Heat and Cold Tolerance of Various Aged Eggs of *Bactrocera dorsalis* and *B. cucurbitae* (Diptera: Tephritidae)

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Abstract: Various aged eggs of Oriental fruit fly, *Bactrocera dorsalis* (Hendel) and melon fly, *Bactrocera cucurbitae* (Coquillett) were subjected to hot water immersion at 44, 45, 46, 47℃ for 5 min or cold treatment at 1 and 3℃ for 5 days to determine the heat and cold tolerance by age of the eggs. The results of hot water immersion test showed that there was a trend towards increased heat tolerance with egg age and there were also significant variations within this trend in both fruit fly species. However, in the case of cold treatment, such a variation was not clear in both species while the trend towards more tolerance of cold with egg age was observed.

Key words: quarantine heat treatment, cold treatment, egg age, *Bactrocera dorsalis*, *Bactrocera cucurbitae*

Introduction

Oriental fruit fly, *Bactrocera dorsalis* (Hendel) and melon fly, *Bactrocera cucurbitae* (Coquillett) that had caused serious damage to agricultural production in the southwestern islands of Japan, was eradicated in 1986 after investment of 18 years and 5 billion yen and in 1993 after over 22 years and more than 20.4 billion yen, respectively (Yoshizawa, 1997). Therefore, a strict watch on Tephritid fruit flies, considered a pest of quarantine significance and a ban on the importation of host plants of these fruit flies from the countries where they exist are maintained by the Japanese quarantine authorities.

However, in accordance with Article VII (2) (a) of the International Plant Protection Convention (FAO, 1997), Japan approves the lifting of import bans when a disinfestation technique with complete kill of these pests has been established by an exporting country and treatment conditions are satisfied (Yoshinaga et al., 2009).

In development of a quarantine disinfestation treatment such as heat treatment and cold treatment, the large-scale disinfestation tests are conducted after determination of the most tolerant stage of the target pests to the treatment (Corcoran et al., 1993; Couey and Chew, 1986; Heather and Hallman, 2008; Heather et al., 1997; Hill et al., 1988; Jessup et al., 1993; Santaballa et al., 2009).

As for the fruit fly eggs, it is reported that heat-susceptibility of young (early-aged) eggs differed from that of mature (late-aged) eggs (Heard et al., 1991; Sales et al., 1996; Foliaki and Armstrong, 1996; Waddell et al., 1997). Furthermore, heat tolerance of egg varied during egg maturation in *Ceratitis capitata* and *Bactrocera tryoni* (Moss and Jang, 1991; Corcoran, 1993). Thus, age of egg tested is an important consideration in the determination of the most tolerant stage among the test insects.

In the present study, we investigated the relationship between egg age of *B. dorsalis* and *B. cucurbitae*, and the mortality caused by hot water immersion or cold treatment.
Materials and Methods

1. Test insects

Laboratory colonies of *B. dorsalis* and *B. cucurbitae* maintained at Naha Plant Protection Station in Okinawa were used. The colonies of *B. dorsalis* (Permit No.63Y2152) and *B. cucurbitae* (Permit No.3N139) were originally collected in Okinawa island before their eradication in 1986 and 1993, respectively. Flies were kept at 27 ± 1°C, 70 ± 10% RH with a photoperiod of 14L:10D and given an artificial diet and water in the adult cage (45×30×30 cm).

Eggs were collected with two polyethylene receptacles (7 cm in diameter, 17 cm in height, 88 oviposition holes) which were placed into the adult cage containing about 2,000 flies for 1 hour. The inner surface of the receptacle was moistened with lemon juice. Eggs were washed out using tap water and transferred onto black gauze using funnel. The black gauze with eggs was then placed on moist filter paper in sterile plastic petri dishes (9 cm in diameter) and kept in a constant temperature chamber (Hitachi Appliances, Inc., Cosmopia EC-42HHD) at 27.0 ± 0.3°C to allow embryonic development until required for treatment. Temperature inside the petri dish was monitored by sensor (Pt 100Ω) and recorded with automatic print recorder (Chino Co., LE2000).

Preliminary test revealed that eggs held at 27.0 ± 0.3°C started to hatch approximately 31 hours and 24 hours after laying for *B. dorsalis* and *B. cucurbitae*, respectively. Based on the mid-point of the oviposition exposure, a nominal collection time, representing zero h (hour)-old egg, was assigned (Corcoran, 1993). Every 3 hours, eggs held at 27.0 ± 0.3°C were removed from the chamber and subjected to hot water immersion or cold treatment.

2. Hot water immersion

Various aged eggs, i.e. 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 h-old eggs of *B. dorsalis*, and 3, 6, 9, 12, 15, 18, 21 and 24 h-old eggs of *B. cucurbitae* were subjected to hot water immersion. Eggs in a glass tube (2.5 cm in diameter, 15 cm in height with a fine wire mesh on the bottom) were immersed into hot water at either 44, 45, 46 or 47°C for 5 min. Treatment was applied in a temperature controlled water bath (Yamato Scientific Co., Ltd., BK-53, 70 liter capacity) after verification of target temperature using a standard precision thermometer (Toa Keiki MFG. Co., Ltd). After treatment, the tubes were taken out of the hot water and immediately immersed into water at 25°C for 5 min in order to prevent further heating. Treated eggs were washed from the tube into funnel and onto black gauze. Untreated control eggs of each species and egg age were handled in the same manner except that they were dipped into water at 25°C for 10 min.

One hundred eggs selected at random from the treated and control of each species, were then transferred to moist black filter paper in plastic petri dishes. These were held in a constant temperature chamber at 27.0 ± 0.3°C for 3 days to assess egg mortality.

These tests were replicated 6 times.

3. Cold treatment

One hundred eggs selected at random from each egg age of each species, i.e. 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 h-old eggs of *B. dorsalis*, and 3, 6, 9, 12, 15, 18, 21 and 24 h-old eggs of *B. cucurbitae*, were then transferred to moist black filter paper in plastic petri dish (9 cm in diameter) and were subjected to cold treatment. Dishes were covered with Parafilm 'M'® to prevent desiccation and then kept in a cold chamber (Hitachi Appliances, Inc., Cosmopia EC-40HHP) set at 1.0°C or 3.0°C for 5 days. After cold treatment, treated eggs in the dishes were held in a constant temperature chamber at 27.0 ± 0.3°C for 3 days.

Untreated control eggs of each species and egg age were handled in the same manner except for cold treatment. After 3 days held at 27.0 ± 0.3°C, egg mortality of treated and control was assessed.

These tests were replicated 6 times.

4. Data Analysis

Egg mortality was assessed at hatching, and all mortality data corrected for mean control mortality using Abbott’s formula (Abbott, 1925). In order to compare the heat or cold tolerance between ages for fruit fly species, data on the corrected mortality from each temperature level in these experiments were subjected to Kruskal-Wallis test and Steel-Dwass test (P > 0.05) using statistical software (Statcel 3).

Results

1. Hot water immersion

The response of *B. dorsalis* and *B. cucurbitae* eggs to heat measured by mean corrected mortality of 6 replicates in each temperature level is shown in Fig. 1 and Fig. 2, respectively. The general trend of both fruit fly species was that eggs become more tolerant of heat as development occurs. In addition, heat tolerance
of *B. dorsalis* eggs decreased twice, i.e. at around 15 h-old and 24 h-old during embryo development, whereas *B. cucurbitae* eggs decreased once at around 15 h-old. This phenomenon was more remarkable at 45°C than at the other temperatures in both species. The corrected mortality of 15 h-old and 24 h-old eggs of *B. dorsalis* or 15 h-old eggs of *B. cucurbitae* treated at 45°C was significant different from before and after the ageing in each species.

**Fig. 1.** Relationship between age and mortality for *Bactrocera dorsalis* eggs immersed in hot water at 44, 45, 46 and 47°C for 5 min. Means in each test followed by the same letter are not significantly different (P > 0.05; Steel-Dwass test).

**Fig. 2.** Relationship between age and mortality for *Bactrocera cucurbitae* eggs immersed in hot water at 44, 45, 46 and 47°C for 5 min. Means in each test followed by the same letter are not significantly different (P > 0.05; Steel-Dwass test).
2. Cold treatment

The response of *B. dorsalis* and *B. cucurbitae* eggs to cold measured by mean corrected mortality of 6 replicates in each temperature level is shown in Fig. 3 and Fig. 4, respectively. The general trend of both fruit fly species was that eggs become more tolerant of cold as development occurs. There was a significant difference in corrected mortality between 3-12 h-old and 18-24 h-old eggs of *B. dorsalis*, and between 3-12 h-old and 18-24 h-old eggs of *B. cucurbitae*, respectively.

Discussion

The results of our hot water immersion test with *B. dorsalis* and *B. cucurbitae* eggs showed that the mortality was dependent on age, with eggs becoming generally more tolerant of heat as embryonic development progressed (Fig. 1 and Fig. 2).

Some authors reported in other fruit fly species such as *Anastrepha ludens* (Tanabe et al., 1994), *Bactrocera curvipes*, *B. facialis*, *B. melanotus*, *B. psidii*, *B. tryoni*, *B. xanthodes* (Sales et al., 1996; Foliaki and Armstrong, 1996; Waddell et al., 1997; Corcoran, 1993; Heard et al., 1991) and *Ceratitis capitata* (Moss and Jang, 1991) that mature eggs were more heat tolerant than young eggs. However, in *Bactrocera passiflorae* eggs, young eggs (<10 h-old) were more tolerant than mature eggs (>40 h-old) against hot water immersion at 44°C (Tora Vueti et al., 1996).

Our results indicated that there was a trend towards increased heat tolerance with egg age and there were also significant variations within this trend. During embryonic development, the points of remarkable decrease in heat tolerance were observed twice at around 15 h-old and 24 h-old in *B. dorsalis* eggs (Fig. 1) and once at around 15 h-old in *B. cucurbitae* eggs (Fig. 2) under hot water immersion at 45°C. Moss and Jang (1991) and Corcoran (1993) reported similar variations in the pattern of tolerance in *Ceratitis capitata* and in *Bactrocera tryoni*, respectively. Remarkable decrease was observed once at around 30 h-old in *B. tryoni* eggs under hot water immersion at 46°C (Corcoran, 1993) and also observed 3 times at around 10 h, 27 h and 42 h-old in *C. capitata* eggs under hot water immersion at 43°C (Moss and Jang, 1991). Corcoran (1993) reported that these variations in tolerance might reflect events in embryonic development during which eggs were more or less affected by heat shock. Such a difference in the point of
decrease in heat tolerance occurred during embryonic development may originate in fruit fly species.

Furthermore, our results indicated the remarkable variations in heat tolerance of eggs with embryonic development was shown at 45°C when eggs were immersed in hot water at 44, 45, 46 and 47°C. In B. tryoni eggs, such remarkable variations in heat tolerance were shown at 46°C when eggs were immersed in hot water at 44, 45, 46 and 47°C (Corcoran, 1993). The difference of temperature level inducing remarkable variations in heat tolerance between our results and Corcoran (1993) might be influenced by storage temperature allowing heat tolerance between our results and Corcoran (1993).

Considering the circumstances mentioned above, it will be necessary to clarify the egg duration under rearing conditions and test mature eggs rather than newly laid eggs to determine the effectiveness of heat or cold disinfestation treatment against B. dorsalis and B. cucurbitae fruit flies. In addition, it will be considered that the variations in heat and cold tolerance during embryonic development depend on fruit fly species and on target temperatures, and that the avoidance of the point of remarkable decrease in heat and cold tolerance at the latter part of egg age.

Further studies will be needed to determine the induction of heat/cold shock protein related to embryonic development of fruit fly and acquisition of thermostolerance in eggs with preheating because Shamsudin et al. (2009) reported that mature eggs (26h-old) of B. tryoni acclimatized at 35 °C or 37 °C for 4-6 hours showed significantly decreased mortality under hot water immersion at 46°C.

References


disinfestation of lemons against *Bactrocera tryoni* and *Ceratitis capitata* (Diptera: Tephritidae) using cold storage. *J. Econ. Entomol.* **86**(3): 798-802.


和 文 摘 要

ミカンコミバエ Bactrocera dorsalis 及びウリミバエ B. cucurbitae の卵の卵期間における熱耐性及び低温耐性の変化（英文）

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ミカンコミバエ及びウリミバエの卵期間において、加熱又は低温に対する耐性の経時的変化を調査するため、温湯処理（44, 45, 46, 47℃のいずれかの温水へ5分間浸漬処理）又は、低温処理（1℃又は3℃で5日間処理）を行った。
温湯処理の結果、両ミバエ種とも、卵齢が進むにつれ熱耐性は高くなる傾向があり、さらに、その過程で卵の熱耐性が変化することが観察された。
低温処理では、卵齢が進むにつれ低温耐性が高くなる傾向は観察されたが、温湯処理で見られたような著しい耐性変化は観察されなかった。

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