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# Basal and induced thermotolerance to heat shocks in *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae)

Termotolerancia basal e inducida a choques térmicos en *Bemisia tabaci* biotipo B (Hemiptera: Aleyrodidae)

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**Abstract:** The whitefly, *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae) is a key agricultural pest and virus vector in diverse crops throughout the world. It is distributed in warm environments with short periods of extreme temperatures, suggesting mechanisms to adapt and expand to new areas. The aim of this study was to determine how temperature affects fitness of *B. tabaci*, particularly during short periods of stressful high temperatures. Thermotolerance curves were constructed at different one-hour heat shocks (37, 39, 41, 43, 44, 45 °C and a control at 25 °C), measuring its effect on three life history traits involved in fitness: survival, fecundity and viability. Then, temperatures were selected to establish an induced thermotolerance or hardening treatment (40 °C/1h, 25 °C/1h and 45 °C/1h) to compare with the basal thermotolerance (45 °C/1h). The heat shocks significantly affected *B. tabaci* survival between 41 °C (82.5%) and 45 °C (2.7%). Fecundity was only affected significantly at 45°C without oviposition. Viability diminished significantly at 44 °C (67%). Thermal hardening increased survival from 2.73% (45 °C/1h) to 10% in males and 33% in females; although it was significantly higher in females, only. These results show high thermotolerance of *B. tabaci* to heat shocks, evidencing plasticity of the species to survive a broad range of temperatures (≥ 25 °C), which contributes to explaining the expansion of biotype B distribution in areas and seasons with extreme high temperatures.

**Key words:** Heat shocks. *Bemisia tabaci* biotype B. Survival. Fecundity. Viability.

**Resumen:** La mosca blanca, *Bemisia tabaci* biotipo B (Hemiptera: Aleyrodidae) es plaga agrícola clave y vector de virus en diversos cultivos alrededor del mundo. Se distribuye en ambientes cálidos con periodos cortos de temperaturas extremas, lo cual evidencia mecanismos para adaptarse y expandirse a nuevas zonas. El objetivo de este estudio fue determinar como la temperatura afecta el "fitness" de *B. tabaci*, particularmente en periodos cortos de temperaturas altas estresantes. Se construyó una curva de termotolerancia a diferentes choques térmicos de 1 hora (37, 39, 41, 43, 44, 45 °C y control a 25 °C), midiendo su efecto sobre tres características de historia de vida implicadas en el "fitness": supervivencia, fecundidad y viabilidad. Luego, se escogieron las temperaturas para establecer un tratamiento de termotolerancia inducida o acondicionamiento térmico (40 °C/1h, 25 °C/1h y 45 °C/1h), que fue comparado con la termotolerancia basal (45 °C/1h). Los choques térmicos afectaron significativamente la supervivencia de *B. tabaci* entre 41 °C (82,5%) y 45 °C (2,7%). La fecundidad solo fue afectada significativamente a 45 °C, sin oviposición. La viabilidad disminuyó significativamente a 44 °C (67%). El acondicionamiento térmico aumentó la supervivencia desde 2,73% (45 °C/1h) hasta 10% en machos y 33% en hembras, aunque este aumento solo fue significativo en las hembras. Estos resultados muestran una alta termotolerancia de *B. tabaci* a los choques térmicos, evidenciando la plasticidad de la especie para sobrevivir a un amplio rango de temperaturas (≥ 25 °C), lo cual contribuye a explicar la expansión de la distribución del biotipo B en áreas y estaciones con periodos de temperaturas altas extremas.

**Palabras clave:** Choques térmicos. *Bemisia tabaci* biotipo B. Supervivencia. Fecundidad. Viabilidad.

## Introduction

To a great extent, temperature defines the abundance and geographic distribution of species, given that each is developed within a defined thermal spectrum where it is able to survive and leave offspring (Laayouni *et al.* 2007; Leather *et al.* 1993). For ectotherm organisms like insects the environmental temperature plays a vital role in survival and reproductive success (fitness), given that body temperature depends largely on the environmental temperature (Hoffmann *et al.* 2003). Thus, short or periodic exposure to extreme temperatures rapidly induces physiological and biochemical responses and behaviors with important evolutionary implications to maintain their populations (Hoffmann and Parsons 1991).

Thermal tolerance or thermotolerance has been defined as an organism's ability and of its cell structures to withstand (survive) destructive heat stress exceeding the range of optimal temperature for its development (Riabowol *et al.* 1988; Solomon *et al.* 1991; Lee *et al.* 1993; Parsell and Lindquist 1993; Krebs 1999). Experimentally, it may be measured in two ways: basal thermotolerance and induced thermotolerance. Basal thermotolerance is defined in terms of the response of a trait related to fitness to direct exposure for a short period of time (measured in hours or minutes) at a high stressful temperature (Lee *et al.* 1993; Krebs 1999; Sorensen *et al.* 2001); while induced thermotolerance, is defined as the increase of thermotolerance after a pre-treatment at high, sub-lethal temperatures or heat hardening, *i.e.*, the organisms are conditioned exhibiting a transient capacity to survive the heat shock, which would kill most unconditioned cells or organisms (Boon-Niermeijer *et al.* 1986; Elliot *et al.* 1996; Krebs and Loeschcke 1996; Krebs and Feder 1997; Krebs 1999; Lansing *et al.* 2000).

The ability of insects to withstand heat is one of the fundamental mechanisms of their invasive trait, which could be the case of the whitefly *Bemisia tabaci* (Gennadius) biotype

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B (Hemiptera: Aleyrodidae), which is highly thermotolerant. This species is one of the invasive pest insects and most important and destructive virus vectors affecting agriculture throughout the world. Besides the direct damage produced by nymphs and adults when feeding, indirect damages occur when they are Begomovirus vectors (Cock 1993; Naranjo *et al.* 2004) and upon excreting a molasses that aids in the development of sooty mold (Cuellar and Morales 2006). *B. tabaci* is highly polyphagous and feeds from over 600 plant species (Secker *et al.* 1998; Cuellar and Morales 2006); it has a high reproductive rate, great capacity to dispersion and resistance to various insecticides (Naranjo *et al.* 2004).

This pest survives and reproduces successfully in zones with extreme thermal characteristics (Oliveira *et al.* 2001)**.** *B. tabaci* can increase its population size during the summer, when temperature frequently exceeds 42 °C and relative humidity is around 10%. Cui *et al.* (2008) found that thermotolerance in *B. tabaci* is much greater compared to other white flies like *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae). These authors also found that the survival rate of females and males of *B. tabaci* biotype B can be affected at temperatures between 41 and 45 °C, with significantly higher survivals for females, while none of the extreme temperatures influenced fecundity (Cui *et al.* 2008).

In Colombia, the biotype B of *B. tabaci* was not an important pest and it was reported in 1997 restricted between 0 and 890 masl (Quintero *et al.* 2001). However, as a consequence of a prolonged dry period, between 1997 and 2003, this insect expanded its geographic distribution in altitude and latitude manner, colonizing new habitats and displacing the biotype A from its habitat in Valle del Cauca (Colombia); these populations of *B. tabaci* promoted more aggressive attacks on diverse hosts, physiological disorder on the maturity of tomato fruit, and virus transmission symptoms (Rodríguez *et al.* 2005; Cuellar and Morales 2006). This expansion may be due to transport of the whitefly by man (Byrne *et al.* 1990) or to its capacity to adapt to high temperatures (Blackman and Cahill 1998).

Contribution of new knowledge on thermotolerance in this insect is necessary to understand more about its behavior and high capacity to disseminate, permitting more adjusted predictions on the variation of its spatial distribution (Blackman and Cahill 1998), and possible areas of expansion of Begomovirus in vegetables in Colombian populations. For this reason, the aim of this study was to determine how temperature affects fitness of *B. tabaci*, particularly during short periods of high stressful temperatures or heat shocks, in one population of species with distribution in Colombia (Rozo-Valle del Cauca). For this, a thermotolerance curve was constructed using a gradient of extreme temperatures, and specifically recording the effects of the heat shocks on survival, fecundity, and viability in this species. In addition, two stress temperatures were selected from the thermotolerance curve to determine if fitness of this insect under induced thermotolerance is greater than in basal thermotolerance. This difference has been broadly proven in model insects like *Drosophila melanogaster* (Feder *et al.* 1996; Krebs and Feder 1997; Dahlgaard *et al.* 1998), given that it is assumed that under conditions of induced thermotolerance, individuals conditioned before the stress exhibit greater transient capacity to survive the heat shock, which would kill most of the unconditioned individuals (basal thermotolerance).

## Materials and methods

**Collection of insects.** Fourth instar nymphs (red-eyed nymphs) of *B. tabaci* were gathered on string bean leaves and leaflets of *Phaseolus vulgaris* (Linneo), melon *Cucumis melo* (Linneo), eggplant *Solanum melongena* (Linneo), tomato *Solanum lycopersicum* (Linneo), and pumpkin *Cucurbita maxima* (Duchesne) in Rozo, Valle del Cauca-Colombia with a altitude between 950 and 1,050 masl (3°37'33.9"N 76°24'41.5"W, annual average temperature: 25°C and annual average RH: 70%).

**Breeding of *B. tabaci* biotype B.** With the individuals collected in the field, breeding of *B. tabaci* biotype B was established on bean plants *Phaseolus vulgaris* ICA-Pijao variety, under controlled conditions of temperature, humidity and photoperiod (25 °C, RH: 65% on average during the day and 12 hours of light/day). The ICA-Pijao variety was chosen because of its tolerance to laboratory conditions.

**Molecular identification of *Bemisia tabaci* biotype B.** The DNA extraction was performed from modifications to the method described by De Barro and Driver (1997). For this, individual whiteflies were homogenized in 20 µl of lysis buffer (50 mM KCl, 10 mM Tris pH 8.4, 0.45% Tween 20, 0.45% Triton X-100, 0.5 mg/ml Proteinase K). Thereafter, each sample was incubated at 65 °C for 20 minutes and then at 90 °C for 10 minutes. Lastly, 40 µl of ultra-pure water was added, completing a 60-µl final volume. The samples were conserved at -20 °C. Identification of *B. tabaci* individuals collected as biotype B was done by using RAPD molecular markers, OPA 04 primer according to Quintero *et al.* (1998). The RAPD obtained were compared to those of the *B. tabaci* biotype B and *T. vaporariorum* rearing in the International Center for Tropical Agriculture (CIAT, for the name in Spanish).

**Thermotolerance curve.** Heat shocks were conducted on adults between 1-24 hours after emerging, by following the method proposed by Cui *et al.* (2008). According this method, we use seven different temperatures (25, 37, 39, 41, 43, 44, and 45 °C) in a climatic chamber (Sanyo, MLR 351H), using 25 °C as control temperature and 65% constant relative humidity. The duration of the heat shocks was 1 hour, sufficient time to cause important changes on the life history traits related to fitness in *B. tabaci* evaluated in this study: survival, fecundity, and viability (Cui *et al.* 2008). For each of these variables a thermotolerance curve was constructed.

*Survival.* Experimental units of 10 adults per sex (10 females and 10 males) were employed individualized in 0.2-ml vials, permitting free passage of humidity before being subjected to each heat shock. Immediately after the heat shock, the individuals were placed at 25 °C for 3 hours to permit their recovery. Thereafter, the number of adults recovered was counted, considering as dead those individuals that did not move their appendages when stimulated by striking the test tubes (Cui *et al.* 2008). The number of experimental units varied between 6 and 12 according to the temperature treatment.

*Fecundity of females.* Fecundity was evaluated in the surviving individuals, three hours after having been subjected to each heat shock. Couples of *B. tabaci* were placed in clamp cages (1 $cm^3$) on the underside of bean leaves (*Ph. vulgaris* ICA- Pijao) at room temperature (25 °C). Five days later, the couples were removed and the number of eggs per leaflet was counted. Not all flies survived the five day period, and in that case time to death after heat shock (in days) was recorded. From 7 to 12 replicates were conducted per temperature treatment.

*Egg-nymph viability of the offspring.* This experiment determined the percentage of nymphs emerging from groups of 20 eggs oviposited by the all females during fecundity tests. The nymphs were counted 17 days after the oviposition and between 10 and 17 replicates were made per temperature treatment.

**Comparison between basal and induced thermotolerance.** *Induced thermotolerance* measures the increase in the thermotolerance after a pre-treatment at high sub-lethal temperatures or heat hardening, prior to exposure of the organisms to a stressful high temperature for the species that would normally cause a very low survival rate (Krebs 1999). To measure this thermotolerance and permit its comparison to the *basal thermotolerance* after direct heat shocks on the thermotolerance curve, it was necessary to determine two types of temperatures; $T_1$: a sub-lethal temperature, that is, the highest temperature from which no significant effects on survival are noted; and $T_2$: a stressful temperature of very low survival, close to 0%. According to these criteria, the induced thermotolerance treatment was conducted with a pre-treatment of the individuals at $T_1$/1h, then at 25 °C/1h, which is known as the acclimation period (Parsell and Lindquist 1993), and – finally – these were exposed at $T_2$/1h (heat shock). The life history traits associated to fitness like survival, fecundity, and viability were measured in the same manner as for the thermotolerance curve. These results were compared to the basal thermotolerance corresponding to the direct heat shock in $T_2$ obtained in the thermotolerance curve.

**Statistical analysis.** Before performing the analyses of variance, the assumptions of homogeneity of variances and data normality were evaluated, and data corresponding to the comparison of survival in basal and induced thermotolerance were transformed via arcsine transformation to comply with the mentioned assumptions.

Survival after heat shocks was analyzed via two-way ANOVA, using survival as dependent variable and temperature and sex of the individuals as categorical variables. For each of the fecundity and viability traits a one-way ANOVA was performed, employing fecundity or viability as dependent variable, and temperature as categorical variable. All the analyses were carried out by using the STATISTICA® 8.0 (StatSoft 2007) statistical package.

## Results

**Identification of *B. tabaci* biotype B.** According to the polymorphism obtained through RAPD, using the OPA04 primer (Quintero *et al.,* 1998) it was possible to corroborate that the samples brought from the field and selected in the laboratory were *B. tabaci* species biotype B (data not shown).

**Thermotolerance curve: Survival.** The differences were significant among temperatures of heat shock (F = 82.11; df = 6; P < 0.001, Table 1), but not between sexes (F = 0.32; df = 1; P = 0.57, Table 1). No temperature*sex interaction effect was noted (F = 1.49; df = 6; P = 0.19, Table 1). Survival diminished with increased temperature, from 98.33% in the control at 25 °C to 2.73% at 45 °C (Fig. 1A). The highest survival percentage obtained for *B. tabaci* adults was at temperatures at 25, 37 and 39 °C, with a survival rate above 90% (Fig. 1A). As of 41 °C, the survival rate began to show significant differences regarding the control temperature (Fig. 1A), going from 70.83 to 2.73% for temperatures of 44 and 45 °C, respectively.

*Fecundity of females.* Fecundity was significantly different among temperatures of heat shock (F = 16.54; df = 6; P < 0.001, Table 1). It was found that these differences were mainly due to the 45 °C treatment, where fecundity was of zero eggs (Fig. 1B). Fecundity varied between 41.33 eggs per female at 25 °C, and 33.78 eggs per female at 44 °C, with a peak of 47.37 eggs per female at 41 °C (Fig. 1B); although this variation was not statistically significant.

*Egg-nymph viability of the offspring.* Viability was significantly different among temperatures of heat shock (F = 9.16; df = 5; P < 0.001, Table 1). It was found that these differences were mainly given by the treatment at 44 °C, where viability was 67% (Fig. 1C). Viability varied between 80% at 25 °C, and 86.25% at 43 °C, with a peak of 87.50% at 37 °C (Fig. 1C), although this variation was not statistically significant (Table 2). Because fecundity at 45 °C was zero (Fig. 1C), it was not possible to record viability data at this temperature.

**Table 1.** Analysis of variance (ANOVA) for survival, fecundity, and viability traits of *B. tabaci* exposed to seven heat shock treatments (data transformed via arcsine transformation).

| Source of variation | Survival | | | Fecundity | | | Viability | | |
|---|---|---|---|---|---|---|---|---|---|
| | **D.F.** | **M.S.** | **P** | **D.F.** | **M.S.** | **P** | **D.F.** | **M.S.** | **P** |
| Temperature | 6 | 4.40 | <0.001*** | 6 | 2679.74 | <0.001*** | 5 | 722.9 | <0.001*** |
| Sex | 1 | 0.02 | 0.57 | NA | | | NA | | |
| Temp. * Sex | 6 | 0.08 | 0.19 | NA | | | NA | | |
| Error | 92 | 4.58 | | 60 | 161.98 | | 72 | 78.9 | |
| Total | 105 | | | 66 | | | 77 | | |

* Significance: P < 0.050. ** Significance: P < 0.010. *** Significance: P < 0.001.

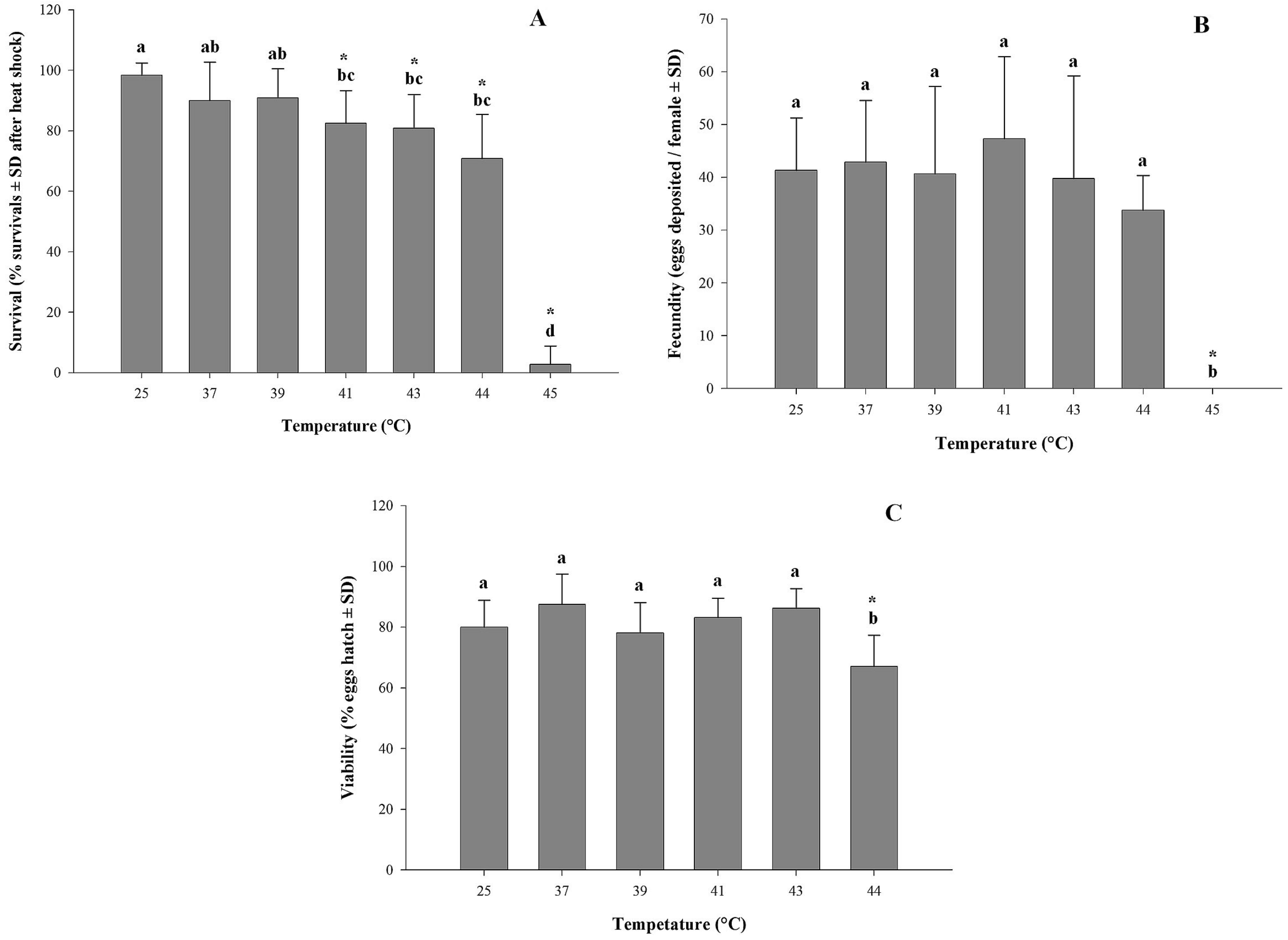

**Figure 1.** Thermotolerance curve for each of the life history traits related to fitness in *B. tabaci*. **A.** Survival: percentage of surviving *B. tabaci* adults after each heat shock. The bars represent the survival mean for the females-males set in each temperature treatment. **B.** Fecundity: number of eggs oviposited per *B. tabaci* female during the five days after having been subjected to heat shock. **C.** Viability: percentage of *B. tabaci* eggs that hatched 17 days after the heat shock. Different letters over the bars indicate significant differences among temperatures according to Tukey's post-ANOVA ($P < 0.05$). On the other hand, symbol * indicate differences between the each treatment and control, according to Dunnett post-ANOVA test ($P < 0.05$). Specific differences mentioned in text comparing different treatments and control, like those for select the heat hardening temperature were evaluated using the Dunnett test. General comparisons between treatments were done using the Tukey test.

**Comparison between basal and induced thermotolerance.** These experiments were carried out by using data from the survival curve, from which 45 °C was chosen as the stressful high temperature to carry out basal and induced thermotolerance measurements, given that at this temperature the survival percentage was close to 0% (Fig. 1A). Survival was significantly affected as of 41 °C (Fig. 1A), which is why 40 °C was selected as the sub-lethal temperature for pre-treatment of thermal conditioning.

Differences between types of thermotolerance were significant ($F = 30.08$, $df = 1$; $P < 0.001$, Table 2); differences were also noted in this case between males and females ($F = 5.55$; $df = 1$; $P = 0.025$, Table 2), as well as temperature *sex interaction effect ($F = 8.36$; $df = 1$; $P = 0.007$, Table 2). The interaction effect indicated that the difference between both types of thermotolerance should be analyzed separately for each sex, and according to this analysis, It was determined by the greater survival of females under induced thermotolerance treatment, compared to males under the same treatment (Fig. 2), and the females and males of basal thermotolerance (Fig. 2), without differences between sex in the basal treatment. Thermotolerance was 2.73% for both sexes in basal thermotolerance, and increased to 10% (without significance) in males and 33% in females receiving thermal conditioning at 40 °C (Fig. 2).

Surviving females in basal thermotolerance and induced thermotolerance did not oviposit, for which fecundity was zero in these treatments, and it was also not possible to record comparative data of viability.

## Discussion

Adult insects can die immediately after a short exposure period to an extremely high temperature, or survive with after effects, which are manifested in the quantity or the development of the offspring (Denlinger and Yocum 1998). According to Somero (1995) and Denlinger and Yocum (1998), the immediate heat damage endured by the insects when subjected to high non-lethal temperatures is produced by dehydration and denaturalization of proteins and enzymes and, as a consequence, survival, fecundity, or viability of the offspring

**Table 2.** Analysis of variance (ANOVA) for the survival variable comparing basal and induced thermotolerance in *B. tabaci* adults (data transformed via arcsine transformation).

| **Source of variation** | **S.S.** | **D.F.** | **M.S.** | **F** | **P** |
|---|---|---|---|---|---|
| Type of thermotolerance | 1.00 | 1 | 1.00 | 30.08 | <0.001*** |
| Sex | 0.18 | 1 | 0.18 | 5.55 | 0.025* |
| Type of thermotolerance x Sex | 0.28 | 1 | 0.28 | 8.36 | 0.007** |
| Error | 1.00 | 30 | 0.03 | | |
| Total | 2.46 | 33 | | | |

* Significance: P < 0.050. ** Significance: P < 0.010. *** Significance: P < 0.001.

is reduced. At extreme temperatures, the effect produced by the heat shock can depend on the accumulated levels of stress, the physiological state of the cells, and the organism in general, as well as on the sex (Krebs and Loeschcke 1994). The temperatures used during heat shocks significantly affected the three life history traits implied in fitness evaluated in *B. tabaci* (survival, fecundity, and viability), understood as the capacity to transmit descent to the following generation. These effects found followed a different pattern for each trait, so survival diminished with increased temperature. Fecundity of the females was only affected significantly at 45 °C (Fig. 1B), where the surviving individuals did not have reproductive potential, *i.e.*, they were incapable of rising, sustaining themselves, and walking on the test tube walls. Viability diminished significantly only at 44 °C (Fig. 1C) with respect to the rest of the temperatures evaluated.

These results indicate that between 41 and 43 °C, although there were direct effects of temperature on survival, the physiological damage produced by said shocks did not affect the development of the oocytes, which is why the posture of the females and the viability of the eggs oviposited occurs without significant effects of heat damage in comparison to the control treatment. Only at 45 °C was the direct effect of the heat shock so intense as to keep the surviving females from reproducing. At 44 °C this type of effect was not noted, but some heat damage seems to be present in the development of the oocytes, given that only at this temperature their hatching percentage diminished.

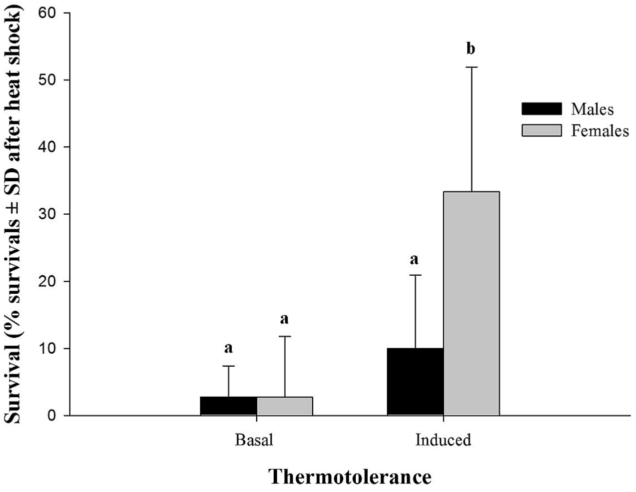

**Figure 2.** Survival of *B. tabaci* adults under basal and induced thermotolerance conditions. Different letters over the bars indicate significant differences among temperatures according to Tukey's post-ANOVA (P < 0.05).

It is clear that survival can be affected by heat shocks due to the direct heat damage on the protein and cell structures in any part of the body, with sufficiently important physiological effects to bring on the organism's death. On the other hand, fecundity and viability are probably affected by more localized effects of the heat damage on oocyte production and their development. If the heat damage affects the female's reproductive organs; hence, affecting the oocyte production process, fecundity is diminished (Scott and Hoffmann 1997; Rinehart *et al.* 2000), as it occurred at 45 °C. But if the damage injures the oocytes, although the number of eggs oviposited is not affected, their consequent development is reduced, manifesting significantly reduced viability with respect to the control treatment (Krebs and Loeschcke 1994).

Also, the thermotolerance curve evidences that *B. tabaci* is a highly tolerant insect species in comparison to other cosmopolitan insects or other species of white flies. For example, some species from the *Drosophila* genus present significant survival reductions at 38 °C, reaching almost zero at 39 °C (Bettencourt *et al.* 1999). *T. vaporariorum*, another species of whitefly that can be distributed in zones along with *B. tabaci,* shows significantly decreased survival as of 39 °C (Cui *et al.* 2008), two degrees less than that registered for *B. tabaci* (Fig. 1A). Fecundity and viability in *T. vaporariorum* diminish as of 37 and 39 °C, respectively (Cui *et al.* 2008), while in the population from Rozo evaluated for *B. tabaci* the temperatures corresponding to said effects were 45 and 44 °C, respectively.

The thermotolerance pattern found for the population from Rozo in *B. tabaci* biotype B differs from those registered on species conducted in other populations. Survival to heat shocks, for example, follows a similar pattern to that reported by Cui *et al.* (2008) for *B. tabaci* populations in Beijing (China). However, unlike the results obtained for *B. tabaci* in Rozo, these authors also found significant differences for survival between sexes, with greater survival for females. Other studies carried out in *B. tabaci* populations in Colombia have revealed differences in survival between sexes at 45 °C (Muñoz-Valencia *et al.* 2011; Juvinao-Quintero *et al.* 2011), which is why this trait seems to also show a high population variation that additionally depends on the temperature evaluated. Cui *et al.* (2008) did not find significant differences for female posture between 37 and 45 °C; although they reported significant effects on viability as of 39 °C. That is, in the whitefly population in Beijing (China), the temperature affecting survival is the same affecting oocyte development, although production in the number of eggs was not affected by any of the temperatures evaluated by these authors.

*B. tabaci* in the Rozo population was not very affected by temperatures between 37 and 43 °C in comparison to that reported by Cui *et al.* (2008) for the Beijing (China) population, and this difference is mainly located in viability, which remains unchanged for the Rozo population, but is diminished in Beijing (China). At 45 °C, this relation is inverted, given that the whitefly in Beijing (China) manages to survive approximately ten times more in proportion to what was registered for Rozo, and –additionally– the survivors manage to reproduce and have viable offspring. This contrast indicates the existence of population differences in tolerance to heat shocks within this species, due to effects of the geographical distance or environmental differences between the distribution zones, which could indicate adaptation to local environments. According to Cui *et al.* (2008) *B. tabaci* proliferates in Beijing (China) with different levels in different seasons with higher populations during the summer, when temperatures are at 30 °C and can increase during the day to 39 °C. Additionally, because it is at sea level (55 masl) this zone easily exceeds the relative humidity of 85%. The Rozo population, besides being in a different continent, is found approximately at 1000 masl in Colombian tropics, for which the temperature and humidity conditions are also different and more constant among months in the year, with average temperature and relative humidity during the month at 25 °C and 70%, respectively. The population in Beijing (China) seems to respond better to extreme temperatures (45 °C), in detriment of fitness at intermediate temperatures compared to the population distributed in Rozo, where extreme temperatures would be less frequent and with low variation in the year.

*Comparison between basal and induced thermotolerance.* Survival was greater under conditions of induced thermotolerance in contrast to basal thermotolerance, which evidences that organisms conditioned exhibit a transient capacity to survive the heat shock, which would kill most unconditioned organisms (Parsell and Lindquist 1993). This agrees with that obtained by Dahlgaard *et al.* (1998), who reported that *Drosophila* adults are benefited by an extended period of increased thermotolerance when they are pre-exposed to high, non-lethal temperatures or heat hardening as acclimation period. Heat hardening can increase fitness of individuals exposed to heat stress, in contrast to individuals exposed to direct heat shock (Levins 1969), and also promotes acclimation of the organism to withstand heat stress, while during basal thermotolerance the organism must face and endure stress without prior preparation.

This study found that the optimal temperature for pre-treatment in induced thermotolerance is 40 °C. It has been observed that at this temperature *B. tabaci* increase synthesis of two polypeptides, corresponding to the Hsp90 and Hsp70 heat shock proteins (Salvucci *et al.* 2000), as well as synthesis of substances with reported functions of heat protection like sorbitol (Wolfe *et al.* 1998; Salvucci 2000), which suggests the existence of a relation between the induction of thermotolerance and increased levels of heat shock protein expression and heat protection substances in this insect. These proteins, as in sorbitol, play an important role in tolerance to heat stress in insects, by protecting the native structure of the thermal denaturing of proteins (Back *et al.* 1979; Henle *et al.* 1982; Salvucci *et al.* 2000). However, according to Salvucci (2000), sorbitol only functions in the presence of food during the time of the heat shock. In this study, tests were conducted individualizing the flies in vials, applying heat shocks without the presence of food, so the increase of survival in induced treatment could be attributed to the expression of Hsp70 proteins instead of other mechanisms like the synthesis of polyhydric alcohols.

Increased survival in induced thermotolerance was only statistically significant for *B. tabaci* females. According to Lu and Wan (2008), resistance under conditions of heat shock is associated to differential gene expression between *B. tabaci* males and females, specifically genes related to metabolism that can be involved in some aspects of heat resistance. This could be explained by the genetic load, given that females are diploids and males are haploids (Price 1996), so expression of important genes for thermotolerance, like Hsp70, can happen faster and in greater numbers in females than in males. Additionally, males are smaller than females, showing a lower surface-to-volume proportion that powers a faster increase of the internal temperature, which makes them more likely to die by desiccation (Dahlgaard *et al.* 1998).

Thermal conditioning does not always lead to increased fitness (survival, fecundity, or viability). Conditioning implies large energy investment, for example, in heat shock protein synthesis, which could cause a *"trade-off*" where greater survival would occur, but fecundity and viability would be reduced (Parsell and Lindquist 1993; Hoffmann 1995; Yu and Wan 2009). This possibly occurred in the Rozo population, as expected, under conditions of induced thermotolerance survival increased, but fecundity and viability were seriously affected, given that none of the surviving individuals had reproductive potential. This suggest that this possible *trade off* in the Rozo population is not a general characteristic of the species but of the population evaluated because it has been found that *B. tabaci* can survive and reproduce even at 45 °C under basal thermotolerance conditions (Cui *et al.* 2008; Saldaña *et al.* 2011) and induced thermotolerance conditions (Muñoz-Valencia *et al.* 2011) in other populations of the current distribution of *B. tabaci*, like Beijing (China), Retiro de los Indios, and Candelaria (Colombia), respectively.

## Conclusion

Finally, it may be stated that the *B. tabaci* biotype B population studied exhibits great heat tolerance within a broad range of temperatures, above those reported in literature for other cosmopolitan species like *Drosophila*, as well as other species of white flies. In addition, increased survival of adults is noted when they are pre-treated at sub-lethal temperatures, which could indicate the presence of important heat protection mechanisms (Dahlgaard and Loeschcke 1997). This could benefit organisms like *B. tabaci* with a prolonged period of acquired thermotolerance, which would protect them against immediate heat stress, as well as against stress experienced some days later (Dahlgaard *et al.* 1998). However, it is important to clarify that thermal conditioning can present a *trade off* in which survival increases as in the Rozo population, but fitness as a whole, understood as the capacity to transmit viable offspring to the following generation, can be affected (Parsell and Lindquist 1993). Ecological implications of the induced thermotolerance depend of the population of the species, which is why the ecological implications of induced thermotolerance depend on the relation between increased survival and the after effect on the reproduction (fecundity and viability) of the surviving individuals. Thus, only when the surviving individuals show reproductive potential, is fitness increased as a whole, making the individuals most suited to colonize environments that are unexplored and inadequate for them. Although the population evaluated in this study could not has reproduction after thermal treatments, the presence of important heat protection mechanisms confirmed by the increase in survive with strong lab conditions (induced thermotolerance) indicate reproductive potential in natural conditions, where stress temperatures are lower and other interactive natural factors are important. Thereby, these results could contribute to explaining the patterns of population dynamics in this species. In Colombia, for example, biotype B has recently expanded both in latitudinal and altitudinal manner, and said expansion coincided with and was possibly facilitated by a dry period (Rodríguez *et al.* 2005). Currently, biotype B has displaced biotype A, proliferating in Colombian tropics.

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