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Quality assessment of three species of commercially produced *Trichogramma* and the first report of thelytoky in commercially produced *Trichogramma*

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Abstract

Weekly shipments of *Trichogramma pretiosum* Riley, *Trichogramma minutum* Riley, and *Trichogramma brassicae* (Bezdenko) (Hymenoptera: Trichogrammatidae) from a single insectary were assessed to determine whether survivorship with and without honey, emergence rate, and sex ratio were consistent over a five-week period in 2000. At least one shipment was misidentified by the insectary. Emergence rates were over 75% in 15 out of 17 shipments, although emergence varied significantly over the experimental period for each species. Mean longevity of honey-fed individuals was 7.16 days for *T. pretiosum*, 6.71 days for *T. minutum*, and 4.02 days for *T. brassicae*; mean longevity of unfed individuals was 2.68 days, 2.42 days, and 1.96 days, respectively. Mean sex ratios (proportion of individuals that were male) were 0.029 for *T. pretiosum*, 0.465 for *T. minutum*, and 0.444 for *T. brassicae*. Seventeen out of 18 virgin *T. pretiosum* females produced daughters, and three out of a subset of six of these females produced sons after being fed the antibiotic tetracycline. These results are consistent with infection by a parthenogenesis-inducing strain of *Wolbachia*.

Keywords: Biological control; Insectary; Natural enemies; Quality control; Thelytoky; *Trichogramma*

1. Introduction

Egg parasitoids in the genus *Trichogramma* are the most intensively produced natural enemies in the world (Li, 1994). Commercially produced species of *Trichogramma* attack the eggs of many lepidopteran species and have been used as biological control agents for a number of agricultural pests. The short generation time of *Trichogramma* wasps, and the fact that they can be reared on factitious hosts, allows these wasps to be produced quickly and affordably relative to other parasitoids (Li, 1994; Smith, 1996). Despite the user-friendliness of *Trichogramma* species, there have been at least as many failures of biological control using *Trichogramma* as there have been successes (Smith, 1996), and the quality of mass-reared wasps has been evaluated as one potential source of variability in the outcome of these biological control programs (Bigler, 1989; Cerutti and Bigler, 1995; Kuhlmann and Mills, 1999; Losey and Calvin, 1995; O'Neil et al., 1998). One aspect of natural enemy quality that is important to biological control programs is whether fitness parameters in commercially produced wasps fluctuate over time.

Trichogramma wasps are haplo-diploid and typically produce male offspring from unfertilized eggs (arrhenotoky). However, certain strains of *Trichogramma* contain thelytokous females, which produce female offspring from unfertilized eggs. In most cases, thelytokous *Trichogramma* are infected with parthenogenesis-inducing (PI) *Wolbachia* (Stouthamer et al., 1993). *Wolbachia* endosymbionts often compromise the fecundity and alter the behavior of infected strains (Silva, 1999; Silva et al., 2000; Stouthamer, 1993). Nevertheless, thelytokous strains of *Trichogramma* may be superior biological

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control agents under conditions of host limitation (Silva et al., 2000; Stouthamer, 1993; Stouthamer and Luck, 1993).

This paper presents the results of experiments that investigated aspects of quality for three species of commercially produced *Trichogramma*. We determined whether the emergence rate, sex ratio, and survivorship of fed and unfed wasps were consistent over time for weekly shipments of *Trichogramma pretiosum* Riley, *Trichogramma minutum* Riley, and *Trichogramma brassicae* (Bezdenko). Also, we demonstrated the presence of "curable" thelytoky in a commercially produced strain of *T. pretiosum*, which is likely the result of *Wolbachia* in this strain.

2. Methods

Shipments containing 6000 *T. minutum*, *T. pretiosum,* and *T. brassicae* were received as loose parasitized *Ephestia kuehniella* Zeller eggs from Beneficial Insectary (Oak Run, CA, 96069) on 18 April, 23 April, 1 May, 15 May, and 23 May 2000. Two additional shipments of *T. brassicae* were also examined on 15 and 17 April. The species identification of several shipments was verified using the taxonomic key published by Pinto (1998) and verified by Dr. J.D. Pinto. Male voucher specimens were mounted in Canada balsam (Carolina Biological Supply Company, Burlington, NC) and deposited in the insect museums at the Illinois Natural History Survey and at the University of Minnesota. For all experiments, wasps were reared in a growth chamber at 75% r.h., 16:8 L:D h photoperiod, and 25 °C. Upon arrival, the parasitized eggs were evenly distributed into two 60 mm diameter plastic petri dishes. For future studies, one dish was given a thin streak of undiluted honey, and the other was not. Both dishes were sealed with Parafilm M (American Can Company, Chicago, IL) to prevent the escape of wasps.

2.1. Assessment of quality parameters

Emergence rate and sex ratio was determined for 100 randomly chosen host eggs from each shipment using dimorphic antennal setation and genitalia features observable at 50×. Eggs that did not yield wasps within one week were scored as unemerged. The emergence rates and the sex ratios were compared among shipments using contingency table analyses.

Survivorship of honey-fed and unfed females was assessed for each shipment, except the 18 April *T. brassicae* shipment. Within 24 h of emergence, 20 female wasps of each species were separated out of the respective treatment dishes and placed individually into 0.5 ml microcentrifuge tubes. Wasps in the honey-fed treatment received a 2 mm diameter drop of honey in the cap of the tubes. Mortality of wasps was recorded daily until all the wasps were dead. Mortality in the fed treatment was categorized as death by natural causes or death by honey. Wasps were counted as 'dead by honey' if they were discovered in the honey during inspection. Survivorship was compared among shipments using a proportional hazards model, and the 'dead by honey' wasps were coded as censored data in the analysis. The mean number of days alive for fed and unfed wasps of each species was compared using Kruskal–Wallis rank sum tests.

2.2. Thelytoky and antibiotic treatment

To test for thelytoky and the presence of parthenogenesis-inducing bacterial endosymbionts, parasitized *E. kuehniella* eggs ($n = 100$ for each *Trichogramma* species) were placed individually into 0.5 ml microcentrifuge tubes, and a 2 mm diameter drop of honey was deposited into the cap of each tube. Unmated females arising from these parasitized eggs ($n = 20$ for each *Trichogramma* species) were placed individually into 0.5 ml microcentrifuge tubes containing ten irradiated *E. kuehniella* eggs (obtained from Beneficial Insectary) and a droplet of honey in the cap. Host eggs were removed from the arena after 24 h and placed singly in separate microcentrifuge tubes. At this point, females were separated into control and antibiotic treatments ($n = 10$ females per treatment per species). For the antibiotic treatment the caps with plain honey were replaced with caps containing honey mixed with antibiotic (100 mg tetracycline per 1 ml honey, as in Stouthamer et al., 1990), and the caps in the control treatment were replaced with new caps containing unaltered honey. After 24 h, 10 more unparasitized *E. kuehniella* eggs were added to each arena, and after an additional 24 h, the eggs were removed and placed singly into separate microcentrifuge tubes.

3. Results

3.1. Species confirmations

The 18 April shipment reported to be *T. pretiosum* by the insectary was identified as part of the *T. minutum* complex (J.D. Pinto, personal communication), and therefore, was not included in the quality assessment analyses. The *T. brassicae* specimens were correctly identified by the insectary, and the identity of *T. minutum* specimens were impossible to substantiate from the mounted material (J.D. Pinto, personal communication).

3.2. Assessment of quality parameters

Emergence rates were significantly different over time for each species (Table 1) (*T. brassicae*: $\chi^2 = 80.02$, df = 6, $P < 0.0001$; *T. minutum*: $\chi^2 = 23.48$, df = 4,

Table 1
Emergence rates and sex ratios for commercially produced *T. brassicae*, *T. minutum*, and *T. pretiosum*[a]

	Proportion of eggs with emergence holes			Male proportion		
	T. brassicae	*T. minutum*	*T. pretiosum*	*T. brassicae*	*T. minutum*	*T. pretiosum*
15 April	0.85	NA	NA	0.494	NA	NA
17 April	0.86	NA	NA	0.591	NA	NA
18 April	0.51	0.71	Mis-identified	0.314	0	Mis-identified
23 April	0.95	0.84	0.89	0.579	0.553	0.022
1 May	0.94	0.95	0.94	0.404	0.516	0.020
15 May	0.82	0.83	0.77	0.439	0.602	0.054
23 May	0.79	0.87	1.00	0.438	0.551	0.020
Mean	0.818	0.841	0.900	0.465	0.444	0.029
(SEM)	(0.057)	(0.039)	(0.048)	(0.037)	(0.112)	(0.008)

[a] Only *T. brassicae* was ordered on 15 and 17 April. On 18 April, *T. pretiosum* was misidentified by the insectary, and no *T. minutum* males were available to substantiate the identity of specimens in this shipment.

$P = 0.0001$; *T. pretiosum*: $\chi^2 = 36.44$, df = 3, $P < 0.0001$). Nevertheless, emergence rates were greater than 75% in 15 out of the 17 shipments (Table 1). There were also significant differences in the sex ratios of the different *T. brassicae* and *T. minutum* shipments (Table 1) (*T. brassicae*: $\chi^2 = 17.18$, df = 6, $P = 0.01$; *T. minutum*: $\chi^2 = 105.01$, df = 4, $P < 0.0001$); however, when the 18 April shipment was excluded from the *T. minutum* analysis for sex ratio, the sex ratio did not vary significantly among the remaining shipment dates ($\chi^2 = 1.36$, df = 3, $P = 0.72$). Sex ratios did not differ among shipments of *T. pretiosum* ($\chi^2 = 2.14$, df = 3, $P = 0.54$). The 18 April shipment contained no male *T. minutum* wasps (Table 1), and consequently, verification of the identity of these species was not possible.

Survivorship was increased by the provision of honey, and it varied among species and shipment dates. For all three species, survivorship was affected by shipment date and diet (results of the proportional hazards model: *T. brassicae*: $\chi^2_{\text{shipment}} = 70.91$, df = 5, $P < 0.0001$; $\chi^2_{\text{date}} = 48.41$, df = 1, $P < 0.0001$; $\chi^2_{\text{shipment}*\text{date}} = 17.42$, df = 5, $P = 0.004$; *T. minutum*: $\chi^2_{\text{shipment}} = 67.09$, df = 4, $P < 0.0001$; $\chi^2_{\text{date}} = 45.60$, df = 1, $P < 0.0001$; $\chi^2_{\text{shipment}*\text{date}} = 32.64$, df = 4, $P < 0.0001$; *T. pretiosum*: $\chi^2_{\text{shipment}} = 48.71$, df = 3, $P < 0.0001$; $\chi^2_{\text{date}} = 75.33$, df = 1, $P < 0.0001$; $\chi^2_{\text{shipment}*\text{date}} = 5.39$, df = 3, $P = 0.15$). Honey more than doubled the mean life span for all three species, and differences between fed and unfed wasp longevity were significant for all three species (mean days ± SEM; *T. brassicae*: fed $= 4.02 \pm 0.31$, unfed $= 1.96 \pm 0.07$; *T. minutum*: fed $= 6.71 \pm 0.74$, unfed $= 2.43 \pm 0.10$; *T. pretiosum*: fed $= 7.16 \pm 0.63$, unfed $= 2.68 \pm 0.14$). Maximum longevity was 18 d for *T. brassicae*, 24 d for *T. minutum* and 24 d for *T. pretiosum* (Fig. 1a–c). For fed wasps, $27.8 \pm 14.1\%$ (mean ± SEM) of *T. pretiosum*, $30.2 \pm 10.2\%$ of *T. brassicae*, and $24.5 \pm 7.3\%$ of *T. minutum* were 'dead by honey.' There was a significant interaction between diet and shipment for *T. brassicae* and *T. minutum*; a portion of the fed wasps in the 23 April (both species) and 17 April (*T. brassicae*), and 18 April (*T. minutum*) shipments lived considerably longer than did wasps from other shipments.

3.3. Thelytoky and antibiotic treatment

For the three species tested, only virgin *T. pretiosum* produced female offspring. Of the 18 virgin females that reproduced before antibiotic treatment, one produced only males, 15 produced only females, and two produced both male and female offspring. Three of the six females producing only daughters produced sons after being fed tetracycline, while the untreated females ($n = 10$) produced no male offspring. Two females produced no offspring and are not considered here.

4. Discussion

We observed fluctuations in all of the fitness parameters assessed for commercially produced *Trichogramma*, but it is difficult to predict how these inconsistencies will affect a biological control program. The longevity of *Trichogramma* spp. in our study was increased 2- to 3-fold by the provision of honey, and this finding is consistent with previous research on this topic (e.g., Bai and Smith, 1993; Hohmann et al., 1988; Kuhlmann and Mills, 1999; Leatemia et al., 1995; McDougall and Mills, 1997). The variability in the longevity among shipments could have important implications for biological control programs. Inundative releases of *Trichogramma* should be timed such that the number of releases is minimized to reduce the cost of pest suppression. Both the longevity of wasps and period of host susceptibility affect optimal release strategies, and if wasp longevity fluctuates with each shipment, there may be a group of hosts that escapes parasitism.

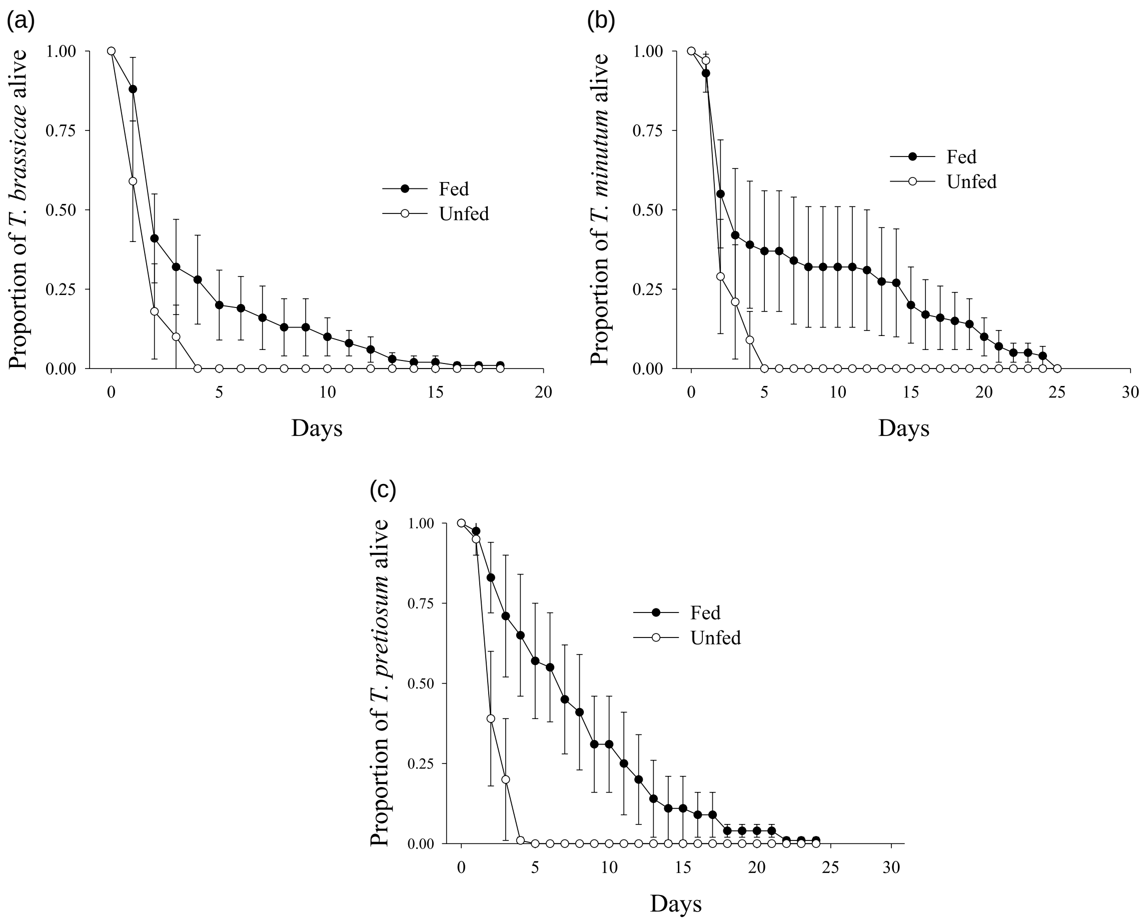

Fig. 1. The mean number of honey fed and unfed *Trichogramma brassicae* (a), *T. minutum* (b), and *T. pretiosum* (c) adults alive per day after emergence. Means are based on multiple sampling dates ($n_{T.\ brassicae} = 6$, $n_{T.\ minutum} = 5$, and $n_{T.\ pretiosum} = 4$) and bars represent the SEMs.

Provision of food may dramatically improve biological control programs if the increases in longevity that are observed in the laboratory are transferable to the field (i.e., Baggen and Gurr, 1998; Cañas and O'Neil, 1998; Smith et al., 1986). The method of delivery and selection of the most appropriate food source will be important for conserving *Trichogramma* in agroecosystems. For example, Lundgren et al. (2002) applied a sucrose solution to cabbage weekly during an augmentation program involving *T. brassicae* and found no increases in egg parasitism, whereas Treacy et al. (1987) found increases in egg parasitism by *T. pretiosum* resulting in nectaried cotton versus nectariless cotton.

Literature on the sex ratios of the *Trichogramma* species used in our study shows a high degree of variability and represents the plasticity of sex ratios within this genus. The proportion of male wasps in *T. brassicae* was higher than those found in non-commercial insectaries (Bai et al., 1995; Cerutti and Bigler, 1995; Wajnberg, 1993), but consistent with those found for commercially produced *T. brassicae* (Hassan and Zhang, 2001; Heimpel and Lundgren, 2000). The sex ratio of *T. minutum* in our study is consistent with much of the research on this species (Kuhlmann and Mills, 1999; Leatemia et al., 1995; Losey and Calvin, 1995); however, there are reports of thelytokous strains of un-vouchered *T. minutum* in the field and in insectary culture (Heimpel and Lundgren, 2000; Wang and Smith, 1996).

The sex ratio of our *T. pretiosum* had a lower proportion of males than has previously been discovered from commercial insectaries (Kuhlmann and Mills, 1999; Losey and Calvin, 1995; O'Neil et al., 1998; but see Heimpel and Lundgren, 2000). In our study, the production of female offspring from virgin mothers is consistent with the presence of *Wolbachia*. The incomplete level of PI-*Wolbachia* infection observed in our strain of *T. pretiosum* (i.e., the two females that produced both male and female offspring as untreated virgins) is apparently commonplace in infected *Trichogramma* populations (Stouthamer, 1997; Stouthamer et al., 2001), and may be the result of imperfect vertical transmission of *Wolbachia*, the presence of genes in some infected wasps that suppress the effects of *Wolbachia*, or the presence of paternal sex-ratio factors such as B chromosomes that convert diploid eggs into haploid ones by destroying the paternal chromosomes (Stouthamer et al., 2001).

It has been proposed that thelytokous strains of *Trichogramma* may be better biological control agents than their arrhenotokous counterparts under conditions of host limitation (Silva et al., 2000; Stouthamer and Luck, 1993). However, egg maturation rates, cold tolerance, mobility and dispersal, longevity, and egg and pupal survival rates are compromised in some thelytokous strains of *Trichogramma* relative to arrhenotokous conspecifics (Wang and Smith, 1996; Silva, 1999; Silva et al., 2000). These differences in behavior and physiological requirements between thelytokous and arrhenotokous strains result in a situation where sexual and asexual forms may be better suited to different biological control programs (Aeschlimann, 1990).

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