

## BIOLOGICAL CONTROL

### Effect of *Wolbachia* on the Survival and Reproduction of *Trichogramma kaykai* Pinto & Stouthamer (Hymenoptera: Trichogrammatidae)

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*Neotropical Entomology* 30(4): 607-612 (2001)

Efeito de *Wolbachia* na Sobrevivência e Reprodução de *Trichogramma kaykai*  
Pinto & Stouthamer (Hymenoptera: Trichogrammatidae)

RESUMO – Infecções causadas por *Wolbachia* resultando em indivíduos partenogênicos são comuns dentro do gênero *Trichogramma*. Contudo, os efeitos do microrganismo sobre a fecundidade e sobrevivência dos parasitóides têm sido relativamente pouco estudados. O objetivo desse estudo foi determinar os efeitos da bactéria na sobrevivência e reprodução de *Trichogramma kaykai* Pinto & Stouthamer, comparando indivíduos infectados (telítocos) com indivíduos curados através de antibiótico (arrenótocos), ambos criados em ovos de *Trichoplusia ni* Hübner. Fêmeas curadas produziram significativamente mais progênie, além de maior número de filhas que as fêmeas infectadas. Um número significativamente maior de indivíduos emergiu de ovos parasitados por fêmeas arrenótocas do que de ovos parasitados por fêmeas telítocas. As fêmeas não infectadas, contudo, viveram menos que as fêmeas infectadas. Fêmeas curadas apresentaram maturação de ovos mais rápida que fêmeas infectadas.

PALAVRAS-CHAVE: Insecta, parasitóide de ovos, partenogênese induzida por bactéria, sucesso reprodutivo.

ABSTRACT – Parthenogenetic inducing *Wolbachia* infections are particularly common in the genus *Trichogramma*, but the influence of the microorganism on parasitoids' fitness has received relatively little attention. The aim of this study was to determine the effects of the bacteria on the survival and reproduction of *Trichogramma kaykai* Pinto & Stouthamer, comparing an infected line (thelytokous) with an antibiotically cured (arrhenotokous) line, both reared on *Trichoplusia ni* Hübner eggs. Cured wasps produced significantly more progeny and more daughters than *Wolbachia*-infected wasps. Significantly more wasps emerged per host when parasitized by the arrhenotokous females than by the thelytokous females. Cured females, however, lived significantly less than infected females. Eggs of cured females matured faster than infected ones.

KEY WORDS: Insecta, egg parasitoid, parthenogenetic induced bacteria, reproductive success.

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As characteristic for Hymenoptera, most species of *Trichogramma* reproduce by arrhenotoky, i.e., males originate from unfertilized eggs and females from fertilized eggs. However, there are some populations within species of *Trichogramma* where daughters are produced from unfertilized eggs (thelytoky). Thelytoky in most species of *Trichogramma* is associated with the presence of a maternally inherited extrachromosomal element, i. e., a non-Mendelian element (*Wolbachia*) that controls reproduction in the cytoplasm of the eggs (Stouthamer *et al.* 1990, Stouthamer & Werren 1993). *Wolbachia*-infected individuals revert to arrhenotokous form when exposed to heat or antibiotic

treatment (Stouthamer *et al.* 1990).

In contrast to most other Hymenoptera taxa in which the microorganism infection has gone to fixation and only females exist (Zchori-Fein *et al.* 1992, Pijls *et al.* 1996), several species of *Trichogramma* are polymorphic, having sympatric parthenogenetic and bisexual populations (Luck *et al.* 1993, Stouthamer & Kazmer 1994, Stouthamer 1997). One of these species, *Trichogramma kaykai* Pinto & Stouthamer, is a parasitoid of *Apodemia mormo deserti* (C. and R. Felder) (Lepidoptera: Riodinidae) eggs. It occurs naturally over an extensive area of the Mojave Desert of southern California (Pinto *et al.* 1997). The frequency of parthenogenetic females

in these populations varies from 6 to 26% (Stouthamer & Kazmer 1994).

The occurrence of microbe-associated thelytoky in several species of *Trichogramma* opens a wide array of possibilities for studying the relationships between parthenogenetic inducing *Wolbachia* and their hymenopteran hosts, from both an evolutionary and applied standpoint. The biology of *Trichogramma* and their mass rearing are well known, making them ideal insects to be used experimentally.

In the present study we compare life history attributes of *T. kaykai* lines that are infected (thelytokous) and antibiotically cured (arrhenotokous), and determine whether the patterns of progeny production of the reproductive forms of *T. kaykai* are similar to those reported for other *Wolbachia*-infected and uninfected *Trichogramma* species (Stouthamer & Luck 1993, van Meer 1999). The influence of *Wolbachia* on other aspects of the parasitoid's reproductive biology such as egg load, survival, sex ratio, and clutch size was also investigated. The outcome of these studies can provide a preliminary measure of the costs and benefits to the wasp of carrying the microorganism in competition with its uninfected counterpart.

## Material and Methods

**Host Culture.** *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae) eggs were obtained from a colony maintained at the University of California, Riverside, on an artificial diet developed by Shorey & Hale (1965) and modified by Pak & Oatman (1982). *T. ni* laid their eggs on paper toweling that lined the inside of an oviposition unit (Knott et al. 1966). The paper toweling was collected daily, irradiated with a cobalt 60 source and stored for up to 3h in a refrigerator at  $6\pm 1^\circ\text{C}$  before use. Irradiation of the eggs prevented cannibalism by moth larvae emerged from the unparasitized eggs.

**Parasitoid Cultures.** Two *T. kaykai* cultures were established in The Agricultural University, Wageningen, The Netherlands, from a single *Wolbachia*-infected female collected from an *A. mormo deserti* egg laid on *Eriogonum inflatum* Torr. & Frem. (Polygonaceae) at Last Chance Canyon, El Paso Mountains, Kern County, California. The first culture was established with the single thelytokous female's offspring collected by R. Stouthamer in the spring of 1997. The second culture was initiated with the progeny of these thelytokous females after they were treated with the antibiotic tetracycline. This treatment eliminates the *Wolbachia* infection and causes the thelytokous females to revert to arrhenotoky (Stouthamer et al. 1990). Both infected and cured lines, after being maintained on *Ephesia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs for six months, were then sent to Riverside in January 1998 where they were reared on *T. ni* eggs. The rearing unit consisted of a 9.5 x 2.5 cm glass shell vial with the end sealed with a filter paper disk held in place by a polyethylene lid with a 1.8 cm diam. hole cut in its center. When the parasitoids emerged, strips of toweling containing several hundred *T. ni* eggs were exposed to the parasitoids inside the glass shell vial. Seven cultures, which were started on different days, provided wasps of the same age whenever they were needed to initiate experiments.

**Experimental Protocol.** After emergence, females were randomly assigned to their corresponding treatments. Except for the egg load experiment, in which the females were maintained in gelatin capsules in the absence of hosts, the parasitoids were supplied with hosts *ad libitum* on the appropriated treatment day. In these experiments, strips of toweling were placed in the vials and replaced every 24h with new strips containing irradiated eggs < 30h old. Honey was streaked on the inside of the vials as a carbohydrate source for the female wasps. The strips that were removed from the vials each day were transferred to an empty vial, labeled, and held for parasitoid emergence. After parasitoid emergence was completed, the number and sex of the emerged wasps were determined and the number of parasitized eggs was counted. The life span of each parental female was also recorded.

**Experimental Procedures.** To determine the effect of *Wolbachia* on a wasp's longevity and fecundity, 24 newly emerged (< 6h old) cured (= arrhenotokous), mated and 22 infected (= thelytokous) *T. kaykai* females, were randomly selected at emergence. Each female was then isolated individually within an oviposition unit (= rearing unit) and supplied with at least 50 eggs per day until death. The number of offspring produced by each female each day and the female's life span was determined.

To determine the temporal change in the egg load of cured and infected female over its lifetime, four treatment groups were established from each line (cured and infected) using 20 females per group. Each female was isolated in a gelatin capsule (#000) streaked with honey and randomly assigned to one of the four treatments based on the number of days since the female's emergence: zero, one, three, or five days. On the specific day, the wasps were frozen and their ovarian eggs counted.

To determine the female's egg load, the wasps were killed by freezing and their abdomen was separated from their body in a small drop of Ringer's insect saline, on a microscope slide, using fine dissecting needles. The ovaries were then excised and the eggs were gently separated. As the saline dried, a drop of acid fuchsin (Maple 1947) was added to stain the eggs. After the staining was completed (ca. 1 min.) the eggs were mounted in Hoyer's medium and their number recorded for each female at 200X under a compound microscope.

In all experiments, female size was determined by measuring the length of the hind tibiae (HTL) of adult females mounted on a glass microscope slide in Hoyer's medium. The HTL was used as an index of wasp size (Waage and Ng 1984, Hohmann et al. 1988).

The parasitoid cultures and the oviposition units were maintained at  $28\pm 1^\circ\text{C}$ ,  $50\pm 10\%$  RH, and photophase of 16h.

**Statistical Analysis.** Comparisons of treatment effects on life span, egg load, and lifetime progeny production by arrhenotokous and thelytokous strains were evaluated using analysis of covariance (ANCOVA) (GLM procedure, SAS Institute Inc. 1994). The fecundity data were square root transformed,  $\sqrt{x + 0.5}$ , and the hind tibia length was transformed using natural logarithms,  $\ln(x)$ . The relationship

was also tested for interaction (e.g. fecundity and variables such as HTL, wasp strain, longevity, etc.) before analysis. Sex ratio and clutch size data were analyzed using a one-way analysis of variance (ANOVA) (GLM procedure, SAS Institute Inc. 1994). Sex ratio data were arcsin transformed before analysis (Zar 1984). Comparisons between single infected and uninfected treatment means were made using *t*-Test for the lifetime progeny production and longevity data, and Tukey Multiple Comparisons Test for the temporal egg load data (Zar 1984).

**Results**

**Lifetime Progeny Production and Longevity.** The age-specific progeny production by cured (arrhenotokous) and *Wolbachia*-infected (thelytokous) strains of *T. kaykai*, of similar size, showed the same pattern. The maximum number of offspring emerged from hosts parasitized during the first day post-emergence, with a sharp reduction occurring thereafter (Fig. 1). The number of progeny produced by cured wasps during their lifetime was significantly greater than that produced by thelytokous wasps (ANCOVA,  $F_{[1,43]} = 17.59$ ,  $P < 0.001$ ) (Table 1). Furthermore, in contrast to expectations

(see sex ratio in Table 1), uninfected females also produced significantly more daughters during their lifetimes than did *Wolbachia*-infected females (ANCOVA,  $F_{[1,43]} = 4.66$ ,  $P < 0.01$ ) (Table 1). This occurred because the uninfected female's total progeny (sons + daughters) was much greater than that of the infected females. Cured wasps also produced approximately 25% more wasps per host than infected wasps ( $F_{[1,43]} = 29.56$ ,  $P < 0.001$ ).

Only female progeny emerged from hosts parasitized by infected females during the first day. This pattern, however, changed in the subsequent days, i. e., sons started to be produced (Fig. 2). Although 40 to 60% of the offspring were males by the fifth and subsequent days, by this time, infected females had laid 95% of their egg load (Fig. 1).

Longevity differed between the two reproductive strains. Infected females lived significantly longer than their cured counterparts (ANCOVA,  $F_{[1,43]} = 7.71$ ,  $P < 0.01$ ) (Table 1).

**Temporal Egg Load.** The egg load of both cured and infected female *T. kaykai* increased significantly over the wasp's lifetime (ANCOVA,  $F_{[3,155]} = 50.39$ ,  $P < 0.001$ ) (Fig. 3). Cured females had a higher egg load than their infected counterparts (ANCOVA,  $F_{[1,155]} = 23.05$ ,  $P < 0.001$ ) but the differences were

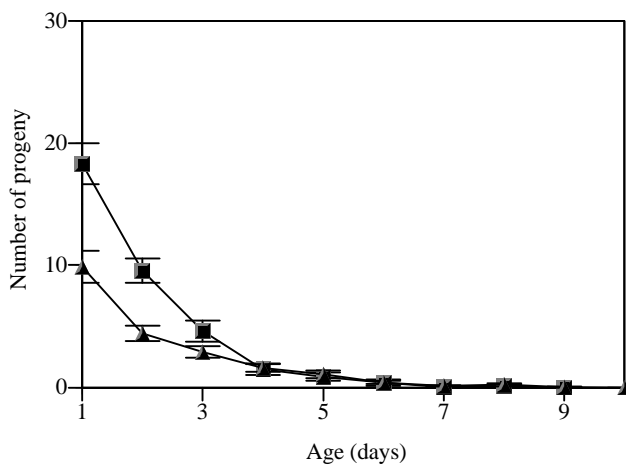


Figure 1. Age-specific fecundity (mean ± s.e.) of *T. kaykai* from Last Chance, California, on *T. ni* eggs. Cured (■), *Wolbachia*-infected (▲).

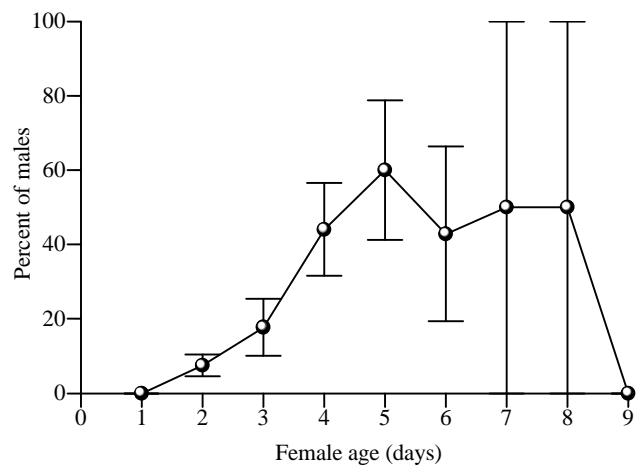


Figure 2. Age-specific male progeny (mean ± s.e.) produced by *Wolbachia*-infected *T. kaykai* from Last Chance Canyon, California, when unlimited *T. ni* eggs were available.

Table 1. Lifetime progeny production and longevity (days) of *Wolbachia*-infected and cured *T. kaykai* from Last Chance Canyon, California, parasitizing *T. ni* eggs.

| Wasp strain | Sample size | HTL parental female (mm) (mean±s.e.) | Total progeny (mean±s.e) | Total no. of daughters (mean±s.e.) | % Females | Brood size (mean±s.e.) | Longevity (mean±s.e.) |
|-------------|-------------|--------------------------------------|--------------------------|------------------------------------|-----------|------------------------|-----------------------|
| Thelytokous | 22          | 0.170 ± 0.03                         | 20.9 ± 2.07 a            | 17.9 ± 1.75 a                      | 89 a      | 1.4 ± 0.05 a           | 8.4 ± 0.46 a          |
| Cured       | 24          | 0.174 ± 0.02                         | 35.4 ± 2.75 b            | 27.0 ± 2.39 b                      | 77 b      | 1.8 ± 0.04 b           | 6.3 ± 0.53 b          |

Means followed by the same letter within a column are not significantly different (*t*- Test).

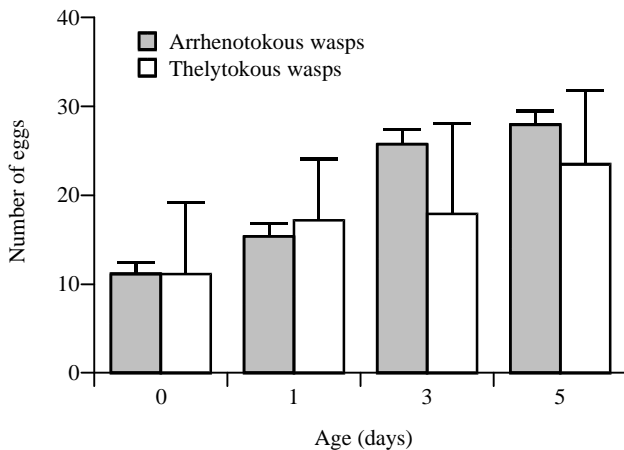


Figure 3. Temporal egg load (mean  $\pm$  s.e.) of *T. kaykai* from Last Chance Canyon, California, on *T. ni* eggs.

significant only three days after emergence (Tukey,  $q = 6.53$ ,  $P < 0.001$ ,  $n = 40$ ). A cured female's egg load increased dramatically after the first day and, by the third day after emergence, it corresponded to 92% of the total lifetime egg complement. This represented more than a two-fold increase when compared to fecundity at emergence. The egg load of infected females increased more slowly than that of cured ones (Fig. 3). It took five days for their egg load to reach 78% of the total egg complement. This also represented more than a two-fold increase over the fecundity at emergence.

## Discussion

Parthenogenesis-inducing *Wolbachia* imposes a significant cost on infected *T. kaykai* females. Infected females produced fewer total progeny and fewer daughters than their uninfected counterparts. A decrease in both total progeny and female progeny has also been reported in several other *Trichogramma* species co-occurring as mixed thelytokous and arrhenotokous populations (Stouthamer & Luck 1993, van Meer 1999). In contrast, a cured *T. pretiosum* line from thelytokous Hawaiian population did not differ from the thelytokous line in the number of daughters it produced although it did differ in total progeny (Stouthamer & Luck 1993). The Hawaiian *T. pretiosum*, however, is only known as a thelytokous population.

A reduction in progeny production in *Wolbachia*-infected females appears to be associated with the infected *Trichogramma* populations that occur sympatrically with uninfected conspecific species (Stouthamer & Luck 1993, van Meer 1999). In the case of *T. kaykai*, gene flow from the uninfected to the infected individuals is known to occur under field conditions (Stouthamer & Kazmer 1994). According to van Meer (1999) this may result in a nuclear-cytoplasmic conflict (*sensu* Cosmides & Tooby 1981) that can be manifested either as "inefficient transmission" of *Wolbachia* or as decreased fecundity. More recently, Huigens *et al.* (2000) suggested that the reduced offspring production by infected females might be a result of opposing adaptations

between *Wolbachia* and the wasps. According to Ewald (1994) in Huigens *et al.* (2000) they should select for conflicting adaptations because the bacterium and the host require opposing adaptations.

Antibiotically cured *T. kaykai* produced more progeny but lived for a shorter period than infected wasps. They were also less fecund and lived for a shorter period than the uninfected, field-collected wasps (Hohmann *et al.* 2001). The shorter longevity of cured wasps may have resulted from selection of traits within the host female that accommodated the *Wolbachia* infection. These traits may have produced physiological and/or behavioral changes in the thelytokous wasps that were retained even after they had reverted to sexual reproduction following treatment with antibiotics. Moreover, an increment in reproduction after treatment could mean a cost for cured wasps, because part of the resources available to the wasp for maintenance was allocated to reproduction. This could explain, at least in part, the reduced longevity of cured versus infected wasps. Van Meer (1999), however, did not find differences in longevity among thelytokous and cured lines in different strains of *T. kaykai*.

Reduced egg production was not the only factor affecting the total number of progeny produced by the *Wolbachia*-infected females. Fewer offspring emerged from the hosts as a result of differential immature mortality (Hohmann 1999). Van Meer (1999) detected preimaginal mortality in several species of *Trichogramma*, including a thelytokous strain of *T. kaykai* from Last Chance Canyon whose pupal and embryo mortality reached 50%. The author suggested that this mortality might be caused by the cytological processes resulting in production of gynandromorphs, or from incompatibility between the host "symbiont" genotype. Even though most of the progeny produced by wasps infected with the parthenogenesis-inducing *Wolbachia* were females (Stouthamer & Werren 1993, Stouthamer & Kazmer 1994), a gradual increase in the frequency of males occurred in those offspring laid after the first day of oviposition. This increase in male offspring, however, had little effect on the overall sex ratio. By the time the male progeny accounted for 60% of the progeny, more than 95% of the wasp's progeny had been produced. A possible explanation for the increasing male progeny is a reduction in the bacterial density in the nurse cells. Van Meer (1999) suggested that male progeny produced by thelytokous females result from a decline in *Wolbachia* titer with mother's age.

Cured wasps appear to be reproductively more successful than their *Wolbachia*-infected counterparts. They parasitize more hosts, produce more progeny, and produce more daughters. All else being equal, uninfected wasps would appear to be fitter than infected wasps in a field situation with unlimited hosts. However, in the southern California desert, *T. kaykai* is likely to be host limited during the summer and fall of most years and during low rainfall years. Under such circumstances *Wolbachia*-infected females may have an advantage because they can devote all of their offspring to the production of daughters.

The fact that both thelytokous and arrhenotokous individuals coexist sympatrically in the desert indicates that the thelytokous subpopulation is managing to persist in this

complex environment. Although the results reported here do not represent the entire *T. kaykai* field population in the southern California desert, and thus cannot lead to generalizations, they do produce insight into potential mechanisms resulting in coexistence. They also suggest that a cost exists in infected wasps when they are compared with their uninfected counterparts and that this cost has potential consequences on the dynamics of these co-existing, conspecific populations. Furthermore, these results are consistent with those reported for different strains of *T. kaykai* and for several others species of *Trichogramma* that have conspecific thelytokous and arrhenotokous populations (Stouthamer & Luck 1993, Volkoff & Daumal 1994, van Meer 1999).

### Acknowledgments

We wish to thank Joseph Morse and John D. Pinto, Department of Entomology, Leonard Nunney, Department of Biology, University of California, Riverside, and Sueli Souza Martinez, Área de Proteção de Plantas, Instituto Agrônômico do Paraná, and two anonymous reviewers of an earlier draft of this manuscript. We also thank J. D. Pinto for identifying the parasitoid lines.

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*Received 29/11/00. Accepted 30/10/01.*

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