



## Acclimation for heat resistance in egg parasitoid *Trichogramma exiguum* Jhon and Plinto

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### ABSTRACT

In recent years, a total area of over 32 million ha in agriculture and forestry in the world has been treated annually with *Trichogramma spp* for controlling insect pests. In the world wide use of *Trichogramma spp*, the former USSR ranked first, followed by China and Mexico. Extensive utilization of this parasitoid was developed for rice, corn, sugarcane, cotton, vegetable and pine. The selection of suitable species, quality parasitoids, reasonable release rate per hectare, climate during release and release methods are the factors that determine the efficiency of *Trichogramma spp*. Therefore, investigations were carried out in the biocontrol laboratory at North Carolina State University, Raleigh, USA, to examine costs (negative fitness effect as well as beneficial effects) for heat resistance acclimation in an egg parasitoid, *T. exiguum* by exposing of immature stages to 40°C for different periods. The focus of the study was on acclimation across life-cycle stages rather than short term hardening of adults. Three questions were considered. Firstly, can adult resistance to a high temperature shock, be increased by pre-pupal acclimation and / or by pupal acclimation in *T. exiguum*?. Secondly, are there other benefits of acclimation?. Thirdly are treatments that increase resistance associated with costs in terms of decreased parasitism or decreased longevity and can these costs be overcome by modifying acclimation treatments. So far, there has been little work in *Trichogramma spp* even through these parasitoids form an important component of IPM strategy in many countries and despite the fact that high temperature decrease *Trichogramma spp.*, parasitism rate. The findings indicates that acclimation can occur without costs in *T. exiguum* and suggest a general phenotypic approach for separating acclimation costs from other fitness costs by sub - lethal exposures.

**Key words :** Acclimation, acclimation costs, longevity, parasitism rate, resistance, temperature, *Trichogramma exiguum*.

### INTRODUCTION

Acclimation is a common phenotypic response to enhance performance and thus adaptive. Acclimation to temperature extremes in insects may occur within a life-cycle or span across stages (Levins, 1969). While it is normally assumed that acclimation is adaptive response increasing fitness under extreme conditions, the direct evidence for this "Beneficial Acclimation Hypothesis is weak (Huey and Berrign, 1997). Several experiments have shown that organisms acclimated under one set of conditions do not necessarily show increased fitness under those conditions. One reason is that costs as well as benefits may be associated with acclimation conditions (Scott *et al.*, 1997). Costs that are following exposure to sub-lethal conditions may be associated specifically with the acclimation process (acclimation costs) or result from general damage unrelated to this process. For instance, insects exposed to high temperatures may suffer water loss from their epicuticular layer is damaged, and effect unrelated to heat acclimation costs such as those

associated with heat shock protein production. One way of identifying acclimation costs is to relate them directly to the physiological mechanism underlying the acclimation response. Another approach for testing acclimation costs is to examine the acclimation response in detail and determine if there are conditions that lead to acclimation without any costs. In this present investigation, the second approach is used to examine costs of heat resistance acclimation in an egg parasitoid, *T. exiguum* Jhon & Plinto. This species has been reared commercially for the control of American boll worm, *Helicoverpa armigera* Hubner, in cotton in particular at North Carolina State University, Raleigh. There has been little work on acclimation in *Trichogramma* despite the fact that high temperatures decrease *Trichogramma* parasitism rates (Chihrane *et al.*, 1991). The focus of the study is on acclimation across life-cycle stages rather than short term hardening of adults. Three questions are considered. Secondly, are there other benefits of acclimation?. Thirdly are treatments that increase resistance associated with

costs in terms of decreased parasitism or decrease longevity and can these costs be overcome by modifying acclimation treatments.

#### MATERIALS AND METHODS

Experiments were undertaken with a mass bred strain of *T. exiguum* which had been initiated by combining 16 iso - female lines started by offspring from single females collected from a number of locations in North Carolina Raleigh, USA. This strain took 9 - 10 days to complete life cycle at 25°C comprising an egg - larval stage lasting 4 - 5 days and a pupal stage lasting 4 - 5 days. Wasps were reared on eggs of the Mediterranean flour moths, *Ephesia kuehniella* Zeller at 25°C. The acclimation treatments involved to sub lethal temperature 33°C for few hr per day over several days. This procedure was favoured over a single continuous acclimation period because i. wasps in North Carolina Raleigh typically encounter maximum temperature for only few hours per day during the afternoon and ii. A long exposure to high temperature induces deformities in offspring as well as sterility. Acclimation was undertaken at early stage of development of after pupal formation at the end of development.

For pre-pupal acclimation wasps were left to parasitize *E. kuehniella* eggs for 24 hr. Parasitized host eggs were then exposed to 33° C for a total of 0, 2, 3 hr day<sup>-1</sup> over the first 4 days of development (*i.e.* 0,8 and 12 hr in total). A longer period of 4 hr day<sup>-1</sup> was also initially considered, but this treatment led to a high percentage (> 75%) of wasps emerging with deformed wings and was therefore abandoned. For pupal acclimation, parasitized host eggs were then exposed to 33°C during the last 4 days of development (7-10 days after parents had parasitized host eggs) for 0, 2,3, or 4 hr day<sup>-1</sup> (*i.e.* 0,8,12, and 16 hr in total). To acclimate pupae in host eggs, the host eggs were placed in plastic 38 ml vials sealed with parafilm and placed in a water bath. In control treatments host eggs were also transferred to vials but were not placed in the water bath.

#### Survival of heat Shock

Females (1 - 20 post emergences) were stressed for 4 h at 40°C because pilot stress tests showed that this stress resulted in intermediate mortality levels.

Wasps were stressed in sealed 12mL glass vials (6 - 10 per container) as for acclimation. Survival was scored after 24 h. A few (<5%) of the wasps in vials were alive but immobile at this time, these were scored as dead because they never recovered. For the experiment on pre-pupal acclimation, each of the treatments was tested at different time against a separate control and data therefore could be combined across controls. There were seven to ten replications per treatment.

#### Fitness Traits

Wasps emerging from host eggs tested for two fitness components, parasitism rate and longevity. Parasitism rate was considered under three temperature conditions: 25°C, 30/25°C, 37/25°C. In the 30/25°C and 37/25°C conditions wasps were placed at the high temperatures for 5 hr day<sup>-1</sup> and at 25°C the rest of the time. Mated females (1-20 hr post emergence) were placed individually in 12 ml glass vials with a drop of honey and at least 100 *E. kuehniella* eggs on a card. For the 25°C and 30/25°C condition, cards were placed after 2 days. For 37/25°C condition, a new card was introduced each day when vials were placed at 37°C because egg cards tended to dry out more quickly in this treatment compared with the others. Because more eggs provided than the wasps could have parasitized over 2 days, replacing cards daily or every 2 days is unlikely to have influenced total parasitism rate. Parasitism was assessed 5 days after cards have been exposed to wasps. Parasitism was quantified by counting the number of black unhatched eggs. For the experiment on pre-pupal acclimation, there were 12 replications per acclimation treatment and 24 replications per unacclimated control for each of the three temperature conditions. For the experiment on pupal acclimation, there were 25 replicates per acclimation treatment and 25 per control for each of the three temperature condition.

Longevity of mated females was scored in similar temperature conditions. However long exposure times to high temperatures were used in an attempt to increase the stress to which wasps were exposed. A longer exposure time could be used than parasitism rate. For 30/25°C condition, wasps were placed for 10 h day<sup>-1</sup> at 30°C in the case of both pre - pupal and

pupal treatments. For 37/25°C condition, wasps were placed for 8 h (pre-pupal acclimation) and for 5 h (pupal acclimation) day<sup>-1</sup> at 37°C to score longevity, wasps were placed individual in 12ml glass vials with honey, and examined daily for mortality. There were 30 wasps were per treatment for pre-pupal acclimation and 25-35 wasps per treatment for pupal acclimation.

## RESULTS

### Heat resistance

Survival following pre - pupal acclimation could not be evaluated with parametric statistics because survival was low for all treatment. In contrast, pupal acclimation influenced resistance. For the 2 h day<sup>-1</sup> exposure, difference between treatments were significant by a t - test on arcsine transformed proportions ( $t = 8.59$ ,  $df = 7$ ,  $p < 0.001$ ). Treatments also differed significantly ( $t = 6.62$ ,  $df = 7$ ,  $p < 0.001$ ) for the 3 h day<sup>-1</sup> but the 4 h day<sup>-1</sup> exposure did not influence adult survival ( $t = 1.17$ ,  $df = 7$ ). Pupal acclimation therefore increased heat resistance but only for exposure periods of 2 h day<sup>-1</sup> and 3 h day<sup>-1</sup>.

### Parasitism rate

In pre-pupal acclimation, exposure of the parasitized eggs to high temperature for 2h day<sup>-1</sup> did not decrease parasitism rate, when compared to the 3 h day<sup>-1</sup>. A two - way ANOVA indicated a significant effect of acclimation ( $F_{2, 135} = 31.28$ ,  $P < 0.001$ ) and a marginally significant effect of temperature condition ( $F_{2, 135} = 3.6$ ,  $P < 0.001$ ) on parasitism rate, while the interaction between these factors was not significant ( $F_{4, 135} = 1.63$ ,  $P < 0.17$ ).

In pupal acclimation, parasitism rates tended to be higher in the 30/25°C condition than the other two conditions. Heat exposure 2 h day<sup>-1</sup> delayed the parasitism. The ANOVA indicated a significant effect of acclimation ( $F_{3, 288} = 9.20$ ,  $P < 0.001$ ) and temperature condition ( $F_{2, 288} = 6.28$ ,  $P < 0.002$ ) on parasitism rate, while the interaction between these terms was not significant ( $F_{6, 288} = 1.04$ ,  $P < 0.40$ ). Hence short pupal acclimation periods do not appear to adversely affect parasitism rates under any of the tested temperature conditions.

### Longevity

In pre - pupal experiment, longevity was reduced by daily exposures to 30°C and particularly 37°C. Unfortunately all the data could not be analyzed in a single ANOVA because of the low longevity scores from wasps placed at 37/25°C fell into a narrow range. The ANOVA on data from the 25°C and 35 / 25°C conditions indicated a significant effect of temperature condition ( $F_{1, 171} = 11.83$ ,  $P < 0.001$ )

but no effect of acclimation ( $F_{2, 171} = 0.27$ ,  $P = 0.76$ ) or interaction between these factors ( $F_{2, 171} = 1.03$ ,  $P = 0.36$ ). For the 30 / 25°C data, there was also no difference between the treatments.

In pupal acclimation with regard to longevity, a single ANOVA could be carried out because longevity scores were higher in the 37/25°C condition than in the previous experiment (the 37°C exposure period was short) and fell into a wide range. To remove an association between means and variances, data were log transformed prior to analysis. The ANOVA indicated a significant effect of temperature condition ( $F_{2, 385} = 134.5$ ,  $P < 0.001$ ) and acclimation ( $F_{3, 385} = 8.05$ ,  $P < 0.001$ ), while the interaction between condition and acclimation was also significant ( $F_{6, 385} = 2.97$ ,  $P < 0.007$ ). Thus there was a beneficial effect of acclimation for 2h day<sup>-1</sup> on longevity. This effect was noticeable under the 30/25°C condition but was small for the other two temperature conditions.

## DISCUSSION

The results indicate that there are beneficial effects of acclimation across life - cycle stages in *T. exiguum*. Survival under heat stress was increased by pupal acclimation for 2 h day<sup>-1</sup>, particularly when wasps were exposed to periods of 30°C. In another *Trichogramma* species, *T. carverae*, exposure to 33°C at the pupal stage increased adult survival (Scott *et al.*, 1997), was reported. In this case although cumulative exposure longer than 8 h was required to produce a beneficial effect. Conditions leading to beneficial effects therefore differ between *Trichogramma* species. Microhabitat significantly impacted average maximum daily temperature, the number of consecutive hours per day at or above 35°C, and *Trichogramma exiguum* emergence percentages. Parasitoid emergence declined significantly in response to increasing number of consecutive hours per day above 35°C (Philip and Orr, 2008). It was also recorded that the lower and extreme limits of temperatures were evaluated as 20 and 35°C with prolonged and reduced developmental period, respectively, causing low parasitism and emergence, whereas rearing at 40°C did not support the survival of *Trichogramma chilonis* (Ishii) (Hymenoptera: Trichogrammatidae) (Nadeem *et al.*, 2009).

Four of the five treatments provided evidence for costs whereas a beneficial effect of acclimation was detected in only two of them. These results generally support the notion that exposures to specific conditions do not necessarily increase fitness under those conditions. (Leroi *et al.*, 1994; Huey and Berrigan, 1997) and contradict the beneficial acclimation hypothesis. Nevertheless in one

treatment (pupal acclimation for 2h day<sup>-1</sup>) there was a beneficial effect without fitness costs as measured by parasitism rate and longevity. Acclimation without costs may also occur in other *Trichogramma*; while Scott *et al.* (1997) found that pupal acclimation in *T.carverae* always resulted in reduced parasitism rate at 25°C, recent data suggest that costs may disappear when acclimation is confined to a late pupal stage.

Although the results suggests that costs are common when organisms encounter sub-lethal conditions. They have implications for the notion that acclimation is invariably associated with fitness costs (Hoffmann, 1995). Instead, the results suggest that it may be possible to identify conditions leading to acclimation without costs, and that costs may be associated general damage rather than the effects of the acclimation responses perceive. Whether this will apply to other insects is unclear. The findings of Krebs and Feder (1997) associating with the decreased larval survival suggest that increased thermo tolerance in *Drosophila* larva often involves a costs because heat-shock protein Hsp 70 expression plays a major role in larval acclimation.

Therefore, the possibility of acclimation without costs has implications for the success of commercially produced *Trichogramma*. The *Trichogramma* are typically released as parasitized host eggs just prior to adult emergence, under air temperatures that can exceed 40° C during summer months. The result of these studies says that parasitism success under such conditions could be improved by exposing pupae to 33°C just prior to release. This simple process could be undertaken by commercial producer prior to shipment or by growers after they have obtained the parasitized eggs.

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