

Potential Use of *Trichogramma pintoii* as a Biocontrol Agent Against *Heortia vitessoides* (Lepidoptera: Pyralidae)

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Abstract

Heortia vitessoides Moore is the most serious insect defoliator of *Aquilaria sinensis* (Lour.) Gilg, an endangered and economically important plant that produces highly prized agarwood. Samples from recently identified indigenous natural populations of *Trichogramma pintoii* Voegelé were collected from *H. vitessoides* eggs in *A. sinensis* forests in Yunnan Province, China. To assess the potential capacity of this parasitoid for use as a biological control agent, its functional response, female reproductive potential, and male insemination capacity were investigated in this study. Females successfully parasitized 1- to 4-d-old eggs of *H. vitessoides* but failed to parasitize 5- to 8-d-old eggs. The parasitoid exhibited a Holling type II functional response, and the estimated maximum numbers of 1- to 4-d-old *H. vitessoides* eggs parasitized by a single *T. pintoii* female were 38.1, 29.8, 26.0, and 22.2 eggs over a 24-h period, respectively. Additionally, the parasitoid's average lifetime fecundity was 89.8 ± 2.5 eggs, of which 66.26% were laid within the first 2 d. The average number of total females that mated with a male in his lifetime (4.70 ± 0.13 d) was 10.4, and the average number of total daughters of a male was 292.1. On day 1 of male adult life, the greatest number of females were inseminated by males, and the most daughters were produced; however, the number of copulations and insemination ability decreased rapidly with male age. These results suggest that *T. pintoii* is a promising candidate for inundative release against *H. vitessoides* in China, and these findings will guide efforts in achieving mass production of this parasitoid.

Key words: *Heortia vitessoides*, *Trichogramma pintoii*, functional response, male insemination capacity, biological control

Native to China and belonging to the family Thymelaeaceae, *Aquilaria sinensis* (Lour.) Gilg is an economically important evergreen tree that is mainly distributed in southern and coastal areas, including Yunnan, Hainan, Guangxi, Guangdong, Fujian, and Taiwan (Wang et al. 2007). *Aquilaria sinensis* is included on the list of rare and endangered species in China (Fu 1992). After *A. sinensis* is wounded by humans or natural causes, resin accumulates in the wood to produce valuable agarwood, which is widely used in religious ceremonies, traditional medicine, and the incense industry (China Pharmacopoeia Commission 2015).

Heortia vitessoides Moore (Lepidoptera: Pyralidae) is the most destructive pest of *A. sinensis* (Qiao et al. 2018). It has been reported that serious damage to *A. sinensis* caused by *H. vitessoides* occurs in China, Malaysia, India, and other countries (Singh et al. 2000, Sajap 2013, Yan and Yue 2019). At present, the application of insecticides remains a major strategy for the control of this pest because they act quickly and are efficient, easy to use, and cost effective (Zhou et al. 2016). However, pesticides may be harmful to the environment

and humans as well as to other nontarget organisms, especially the natural enemies of *H. vitessoides*, and therefore, pesticide use may encourage secondary pest outbreaks. Moreover, an important disadvantage is the occurrence of serious pesticide residues in *A. sinensis* plantations, which severely threaten the high quality and value of agarwood. It is, therefore, essential that biological control agents against *H. vitessoides* be investigated for application in the field. To date, the biological control of *H. vitessoides* has been studied in terms of the use of predatory natural enemies (Qiao et al. 2013) and pathogenic bacteria (Rishi et al. 2016), but to the best of our knowledge, *H. vitessoides* parasitoids have not been evaluated. In December 2017, we first found the parasitoid wasp *Trichogramma pintoii* Voegelé (Hymenoptera: Trichogrammatidae) parasitizing *H. vitessoides* eggs in Jinghong city, Yunnan Province, China, and the natural parasitism of *H. vitessoides* eggs by *T. pintoii* reached an average of 87.73% in the field.

Some of the most common and important natural enemies are wasps of the genus *Trichogramma*, which have been used worldwide

to control pests, particularly Lepidoptera, for many years because of their effective and stable control effects on target pests and ease of large-scale artificial breeding using alternative host eggs (Smith 1996, Khan et al. 2015, Razinger et al. 2016, Wu et al. 2018). *Trichogramma pintoi* parasitized *Ocinara varians* Walker (Ou et al. 2006), *Ephestia kuehniella* Zeller (Robert et al. 2016), and *Cydalima perspectalis* Walker (Göttig and Herz 2016). Currently, the application of *Trichogramma* depends mainly upon inundative releases, which require numerous *Trichogramma* individuals in the short term. The functional response is an important attribute for measuring the efficacy of natural enemies (Li et al. 2007). Holling (1959) classified the functional response into three types based on the shape of the response curve, as characterized by whether the number of prey consumed increases linearly (type I), hyperbolically (type II), or sigmoidally (type III). Holling (1959) modeled the type II response using the 'disc equation'. Female lifespan, egg load, and number of offspring are important indicators of fertility. To assess the efficacy of a parasitoid in controlling a target pest and to determine the optimum amount of parasitic wasps to release as biocontrol agents, knowledge of the functional response, and reproductive biology of the candidate is essential (Ballal and Singh 2003, Mills and Lacan 2004).

When *T. pintoi* is used as a biocontrol agent, another important aspect that should be considered is the sustainability of its ability to prevent *H. vitessoides* attack. The high insemination capacity of males is key to continuous pest control by *T. pintoi*. Regarding this, Martel et al. (2016) proposed the index of insemination strategy (IIS), which represents the ratio of the mean number of total females mated with a male on an emergence patch to the mean number of females available per male at emergence on that patch. *Trichogramma* species are haplodiploid; their unfertilized eggs (haploid) develop into males and fertilized eggs (diploid) into females (Heimpel and de Boer 2008). Thus, the number of female offspring reflects the male individual reproductive capacity to some extent (Godfray 1994). The quality of both the females and males will affect the capacity of *T. pintoi* to control *H. vitessoides*. In addition to female fertility and the functional response, male insemination capacity should be considered in studies evaluating the potential of *T. pintoi* as a biocontrol agent of *H. vitessoides*. Inundative release of *T. pintoi* is a promising alternative for controlling *H. vitessoides* in China, preventing, or at least decreasing, demand for pesticide application. Our aims in this study were to i) evaluate the potential of *T. pintoi* to control *H. vitessoides* and ii) assess the sustainability of its ability to prevent *H. vitessoides* attack to further recommend *T. pintoi* as an integrated pest management tool.

Materials and Methods

Aquilaria sinensis Seedlings

Aquilaria sinensis seeds were sown in trays filled with seed raising mix. After 40 d, the seedlings with four true leaves were transplanted into pots and grown until they were large enough to transplant to the experimental site of the Department of Yunnan Branch, Institute of Medicinal Plant Development, where they were spaced at 1.0 × 0.6 m. The plants were watered and fertilized regularly.

Hosts

Egg masses of *H. vitessoides*, collected from *A. sinensis* leaves in Jinghong city, Xishuangbanna prefecture, Yunnan Province, China (location 22.01°N, 100.79°E), were placed in transparent

plastic containers (length 20 cm, width 13 cm, and height 7 cm) with screened, ventilated lids. Approximately eight fresh leaves of *A. sinensis* taken from the *A. sinensis* plants in the experimental site of the Department of Yunnan Branch, Institute of Medicinal Plant Development, were provided to the larvae as food in each plastic container. The leaves were replaced daily to prevent their desiccation. Soft sands were provided to late-instar larvae for pupation. Then, ~100 newly emerged adult *H. vitessoides* were obtained and transferred into cages (60 × 40 × 35 cm) made of 100-mesh high-density gauze over wood frames that contained one potted *A. sinensis* plant for egg laying. The adults were fed with a 10% sucrose solution. The plants and sucrose solution were renewed every day. The *H. vitessoides* colony was maintained according to these specifications, and fresh eggs were obtained daily and used in the experiments.

Parasitoids

Indigenous populations of *T. pintoi* were obtained in 2017 from *H. vitessoides* eggs on *A. sinensis* in Jinghong city, Xishuangbanna Prefecture, Yunnan Province, China (22.01°N, 100.79°E). The parasitoid was maintained on *Corcyra cephalonica* (Stainton) eggs (Lepidoptera: Pyralidae) (<24 h old) sterilized by UV radiation (254 nm, 30 W, 50 cm from light source for 30 min). The parasitoids were fed a 10% sucrose solution at 25 ± 1°C and 75 ± 5% RH under a photoperiod 12:12 (L:D) h. Eggs of *C. cephalonica* reared on corn flour inside an incubator (Wu et al. 2017) were collected every day. An egg card made of ~100 irradiated *C. cephalonica* eggs glued onto a 1.0 × 1.0-cm piece of graph paper with gum arabic was exposed to ~20 newly emerged and mated female wasps for 8 h in a transparent plastic tube (10 cm in length and 1.3 cm in diameter) with a plug of absorbent cotton. The wasps started to emerge ~10 d later. In this way, the parasitoid colony was established.

The experiments were performed under laboratory conditions of 25 ± 1°C, 75 ± 5% RH, and a photoperiod of 12:12 h (L:D) unless otherwise indicated. None of the females used had been previously exposed to hosts.

Functional Response Experiments

We conducted these experiments for each of the eight ages of *H. vitessoides* eggs. Six densities (5, 10, 20, 40, 80, or 160 per tube) of the hosts were exposed to a single mated *T. pintoi* female aged from 12 to 24 h for 24 h in transparent plastic tubes with plugs of absorbent cotton as described above. Each tube contained an egg card as described above. Each treatment was repeated 10 times. Degreasing cotton soaked with 10% sucrose solution was provided for the parasitoid. The number of black eggs was recorded as the number of parasitized eggs per female.

To fit the functional response of *T. pintoi* to host density, the Holling's type II model (Holling 1959) was used:

$$N_a = \frac{aN_T}{1 + aT_bN}$$

where N_a is the number of eggs parasitized by the parasitoid, N is the initial density of the host eggs, a is the instantaneous attack rate, T is the total time of host-parasitoid exposure (24 h in this study), and T_b is the handling time (h). Nonlinear least squares regression (Wilkinson 1989) was used to estimate the parameters a and T_b in the functional response equation. Moreover, the ratio of exposure time to handling time (T/T_b) represents the maximum number of hosts parasitized by a female (Hassel 1978).

Longevity and Daily Fecundity of Female *T. pinto*

A single, newly emerged and mated female wasp was introduced into a transparent plastic tube that contained a piece of graph paper carrying 80 *H. vitessoides* eggs less than 24-h old and a 10% sucrose solution for 24 h. The graph paper and sucrose solution were renewed daily until the death of the female. Lifetime fecundity and daily fecundity were determined by counting the number of parasitoid larvae and pupae among the exposed host eggs. Longevity was calculated by recording the date of emergence and death of the females. In this experiment, 20 females were tested. Furthermore, to determine egg load, 20 newly emerged virgin females were dissected, and all the mature eggs were counted.

Longevity and Fecundity of Male *T. pinto*

Mature *T. pinto* pupae (red-eyed) were individually placed in transparent plastic tubes, a wet strip of filter paper was added to maintain humidity, and each tube was plugged with cotton. Newly emerged, average-sized individual males were assigned to numbered tubes in which 10% sucrose solution was the source of food. Each male was provided a newly emerged and unmated female in a tube. After copulation occurred, the inseminated female was removed and placed in another tube. Another virgin female was provided to the male immediately. This experiment was repeated each day until the male exhibited no interest in a virgin female for half an hour. After 1 d, the experiment was repeated until the male again showed no interest in a virgin female. This experiment was repeated until the male died. Each inseminated female was introduced into a tube containing 80 *H. vitessoides* eggs less than 24-h old as hosts until the female parasitoid died. The parasitoids were provided with a 10% sucrose solution, and the tubes were plugged with absorbent cotton, labeled, and maintained at $25 \pm 1^\circ\text{C}$ and $75 \pm 5\%$ RH and under a photoperiod of 12:12 (L:D) h. The number of inseminated females and the longevity of male parasitoids were determined, and male insemination, represented by the total number of female offspring, was estimated. The number and sex of the F1 parasitoid offspring that emerged from the hosts at each male age were recorded, and the percentage of F1 females was calculated. This experiment was performed with 20 male wasps.

Statistical Analysis

The effect of female age on daily fecundity and the effect of male age on the number of copulations, female progeny production, and percentage of F1 females were subjected to one-way analysis of variance. The data were examined for normality and homoscedasticity followed by multiple comparisons of means, which were carried out

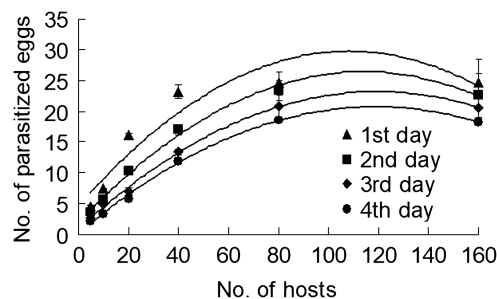


Fig. 1. Functional response of *Trichogramma pinto* to six different initial densities of *Heortia vitessoides* eggs under insectary conditions [$25 \pm 1^\circ\text{C}$; $75 \pm 5\%$ RH; photoperiod of 12:12 (L:D) h]. Symbols: observed mean \pm SE. The lines show the predicted responses based on the model.

with Fisher's protected least significant difference (LSD) test. All analyses were performed using SAS software (SAS Institute 1999). The significance level was 5% for all tests.

Results

Functional Response

Trichogramma pinto successfully parasitized the 1- to 4-d-old eggs of *H. vitessoides* but failed to parasitize 5- to 8-d-old eggs. According to the data shown in Figs. 1 and 2, the functional responses of *T. pinto* to the four host egg ages fit Holling type II. The proportions of parasitized 1-, 2-, 3- and 4-d-old *H. vitessoides* eggs decreased as N increased (Fig. 2). The disc equations of the functional response, instantaneous attack rate (a), handling time (T_h), and maximum number of parasitized hosts (N_{max}) for the 1-, 2-, 3-, and 4-d-old *H. vitessoides* eggs are shown in Table 1. The lowest and highest estimated attack rates were observed for 4- and 1-d-old eggs, respectively. The estimated handling time was shortest for the 1-d-old eggs and reached a maximum for the 4-d-old eggs. The ratio between a and T_h suggested that *T. pinto* prefers to parasitize 1-d-old eggs of *H. vitessoides* rather than 2- to 4-d-old eggs.

Daily Oviposition, Fecundity, and Longevity of Female *T. pinto*

The daily fecundity decreased with *T. pinto* female age, and females laid significantly more eggs on the first day than they did on any of the following 5 d ($F_{5,114} = 278.52$, $P < 0.0001$; Fig. 3). Although the oviposition period was ~ 5 d, 66.26% of the eggs were laid in the first 2 d. The average total fecundity across a female's lifetime (6.00 ± 0.15 d) was 89.8 ± 2.5 . The egg load of a female at emergence was 45.3 ± 3.7 .

Longevity and Fecundity of Male *T. pinto*

Immediately after emergence, the males started to actively pursue and inseminate females. The number of copulations, female progeny production, and percentage of F1 females declined with male age (copulations: $F_{4,95} = 157.57$, $P < 0.0001$; female progeny production: $F_{4,95} = 480.14$, $P < 0.0001$; percentage of F1 females: $F_{4,95} = 449.57$, $P < 0.0001$; Table 2). The mean longevity of *T. pinto* males was 4.70 ± 0.13 d, and a male copulated with 10.4 ± 0.3 females and exhibited a lifetime fecundity of 292.1 ± 9.6 daughters per male. Although the males were alive on day 5, they showed no interest

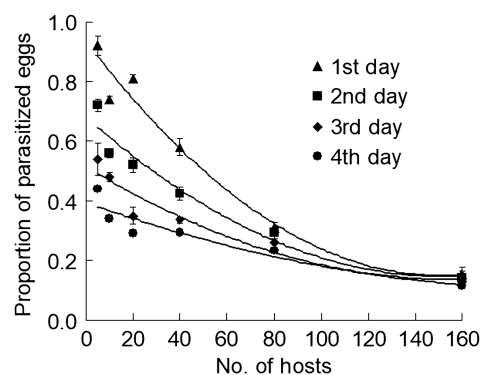
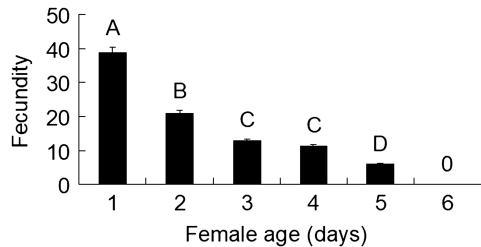


Fig. 2. Proportion of *Heortia vitessoides* eggs of different ages parasitized by *Trichogramma pinto* in relation to host density under insectary conditions [$25 \pm 1^\circ\text{C}$; $75 \pm 5\%$ RH; photoperiod of 12:12 (L:D) h]. Symbols: observed mean \pm SE.

Table 1. Estimates of the functional response parameters for *Trichogramma pintoi* parasitizing 1- to 4-d-old eggs of *Heortia vitessoides*

Age of host eggs	Disc equation of the functional response	Instantaneous attack rate (<i>a</i>)	Handling time (T_h) (d)	Maximum parasitized hosts (Na_{max})	Efficiency parameters (a/T_h)	Correlation coefficient R^2
1 d	$Na = 1.0359N/(1 + 0.0272N)$	1.0359 ± 0.0369	0.0267 ± 0.0013	38.1	39.3433	0.9829
2 d	$Na = 0.8206N/(1 + 0.0266N)$	0.8206 ± 0.0377	0.0335 ± 0.0022	29.8	23.6017	0.9884
3 d	$Na = 0.5958N/(1 + 0.0228N)$	0.5958 ± 0.0362	0.0392 ± 0.0028	26.0	15.4792	0.9923
4 d	$Na = 0.4643N/(1 + 0.0210N)$	0.4643 ± 0.0311	0.0485 ± 0.0070	22.2	10.3583	0.9805

**Fig. 3.** Average daily fecundity of *Trichogramma pintoi* females. All values are expressed as the means \pm SE. Mean values with different letters are significantly different from one another ($P < 0.05$; Fisher's LSD).

in female wasps. They produced a greater percentage of F1 females on day 1 than day 3, followed by day 4, but no females on day 5. Moreover, in our study, mated females produced sons and daughters, but unmated females produced only sons.

Discussion

Functional Response

Many factors affect the type of functional response exhibited by *Trichogramma* wasps, including their strain (Farrokhi et al. 2010), host species and density (Reay-Jones et al. 2006), as well as the temperature and relative humidity (Kalyebi et al. 2005). Furthermore, Montoya et al. (2000) considered that functional response studies conducted in a laboratory cannot represent the field situation. Although more field studies should be conducted to obtain more applicable results, laboratory research can provide a theoretical basis for standardized parasitoid production and has some value in evaluating parasitoid potential.

The genus *Trichogramma* has been reported to exhibit the three types of functional response. For example, the functional response of *Trichogramma minutum* Riley with *E. kuehniella* eggs (type I; Mills and Lacan 2004), *Trichogramma chilonis* Ishii parasitizing *Chilo sacchariphagus* Bojer (type II; Reay-Jones et al. 2006), *Trichogramma brassicae* Bezdenko parasitizing *Sitotroga cerealella* (Olivier) (type II; Farrokhi et al. 2010), and *Trichogramma ostriniae* Pang et Chen on *Ostrinia nubilalis* (Hübner) eggs (type III; Wang and Ferro 1998). In this study, the functional response of *T. pintoi* to *H. vitessoides* eggs was type II. Strand et al. (1986) found that parasitoids are unable to obtain nutrients from older host eggs, as the egg cuticle becomes harder with age. Our results indicate that the handling time of *T. pintoi* for 1-d-old *H. vitessoides* eggs was shorter than that for 2- to 4-d-old eggs, which probably occurred because the *H. vitessoides* egg cuticle hardens with age; thus, a longer oviposition time is necessary. Overall, our results suggest that *T. pintoi* may have stronger control potential against fresh *H. vitessoides* eggs than against old eggs. Similar findings were reported by Tunçbilek

and Ayzav (2003) for *Trichogramma evanescens* Westw. parasitizing *E. kuehniella* and by Pizzol et al. (2012) for *Trichogramma cacoeciae* Marchal parasitizing *Lobesia botrana* Denis and Schiffermüller and by Tian et al. (2017) for four *Trichogramma* species parasitizing *Cnaphalocrocis medinalis* (Guenée). At a temperature of $25 \pm 1^\circ\text{C}$, the maximum numbers of 1-d-old *H. vitessoides* eggs parasitized by 1-d-old *T. pintoi* in our study was higher than the value of 18.6 reported for 1-d-old *T. pintoi* when parasitizing 1-d-old *O. varians* eggs (Ou et al. 2006). However, the handling time of *T. pintoi* was shorter than the 0.0539 d estimated for *T. pintoi* when parasitizing *O. varians* eggs (Ou et al. 2006). Thus, *T. pintoi* appears to have greater control potential against *H. vitessoides* 1-d-old eggs than 1-d-old *O. varians* eggs.

Daily Oviposition, Fecundity, and Longevity of Female *T. pintoi*

In *Trichogramma* species, oogenesis was observed in the prepupal stage, and newly emerged females can oviposit (Volkoff and Daumal 1994). Based on the view of Flanders (1950), *Trichogramma* species can be classified into two types, pro-ovigenic (Volkoff and Daumal 1994) and synovigenic (Kuhlmann and Mills 1999), on the basis of the relationship between oogenesis and oviposition. In this study, females of *T. pintoi* laid more eggs during their lives than the egg load at emergence. This result indicates that *T. pintoi* are synovigenic, continuing to develop eggs throughout their adult life, which helps *T. pintoi* adapt to patchy host distribution and avoid egg depletion during attacks on gregarious hosts (Mills and Kuhlmann 2000). Moreover, the numbers of 1- to 4-d-old *H. vitessoides* eggs parasitized by *T. pintoi* indicated that the egg load of the parasitoid may limit its efficiency. The results showed that the females achieved their maximum reproduction when 40 host eggs were provided. Furthermore, a previous study showed that the daily fecundity of *Trichogramma pretiosum* significantly differed over time, with very high numbers of offspring being produced on the first day and fecundity decreasing over the remainder of the trial (Lindsey and Stouthamer 2017). These results were consistent with the results from our study showing that daily fecundity decreased with *T. pintoi* female age and that females laid significantly more eggs on the first day than they did on any of the latter 5 d (Fig. 3). In short, the high fecundity and rapid oviposition rate of this parasitoid contribute to *H. vitessoides* control.

Longevity and Fecundity of Male *T. pintoi*

The number of copulations, female progeny production and percentage of F1 females declined with male age (Table 2). This may be, and most likely, because of male senescence or sperm depletion. We calculated the IIS proposed by Martel et al. (2016) for *T. pintoi*, obtaining a value >1 (4.08 ± 0.36). The males of *T. pintoi* present generally high insemination ability, as found

Table 2. Insemination potential of *Trichogramma pintoi* males

Male age (d)	Number of females inseminated ^a	Number of daughters produced ^a	Percentage of F1 females (%) ^a
1	4.3 ± 0.2 A	129.6 ± 3.1 A	82.86 ± 1.95 A
2	2.8 ± 0.2 B	71.7 ± 2.6 B	78.35 ± 1.45 AB
3	2.2 ± 0.1 C	61.0 ± 2.2 C	76.24 ± 1.32 B
4	1.1 ± 0.1 D	29.8 ± 1.9 D	65.32 ± 2.36 C
5	0.0 ± 0.0 E	0.0 ± 0.0 E	0.00 ± 0.00 D

^aAll values are expressed as the means ± SE. Mean values with different letters are significantly different from one another ($P < 0.05$; Fisher's LSD).

for many other parasitoid species (Martel et al. 2016). The result indicating that an individual male *T. pintoi* is able to produce 292.1 females on average shows that a male can inseminate more than one female because it has more sperm than the egg supply of a single female. The trend of a reduction in the percentage of female progeny on successive days suggests that males transfer fewer sperm with successive inseminations. This apparent decrease in the number of transmitted spermatozoa noted for *T. pintoi* is different from the result indicating almost constant ejaculate volume with each insemination in males of the eulophid *Dahlbominus fuscipennis* (Wilkes 1965). The first 3 d of adult male age should be the focus of control efforts, as male sperm transmission capacity was found to be strongest during these 3 d. Indeed, males produced more female progeny over these 3 d. Males can produce 292.1 female offspring during their lifetimes, while the lifetime fecundity of females is 89.8 eggs. A male can copulate with and inseminate multiple females. The differences in intrinsic reproductive potential between female and male individuals of *T. pintoi* may be one of the reasons why the sex ratio of *T. pintoi* populations is often biased toward females. The status of the males is not considered to be as important as that of the females, but it plays an important part in the offspring sex ratio. Some scholars have proposed that the biased sex ratio is because of a physiological constraint due to sperm limitation (Chirault et al. 2018), and further exploration is needed regarding the contribution of males to the sex ratio.

In summary, this research has helped to enhance our knowledge of *T. pintoi*-*H. vitessoides* interactions and the reproductive capacity of *T. pintoi* females and males in the laboratory. The results indicate the potential capacity of *T. pintoi* to serve as an effective biological control agent against *H. vitessoides*. According to our results regarding functional response, a protocol involving 40 one-day-old eggs per tube exposed to an individual *T. pintoi* female for 24 h may be applied for mass-rearing purposes. Furthermore, to obtain a better control effect, inundative field releases of mass-reared 1-d-old mated *T. pintoi* every 5 d should be performed when most of the population of *H. vitessoides* is in the 1- to 4-d-old egg stage. Although the fecundity and offspring sex ratio are good predictors of field success in *T. pintoi*, the control effect of *T. pintoi* against *H. vitessoides* should be evaluated in the field.

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