 am on the day of release. The number of wasps was investigated twice (26 June and 26 July). Wasps were collected at 6 pm on the same day and identified under the stereoscope. The change in the number of wasps between the two investigation times was calculated.

2.3 Survey of oriental fruit moth (OFM) and related damages

To trap adult OFM moths, a trapping basin was designed with a lure. A trap without the lure was used as a control. A total of 3 trapping basins with lure and a control trap were placed at each experimental plot 5 days before releasing the parasitic wasps. The survey was conducted twice, and the change in the number of moths was calculated.

2.3.1 Survey of wilting shoots

A total of 100 wilting shoots in each plot were broken off to investigate whether the trees were damaged by OFM. Five

sampling points around the release points were selected. Wilting shoots with OFM larva or signs of OFM damage (such as fecula and gummosis) were considered effective wilting shoots, and their number was recorded. This survey was also conducted twice, and the change in the number of effective wilting shoots was calculated.

2.3.2 Survey of insect infested peaches

The insect-infested peaches were investigated by a similar method to the wilting shoot survey. Peaches with OFM larva or signs of OFM damage were considered infested peaches. In addition, the change in the number of insect-infested peaches was calculated.

2.4 Statistical analysis

One way analysis of variance (ANOVA) was performed after homogeneity of variance testing of all the data, followed by means comparison using Duncan's multiple range test. P-value < 0.05 was considered to represent a significant difference.

3 Results and discussion

3.1 Dominant natural enemies

OFM is among the most important pests causing economic losses in many countries. Compared with other management strategies, biological control is cost-effective and eco-friendly and is widely applied in agricultural production. In this study surveying OFM-parasite enemies, 6 enemy species of *Grapholita molesta* were identified: *Trichogramma dendrolimi*, *Coccygomimus disparis*, *Pristomerus* sp., *Braconidae* sp., *Trichogramma ostriniae*, and *Elodia morio*. *T. dendrolimi* was the dominant species and accounted for 54.7 ± 0.07% of all these species.

Feng *et al.* [18] performed an isotope-labeling experiment by releasing *T. dendrolimi* labeled with P^{32} at a dosage of 25 μci mL^{-1} to an orchard and found that the parasitism rate of *T. dendrolimi* in *Adoxophyes orana* eggs was 93%, suggesting a high parasitism rate of *T. dendrolimi* in the orchard. In the current study, parasitism was detected by randomly rearing wild OFM eggs in the laboratory. This experiment confirmed that the OFM eggs were mainly parasitized by *T. dendrolimi*, which suggested that the chalcidoid wasp *T. dendrolimi* is a dominant natural enemy of *T. dendrolimi* in the peach orchard at Xishandi village of Houcheng town, Taigu county, Shanxi province, China.

3.2 Effects of the release distance on the density of *T. dendrolimi*

The release strategies of natural enemies, including time and distance, are important for pest control [19]. It has been reported that the release time should be in accordance with the oviposition period of the host [19]. Usually, more than one release time is chosen for pest management according to the cycle of generations occurring in a year. One limitation of the field experiment in our study was that we only evaluated the short-term efficacy of *T. dendrolimi* for OFM control, as the release conducted was based on one generation of OFM. However, we chose 2 times per day to release the *T. dendrolimi*: 10 am and 4 pm. According to our previous experience, very early release is not beneficial for wasp diffusion because of high humidity, whereas a very late release is unsuitable for finding their hosts; in addition, release during a hot noon will reduce the wasps' activity.

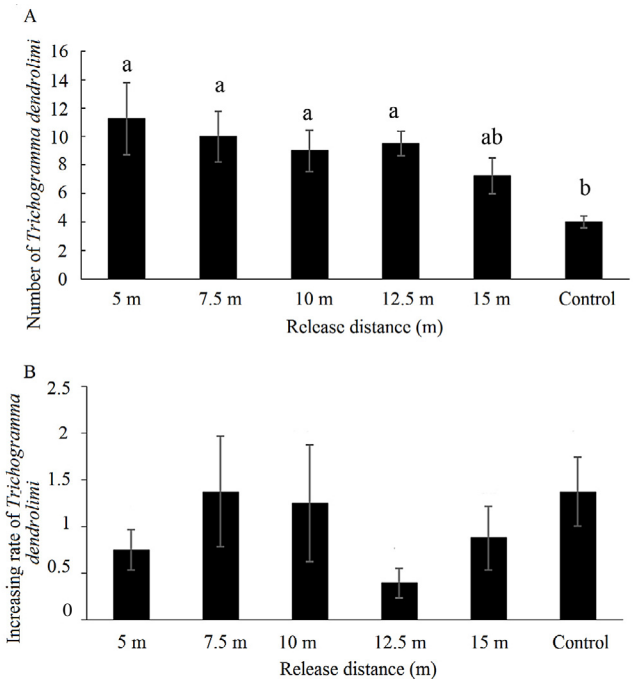

Figure 1. Effects of different release distances on (A) the mean density and (B) the rate of increase of *Trichogramma dendrolimi.* The control plot is the plot where no *T. dendrolimi* is released. Significant differences are marked on the error bar of each column. Replicates = 3, sample size = 100.

The number of *T. dendrolimi* in the different release distance plots was recorded. As shown in *figure 1A*, the population density was much higher in the wasp release plots than in the control plot ($P < 0.05$). It appeared that the population density decreased in a release distance-dependent manner. The differences among these treatment groups were not significant. It is not difficult to explain this phenomenon: once the wasps are released, they spread in an unknown distribution pattern [20] to avoid high inner population density in favor of finding hosts.

Compared with the first survey, the *T. dendrolimi* density changed irregularly. Different release distance did not significantly affect the increase in wasps ($P > 0.05$, *figure 1B*), indicating that the population of wasps was stable during the two surveys. A stable population of these enemies is beneficial for reproduction; thus, all the release densities or release distances of *T. dendrolimi* in our strategy were feasible from this perspective.

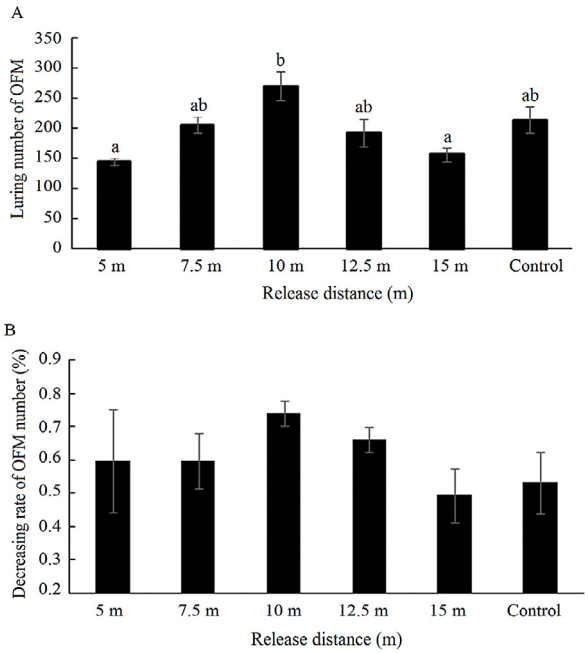

Figure 2. Effects of different release distances on the number of OFM adults (A) and its rate of decrease (B). The control plot is the plot where no *Trichogramma dendrolimi* is released. Significant differences are marked on the error bar of each column. Replicates = 3, sample size = 100.

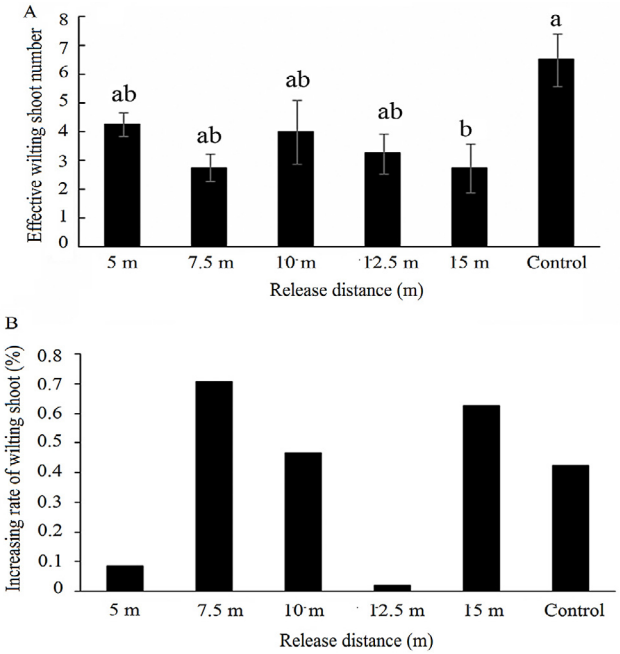

Figure 3. Effects of different release distances on (A) the wilting shoot number, and (B) its rate of increase. The control plot is the plot where no *Trichogramma dendrolimi* is released. Significant differences are marked on the error bar of each column. Replicates = 3, sample size = 100.

3.3 Effect of the release distance on the damaged trees

To detect the effect of different release distances of *T. dendrolimi* on the management of OFM, first, the effect on the number of OFM was evaluated. Different release distances were found to be significantly associated with the number of OFM (*figure 2A*). More OFM adults were lured in the 10 m release distance plot than in the other treated groups ($P < 0.05$). Different treatment plots did not significantly affect the number of OFM (*figure 2B*).

It was reported that approximately 4 generations of OFM adults occur per year in central China, generally with one month per generation [21]. The first emergence period is in late May. Based on this theory, our two prior surveys were conducted for the 2nd and the 3rd emergence generations. The 3rd generation of OFM adults was found to be significantly suppressed compared to the 2nd generation after the wasps were released. This result might be explained by the parasitic position and release time of *T. dendrolimi*. That is, the release of *T. dendrolimi* in our study from June to July might not affect the 2nd generation of OFM.

3.4 Effect of the release distance on the wilting shoot number damaged by OFM

We then analyzed whether different release distances significantly affected the damage by OFM larvae to the 3rd generation. The number of effective wilting shoots showed no significant difference among different release plots. However, the number of effective wilting shoots was significantly reduced at the release distance of 15 m compared to the control plot ($P < 0.05$, *figure 3A*).

The number of effective wilting shoots in the second survey was elevated compared with the first survey (*figure 3B*). The rate of increase of effective wilting shoots was not significantly different between different groups ($P > 0.05$).

3.5 Effect of the release distance on the OFM-infested peaches

Data from the damage of OFM larvae to peaches showed that (*figure 4A*) the wasps significantly reduced the number of OFM-damaged peaches. However, different release approaches did not significantly affect the rate of decrease of the damaged peach number (*figure 4B*).

In China, the release frequency of *Trichogramma* wasps for Asian corn borer is 3 to 2 releases per generation. From an economic perspective, a release of wasps every two weeks may also be efficient in inhibiting OFM occurrence in peach orchards. However, it is well known that the wasps manage pests by parasitizing the eggs of their hosts. Thus, the control may be limited to the egg stage. Therefore, in practice, this type of biological management should be conducted in coordination with other strategies, such as sex pheromone composition for adult control [22]. Sex pheromone composition is suggested for use in monitoring overwintering OFM adults prior to peach bloom. When there are sufficient adults to require management, the *T. dendrolimi* species should be released at a distance of 15 m.

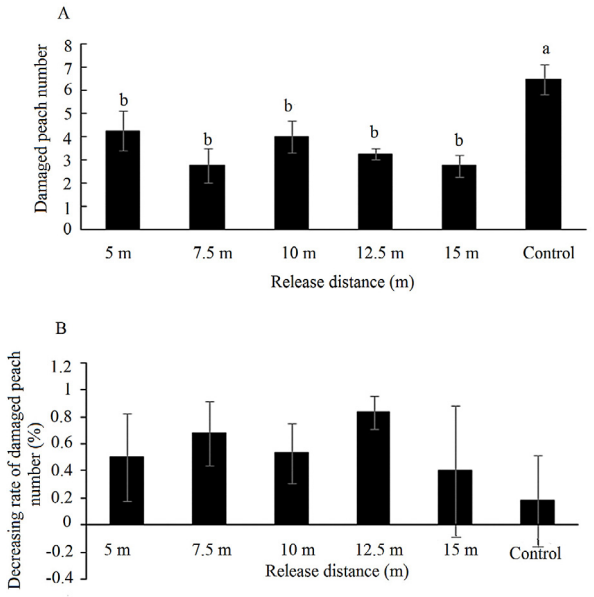

Figure 4. Effects of different release distances on (A) the damaged peach number, and (B) its rate of decrease. The control plot is the plot where no *Trichogramma dendrolimi* is released. Significant differences are marked on the error bar of each column. Replicates = 3, sample size = 100.

During this process, the wasp density and release frequency should be adjusted according to the OFM amount.

In this study, the effect of different release distances on the biological control of OFM was evaluated. Survey data suggested that the density of wasps in the release plots was markedly elevated compared to the control plot. Although the densities in different treatment plots were not significantly different, the density changed in a distance-dependent manner, and the population was stable during the survey times. The control effect of *T. dendrolimi* on the damage of wilting shoots and fruits caused by OFM larvae was then analyzed. It was found that the effective wilting shoot, damaged peach and OFM numbers were significantly reduced in the release plots compared to the control plot, especially at the release distance of 15 m, indicating that this release distance is superior for OFM control. Moreover, the study found no significant differences in the changing rate of wasps during the two surveys. In addition, the rate of increase in the number of effective wilting shoots and the rate of decrease in damaged peach number showed no significant difference. This result indicated that the efficacy of this control strategy required a relatively long time. Taken together, the study showed that the most effective *T. dendrolimi* release approach for OFM management in peach orchards is a release distance of 15 m (0.9×10^4 wasps ha^{-1}).

4 Conclusion

This study based on natural enemies, pests and host plants suggests that the inoculative release of *T. dendrolimi* may be an effective and safe approach to inhibiting the OFM population in peach orchards. Moreover, releasing the wasps at a distance of 15 m is strongly recommended in practice. However, this approach is recommended for use in combination with other management strategies for the long-term control of OFM.

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