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Thermosensitivity and the acquisition of thermotolerance in eggs of *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae)

[Kepekaan haba dan perolehan ketahanan haba bagi telur *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae)]

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Abtract

Young (2-h-old) and mature (26-h-old) eggs of Queensland fruit fly, *Bactrocera tryoni* (Froggatt) were tested for development of thermotolerance by acclimatising them at a range of temperatures (30, 35, 37 and 40 °C) for 1–6 h, then challenging them at 46 °C for a time needed to achieve the LT_{50}. Both young and mature eggs had the ability to develop thermotolerance and showed variable and differing responses, depending on the temperature and duration of acclimation. Young eggs acclimatised at 30 °C for 3 h or 5 h or at 35 °C for 6 h showed significantly decreased mortality ($p \leq 0.05$) when challenged compared to controls held at 26 °C, indicating development of thermotolerance. Mature eggs acclimatised at 35 °C or 37 °C also showed significantly decreased mortality ($p \leq 0.05$). When acclimatised at 40 °C, both young and mature eggs showed higher mortality than controls. Mortality in mature eggs was significantly different ($p \leq 0.05$) only after 6 h of acclimation, but mortality for young eggs was always greater ($p \leq 0.05$) than controls.

Introduction

Queensland fruit fly, *Bactrocera tryoni* (Froggatt), is a major international quarantine pest endemic to coastal regions of northern and eastern mainland states of Australia. Fruits and vegetables that are hosts to this fly may need to be subjected to quarantine disinfestation treatments before they can be traded to other parts of Australia and internationally. Export markets such as Japan, US and New Zealand prohibit importation of fruits and vegetables which are hosts of Queensland fruit fly, without approved disinfestation treatments (Heather 1996).

Some of the postharvest disinfestation treatments recently accepted internationally use fumigation with methyl bromide (MB), or residue free physical treatment with heat, cold or irradiation. Treatment options depend largely on their acceptability to the importing country, tolerance of the produce to treatments, and the degree of consumer preference for residue free physical treatments. Heat treatment is highly appropriate to tropical and temperate fruits being well tolerated physiologically and having logistical flexibility.

The most popular and commonly used heat treatments are hot water dipping and circulated hot air. Hot water dipping is

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approved by the US quarantine authorities for the export of papaya from Hawaii to the mainland (Couey and Hayes 1986) and the import of mangoes from Peru and the Caribbean (Sharp and Pico-Martinez 1990). The most favoured method currently is hot air treatment including vapour heat and other related methods which are used for the export of papaya from Hawaii to mainland USA and mangoes from the Philippines, Thailand and Australia to Japan (Merino et al. 1985; Armstrong et al. 1989; Unahawutti et al. 1993; Heather et al. 1997).

With the banning of the fumigant, ethylene dibromide (EDB) in 1984 in the US and elsewhere and the agreement that methyl bromide be progressively withdrawn from use by many countries from the year 2000, it will be necessary for disinfestation research to look actively to physical treatments, especially heat for future quarantine requirements.

The purpose of this study was to examine the lethal response of eggs of Queensland fruit fly to heat applied by hot water immersion in terms of the acquisition of thermotolerance that could modify the efficacy of quarantine treatments.

Materials and methods

Insect rearing

Populations of *B. tryoni* were maintained in laboratory cages containing about 25,000 flies of mixed sex, using the rearing procedures of Heather and Corcoran (1985).

Egg collection

Eggs were collected from mature females (>1 month old) using hollow apple domes prepared as described by Heather and Corcoran (1985). These were exposed for oviposition in a holding cage for 1 h. Eggs were then washed from the apple dome using a stream of water and transferred onto black filter paper using a Büchner funnel connected to a vacuum pump. This removed free moisture and spread the eggs evenly on black filter paper for optimal visibility. The black filter paper with eggs was then placed on a moist sponge and held for a maximum of 26 h at a temperature of 26 ± 1 °C until required for treatment.

Based on the mid-point of the oviposition exposure, a nominal collection time, representing zero development, was assigned (Corcoran 1993). Young eggs were those treated 2 ± 0.5 h after this assigned oviposition time and mature eggs were those treated 26 ± 0.5 h after oviposition.

Hot water immersion

Several hundred eggs were collected by cutting portions of filter paper with eggs and washing the eggs into a 20 mm diameter glass tube which was open at both ends but gauzed with muslin cloth at the dipping end to allow direct exposure to the hot water of the water bath. For treatment, tubes with eggs were immersed in water in a Grant W38 water bath of 38 litre capacity with a controller specification of ± 0.05 °C at 26, 30, 35, 37, 40, or 46 °C. The water bath temperature was calibrated and confirmed with a standard mercury-in-glass thermometer.

Three replicates of eggs at each conditioning temperature (30 through 40 °C) were immersed simultaneously. Treatments were removed from the water bath after 1, 2, 3, 4, 5 or 6 h exposure time. After exposure, eggs were immediately cooled in ambient water for 1 min, to dissipitate the heat, before being challenged at a lethal temperature of 46 °C for a time needed to achieve the LT_{50} mortality found in preliminary trials to be 45 sec for young eggs (2-h-old) and 4.7 min for mature eggs (26-h-old).

Treated eggs were washed from the test tube into the Büchner funnel and on to black filter paper. One hundred eggs from each treatment were then transferred to carrot medium in 90 mm diameter plastic petri dishes. These were held in a controlled temperature room at 26 ± 1 °C and 70 ± 2% RH. Egg mortality was assessed 96–120 h after oviposition.

Control eggs were treated in the same manner by dipping in water at 26 °C but were not challenged at the lethal temperature.

Data analysis

All mortality data were corrected for natural mortality using Abbott's formula (Abbott 1925). Where appropriate, arcsin transformation was done to facilitate analysis of data. This is a standard transformation for data expressed as percentages (Steel and Torrie 1980). Data were then analysed by ANOVA using Biometry Statistical Software, Queensland Department of Primary Industries, Australia, 1992.

Results and discussion

Young eggs

Young eggs acclimatised at 30 °C showed significantly lower mortality than controls at the third and fifth hours of acclimation (*Figure 1*). At 35 °C acclimation, although acclimatised eggs had higher survival than controls, only the sixth hour of acclimation showed a statistically significant reduction in mortality ($p \leq 0.05$). At 37 °C, significant differences were apparent ($p \leq 0.05$) after the fifth and sixth hours of acclimation, but the mortalities were higher for the acclimatised eggs than for controls, indicating thermo-intolerance. At 40 °C, there were significant differences in mortality between the acclimatised eggs and the controls for all the

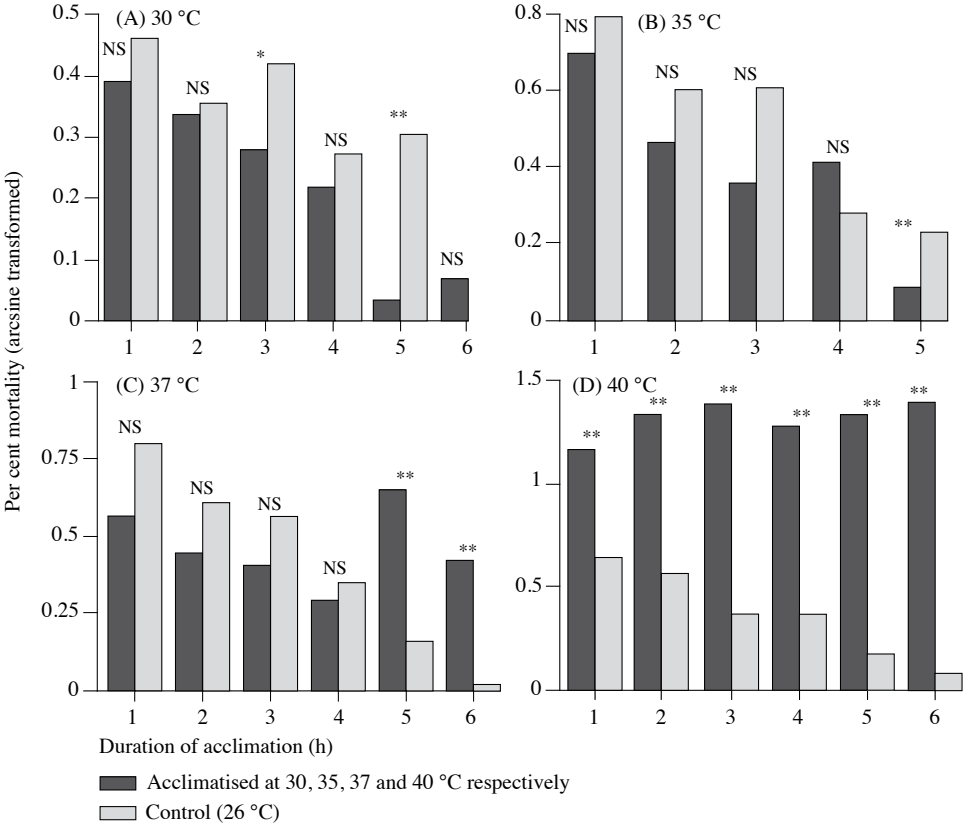

*Figure 1. Comparison of mortality of young eggs at 30, 35, 37 and 40 °C and at 46 °C for LT_{50} level. Asterisk(s) or letters above indicate the level of significance in per cent mortality between acclimatised and control groups. (ANOVA, a = 0.05): * = p ≤0.05, ** = p ≤0.01, NS = Not Significant*

treatment times (1–6 h), again with higher mortality in the preconditioned eggs. This high level of mortality is likely to be due to thermo-intolerance at 40 °C.

However, control eggs showed a decreasing trend in mortality as duration of acclimation was prolonged, but this could be due to developmental changes which occurred with ageing (*Figure 1*). Moss and Chan (1993) reported that mortality of eggs of Mediterranean fruit fly, *Ceratitis capitata* (Wiedemenn) subjected to hot water immersion was dependent on age, with decreasing mortality with age. However, to the contrary, Ashburner and Bonner (1979) found that eggs of *Drosophila melanogaster* at the embryonic developmental stage could be easily killed by exposure to extreme heat.

Mature eggs

For mature eggs, no significant difference was observed between acclimatised and control eggs at 30 °C for any duration (*Figure 2*). However at 35 °C and 37 °C, significant differences were observed for both temperatures when eggs were acclimatised for 1, 4, 5, or 6 h. At 35 °C alone, mortality was reduced markedly from 3 h to 6 h of acclimation and survival of acclimatised eggs after sixth hour almost reached that of natural mortality. Inducing thermotolerance by conditioning eggs at

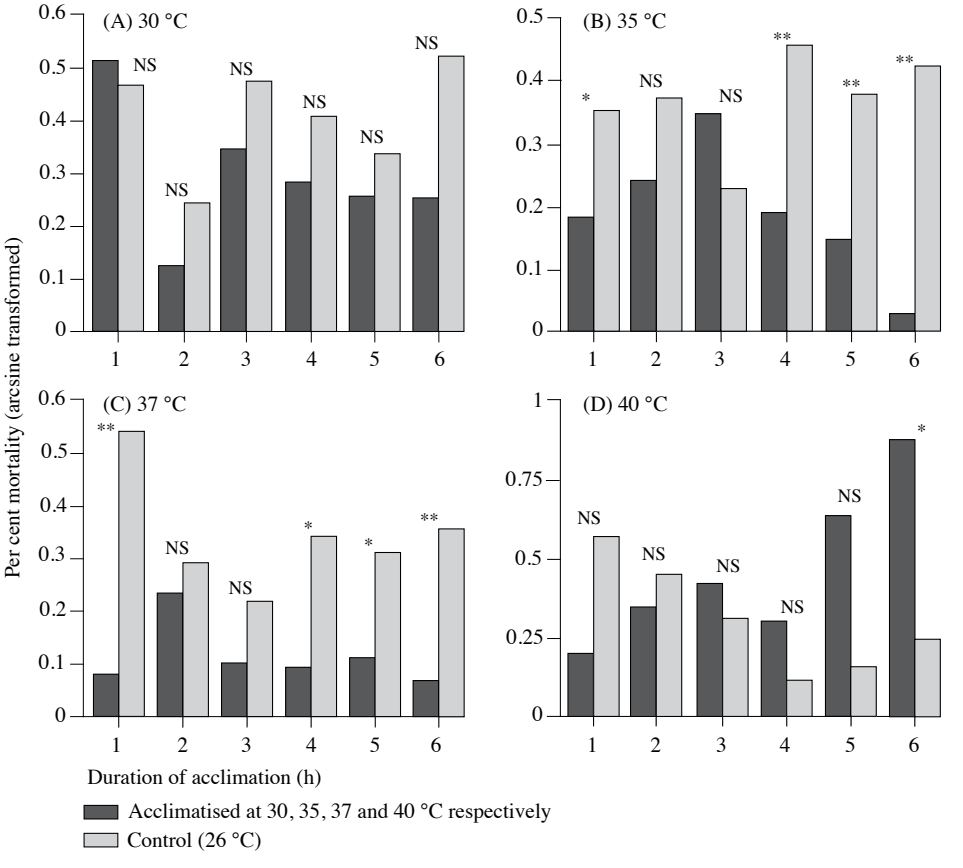

*Figure 2. Comparison of mortality of mature eggs acclimatised at 30, 35, 37 and 40 °C and challenged at 46 °C for LT_{50} level. Asterisk (s) or letters above bars indicate the level of significance in per cent mortality between acclimatised and control groups. (ANOVA, a = 0.05): * = p ≤0.05, ** = p ≤0.01, NS = Not Significant*

35 °C and 37 °C for certain times increased survival of the eggs when later subjected to a more severe challenge temperature. The authors hypothesise that survival of preconditioned eggs is the result of the synthesis of heat shock proteins during preconditioning as was shown for eggs of *Ceratitis capitata* by Jang (1992). The presence of heat shock proteins in the heat tolerant eggs compared to control is currently being investigated in a separate study using electrophoretic techniques to determine whether correlation can be found.

The mortality of eggs acclimatised at 40 °C was lower than for the control during the 1 h and 2 h, and but the reverse occurred after 3 h and until the maximum duration of 6 h of acclimation. At 40 °C therefore, thermotolerance could only be induced during the first 2 h of acclimation and beyond this point the process was reversed. At 6 h, there was a significant difference ($p \leq 0.05$) between the acclimatised and the controls, but the mortality rate was lower in controls than in the acclimatised eggs (*Figure 2*). Acclimation at 40 °C was related to high mortality regardless of the age of the eggs. Long duration of exposure (more than 3 h) at 40 °C were sufficient to kill eggs even before exposing them to the challenge temperature of 46 °C. Therefore, exposing eggs for more than 2 h can be considered a lethal treatment.

It is concluded that young and mature eggs acclimatised at 30, 35, 37 or 40 °C responded differently in their mortality. The control temperature of 26 °C may have been sufficient to provide some level of thermotolerance both for the young and the mature eggs in a similar way to that provided by exposure to 30, 35, or 37 °C. However, this could also have been an age effect particularly for the young eggs as discussed above. An acclimation temperature of 35 °C and 37 °C was judged to be the best temperature for conferring thermotolerance in mature eggs. It achieved the lowest levels of mortality as compared to the control at 1, 4, 5 and 6 h of acclimation (*Figure 2*). Acclimation for more than 2 h at 40 °C caused increased mortality even without challenge.

Thermotolerance is shown to be linked to age. The correlation between thermotolerance in eggs with their corresponding level of heat shock proteins synthesised warrants further investigation. Because of relatively short heating times (<2 h) for most modern hot air or hot water treatment systems, this phenomenon of heat acclimation seems unlikely to affect hot water or hot air disinfestation treatment efficacy unless fruits are pre-conditioned with heat during treatment.

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Abstrak

Telur muda (2 jam) dan telur matang (26 jam) lalat buah Queensland, *Bactrocera tryoni* (Froggatt) telah diuji untuk mendapatkan ketahanan terhadap suhu panas dengan mendedahkan telur-telur ini pada pelbagai suhu (30, 35, 37 dan 40 °C) selama 1-6 jam sebelum dicabar pada suhu 46 °C untuk mencapai LT_{50}. Kedua-dua telur muda dan telur matang mampu mencapai ketahanan terhadap suhu panas, dan menunjukkan tindak balas yang berbeza mengikut suhu dan tempoh masa didedahkan. Telur muda yang dipradedahkan pada suhu 30 °C selama 3 jam atau 5 jam atau pada suhu 35 °C selama 6 jam menunjukkan kematian menurun yang signifikan ($p \leq 0.05$) apabila dicabar pada 46 °C berbanding dengan telur kawalan yang dipradedahkan pada suhu 26 °C, membuktikan berlaku ketahanan terhadap suhu panas (*thermotolerance*). Telur matang pula yang dipradedahkan pada 35 °C atau 37 °C juga menunjukkan kematian menurun yang signifikan ($p \leq 0.05$). Apabila dipradedahkan pada suhu 40 °C, kedua–dua telur muda dan telur matang menunjukkan kematian tinggi yang signifikan ($p \leq 0.05$) berbanding dengan kawalan. Kematian pada telur matang menjadi signifikan ($p \leq 0.05$) hanya setelah 6 jam prapendedahan, tetapi untuk telur muda kematian senantiasa melebihi ($p \leq 0.05$) kawalan.

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