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Comparative Heat Tolerance of Third-Instar Larvae, the Oriental Fruit Fly (Diptera : Tephritidae), Reared at Different Temperatures and Exposed to Hot Water Immersion

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Abstract : Mature third instar larvae of the oriental fruit fly, *Bactrocera dorsalis* (HENDEL), were reared from eggs in an artificial diet at the different temperatures (20, 25, 30 and 35°C), and then exposed to hot water immersion (43°C) to compare heat tolerance. No survivors were obtained at 40 min-exposure time from larvae reared at 20°C, while in the same exposure time the corrected mortality was 81.4%, 72.1% and 64.1% for the larvae reared at 25°C, 30°C and 35°C, respectively. Rearing temperature (20-35°C) affected the tolerance of the oriental fruit fly larvae to 43°C and they were more tolerant when reared at higher temperatures between 20-35°C.
Key words : *Bactrocera dorsalis*, quarantine treatment, heat tolerance, rearing temperature

Introduction

Hosts of fruit flies such as the Mediterranean fruit fly, *Ceratitis capitata* (WIEDEMANN), the oriental fruit fly, *Bactrocera dorsalis* (HENDEL) or the melon fly, *B. cucurbitae* (COLLIQUETT) etc. which are considered pests of quarantine significance, shipped to areas where the pest does not exist must be subjected to quarantine treatment for disinfestation. Heat and cold treatments have been developed and applied for disinfestation of Tephritid fruit flies from many commodities (ARMSTRONG, 1994 ; HALLMAN and ARMSTRONG, 1994 ; JESSUP *et al.*, 1993 ; PAUL and MCDONALD, 1994 ; WADDELL *et al.*, 1997b).

Establishment of quarantine heat treatment protocols is based on thermal mortality data for fruit flies. These thermal mortality studies do not only contribute the development of quarantine treatments but also reveal the heat tolerance of fruit flies more clearly. Species, life stages, life stages within same species, and aging within life stages influence heat tolerance (JANG, 1986, 1991 ; TANABE *et al.*, 1994 ; WADDELL *et al.*, 1997a). In addition, conditions such as rearing temperature or larval density influence their heat tolerance (HALLMAN, 1994 ; HANSEN and SHARP, 1997). HALLMAN(1994) reported that rearing temperature of immatures of the Caribbean fruit fly, *Anastrepha suspensa* (LOEW), affected the heat tolerance when third instar larvae were exposed to hot water immersion.

In the present study, we investigated whether the rearing temperature affected the heat tolerance of third instar larvae of the oriental fruit fly, *B. dorsalis*.

Materials and Methods

1. Test insects

Mature third instar larvae were obtained and used from a laboratory colony of *B. dorsalis* at Naha Plant Protection Station in Okinawa. The *B. dorsalis* colony was originally collected in Okinawa island before its eradication in Japan in 1986 (Import Permit No. 63Y2152). Flies were kept at 27±1°C, 70±10%RH, and a photoperiod of 14L: 10D and given artificial diet and water in the adult cage (30×30×45 cm). Eggs were obtained from gravid females using a polypropylene oviposition receptacle (7 cm in diameter, 17 cm in height). The receptacle, punctured 88 times using 0.5 mm diameter pin, internally coated with lemon juice (10 cc) was placed in the adult cage for 4 hours. Eggs were washed out using tap water and collected. Approximately 16,000 eggs (1.6 ml) were spread on 957 g of the larval artificial diet in a polypropylene container (26×19×8 cm). The container was placed in a large container (34×26×11 cm) with mesh lid, and inside of the larger one put water for the collection of mature larvae. The containers were then kept in a multi thermo incubator (Tokyo Rikakikai Co., Ltd. EYELA MT1-202) at 20±0.6°C, 25±0.1°C, 30±0.1°C and 35±0.1°C. Under these rearing temperatures, most third instar larvae jumped from the diet into the water after 11 days, 7 days and 6 days after egg inoculation for 20°C, 25°C and 30°C, respectively. At 35°C, the larvae did not jump into the water and they were collected when some pupae were observed inside the diet. Thus, only mature larvae were collected and used for hot water immersion.

2. Hot water immersion

One hundred mature larvae 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 43±0.1°C for 0-90 min at 5 min intervals. Treatment was applied in a temperature controlled water bath (Yamato Science BK-53, 70 liter capacity) after verification of target temperature using a standard precision thermometer (Toa Keiki MFG. Co., Ltd.).

Immediately after treatment, the larvae in tube were cooled by dipping into water at 25°C for 1 min to remove latent heat from the insects. Then, the larvae were held on 200 g moist sand in polypropylene container (12 cm diameter, 10 cm height) for their pupation. The containers were held for 30 days under 27±1°C, 70±10% RH and a photoperiod of 14L : 10D, and survivorship was determined based on adult emergence.

In each exposure time and rearing temperature, 200 larvae were treated. Four hundred larvae for control in each rearing temperature were immersed into water at 25°C until the end of treatment for the longest exposure time. These tests were replicated four times.

3. Data analysis

Data were subjected to probit analysis and logit analysis to compare the thermotolerance of larvae which were reared at different temperatures using the computer program, POLO-PC (LeOra Software, 1987). This program estimates lethal time for 50% (LT_{50}), 95% (LT_{95}) and 99% (LT_{99}) mortality, and upper and lower 95% confidence limits for each lethal time estimate.

Results

Percentage of corrected mortality of third instar larvae versus exposure time for each of the 4 replications was calculated using ABBOTT's (1925) correction and is plotted in Fig. 1. No survivors were observed after 35 min-, 65 min-, 70 min- and 90 min-exposure time to 43°C hot water immersion when insect were reared at 20°C, 25°C, 30°C and 35°C, respectively (arrows in Fig. 1). No survivors were found at 40 min-exposure time from the

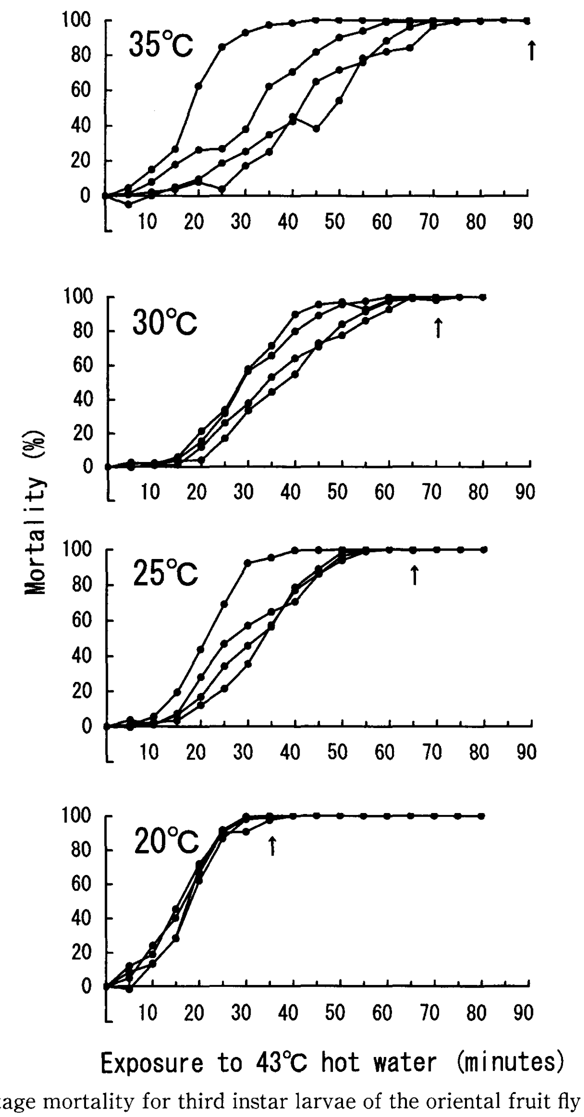

Fig. 1. Percentage mortality for third instar larvae of the oriental fruit fly reared at 20, 25, 30 and 35°C, after immersion in water at 43°C. Arrows indicate the point most tolerant of heat.

Table 1. Comparison of estimated thermal death times 95% CL on mortality of *B. dorsalis* third instar larvae reared at different temperature to hot water treatment at 43°C, modeled with probit and logit analysis

Analysis	Temp	χ^2	LT_{50} (95%CL)	LT_{95} (95%CL)	LT_{99} (95%CL)
Probit	20°C	92.74	16.47 (15.28-17.51)	29.15 (27.27-31.71)	36.93 (33.68- 41.73)
	25	105.04	27.22 (25.75-28.56)	49.53 (46.77-53.07)	63.48 (58.65- 70.04)
	30	55.75	31.55 (30.49-32.55)	57.05 (54.78-59.73)	72.92 (68.96- 77.78)
	35	174.64	32.26 (29.18-34.87)	68.16 (62.87-75.71)	92.91 (82.58-109.22)
Logit	20°C	117.01	16.75 (15.34-17.92)	28.93 (26.77-32.19)	39.30 (34.83- 46.96)
	25	159.06	27.30 (25.45-28.95)	50.22 (46.50-55.49)	70.67 (62.83- 82.92)
	30	89.31	31.57 (30.21-32.83)	57.89 (54.68-62.00)	81.33 (74.53- 90.60)
	35	213.81	32.49 (28.97-35.38)	70.60 (63.80-81.44)	109.08 (92.36-139.76)

larvae reared at 20°C, while in the same exposure time (40 min) the corrected mortality was 81.4±6.3% (average±SE), 72.1±7.9% and 64.1±13.1% for the larvae reared at 25°C, 30°C and 35°C, respectively.

There was a tendency for the larvae reared at higher temperature to be more tolerant and the most tolerant individual was obtained from the larvae reared at 35°C. However, the tolerance of larvae reared at 35°C, was variable because one of 4 replications showed high corrected mortality, 98.3% in 40 min-exposure time and no survivor in more than 45 min-exposure time, which was similar to thermotolerance of larvae reared at 20°C. The survival rate or adult emergence of control larvae reared at 20°C, 25°C, 30°C and 35°C was 96.4±0.8%, 97.3±0.4%, 98.1±0.4% and 91.1±1.1%, respectively.

The two dose-response models (probit and logit) were compared to identify which provided better fit to the data. Probit model provided better fit although chi-square values of both models showed poor fit of data. In each model, estimated exposure time to prohibit 50%, 95% and 99% adult emergence (LT_{50}, LT_{95} and LT_{99}) for the larvae in each rearing temperature (20-35°C) are shown in Table 1. Significant differences in mortality for larvae between 20°C, 25°C and 30-35°C were observed based on non-overlap of the LT_{50} values at the 95%CLs. Similarly, significant differences in LT_{95} were observed in each rearing temperature by probit analysis. In LT_{99}, significant differences were observed between 20°C, 25-30°C and 35°C.

Discussion

Our experiments indicated that the third instar larvae of *B. dorsalis* were more thermotolerant when they were reared at higher temperatures than at lower temperatures in the range of 20-35°C. This result suggests that the larvae seemed to be acclimated by high temperatures rearing at the egg and the larval stage, and the rearing temperature had exerted the influence on the heat receptivity of *B. dorsalis* larvae.

HALLMAN (1994) showed that third instar larvae of the Caribbean fruit fly, *Anastrepha suspensa* (LOEW) subfamily Trypetinae, exposed to hot water immersion between 43-46°C, were more tolerant to temperatures when reared at higher temperatures than low temperatures between 20–30°C. Because *B. dorsalis* belongs to different subfamily Dacinae showed similar property in our study, it seems that this property is generally applicable in Tephritid fruit flies.

Also, HALLMAN (1994) reported when the third instar larvae of *A. suspensa* were immersed in water at 43°C for 43 min, 0 of 17,074, 3 of 33,058, and 25 of 33,998 larvae reared at 20, 25 and 30°C, respectively, survived to the adult stage. In our study, when the larvae of *B. dorsalis* treated at 43°C for 45 min, 0 of 800, 77 of 800, 142 of 800 larvae reared at 20, 25 and 30°C, respectively, grew to the adult. These results suggest that in third instar larva, *B. dorsalis* is more tolerant than *A. suspensa*, though both treatment methods were not exactly the same.

We reared *B. dorsalis* from egg to third instar larva in an incubator set at 35°C as well as at 20°C, 25°C and 30°C. However, this temperature was higher than the average of maximum temperature 31°C in summer at Okinawa island. Actually, temperatures of the artificial diet in the period of the latter half of third instar stage, seemed to be higher than 35°C induced by the larval metabolic heat. In larvae reared at 35°C, there were individuals with very strong heat tolerance, while the individuals without strong heat tolerance were seen, and their thermotolerance was unstable. They did not show jumping behavior before the pupation, and the emerging rate of untreated larvae for control was lower than those of larvae reared at the range of 20–30°C. These results suggest the larvae reared at 35°C suffered the physiological damage under an extreme high temperature.

When the disinfestation examinations of immature fruit flies in fruits are conducted, in order to decide on the standard of the quarantine treatment by heat, the temperatures of fruit fly's habitat should be taken into consideration.

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Literature Cited

ABBOTT, W.S. (1925) A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* **18** : 265–267.

ARMSTRONG, J.W. (1994) Heat and cold treatments, pp. 103–119. In R.E. PAULL and J.W. ARMSTRONG [eds.], Insect pests and fresh horticultural products : treatments and responses. CAB, Wallingford, UK.

HALLMAN, G.J. (1994) Mortality of third-instar Caribbean fruit fly (Diptera : Tephritidae) reared at three temperatures and exposed to hot water immersion or cold storage. *J. Econ. Entomol.* **87** : 405–408.

HALLMAN, G.J. and J.W. ARMSTRONG (1994) Heated air treatments, pp. 149–164. In J.L. SHARP and G.J. HALLMAN [eds.], Quarantine treatments for pests of food plants. Westview, Boulder, CO.

HANSEN, J.D. and J.L. SHARP (1997) Thermal Death in third instars of the Caribbean fruit fly (Diptera : Tephritidae) : Density relationships. *J. Econ. Entomol.* **90** : 540–545.

JANG, E.B. (1986) Kinetics of thermal death of eggs and first instar larvae of three species of fruit flies (Diptera : Tephritidae). *J. Econ. Entomol.* **79** : 700–705.

JANG, E.B. (1991) Thermal death kinetics and heat tolerance in early and late third instars of the oriental fruit fly (Diptera : Tephritidae). *J. Econ. Entomol.* **84** : 1298-1303.

JESSUP, A.J., C.P.F. De LIMA, C.W. HOOD, R.F. SLOGGETT, A.M. HARRIS and M. BECKINGHAM (1993) Quarantine disinfestation of lemons against *Bactrocera tryoni* and *Ceratitis capitata* (Diptera : Tephritidae) using cold strage. *J. Econ. Entomol.* **86**:798-802.

PAULL, R.E. and R.E. McDONALD (1994) Heat and cold treatments, pp. 191-222. In R.E. PAULL and J.W. ARMSTRONG [eds.], Insect pests and fresh horticultural products : treatments and responses. CAB, Wallingford, UK.

TANABE, K., T. DOHINO, M. KUMAGAI, R. IWAIZUMI and M. IWATA (1994) Thermal death of immature stages of Mexican fruit fly, *Anastrepha ludens* LOEW (Diptera : Tephritidae). *Res. Bull. Pl. Prot. Japan.* **30** : 35-41.

WADDELL, B.C., G.K. CLARE and J.H. MAINDONALD (1997a) Comparative mortality responses of two Cook Island fruit fly (Diptera : Tephritidae) species to hot water immersion. *J. Econ. Entomol.* **90** : 1351-1356.

WADDELL, B.C., G.K. CLARE, R.J. PETRY, J.H. MAINDONALD, M.PUREA, W.WIGMORE, P. JOSEPH, R.A. FULLERTON, T.A. BATCHELOR and M.LAY-YEE (1997b) Quarantine heat treatment for *Bactrocera melanotus* (Coquillett) and *B. xanthodes* (Broun) (Diptera : Tephritidae) in Waimanalo papaya in the Cook Islands, pp. 251-255. In A.J. ALLWOOD and R.A.I. DREW [eds.], Management of fruit flies in the pacific : A regional symposium, Nadi, Fiji 28-31 Oct. 1996. ACIAR Proceedings No. 76.

和 文 摘 要

異なる温度で飼育したミカンコミバエ3齢幼虫の温湯浸漬による耐熱性の比較

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熱処理による輸入禁止生果実解禁試験の基礎的資料を得るため，発育温度がミカンコミバエ3齢幼虫の耐熱性に影響を与えるか調査した。

那覇植物防疫事務所で累代飼育しているミカンコミバエを卵から蛹化直前の3齢幼虫まで，20, 25, 30, 35°C の各温度条件下で飼育した。これらの幼虫を裸虫温湯浸漬法により 43.0°C の温水に1処理区 200 頭ずつ5分間隔最長 90 分間処理した。試験は各飼育温度ごと4反復実施した。生死判定は成虫の羽化をもって行い，死亡率を補正した。

生存虫が認められた最長処理時間は，飼育温度が高いほど長く，20°C 飼育虫で 35 分間，25°C 飼育虫で 65 分間，30°C 飼育虫で 70 分間，35°C 飼育虫で 90 分間であった。

これらの結果をプロビット法及びロジット法で解析し 50%，95% 及び 99% 殺虫するのに必要な処理時間（LT_{50}, LT_{95}, LT_{99}値）を推定した結果，プロビット法の方が適合度は高かった。本解析法では 20°C 飼育虫，25-30°C 飼育虫及び 35°C 飼育虫の間で，LT_{99}値において有意差が認められた。

以上の結果から，発育温度はミカンコミバエ3齢幼虫の耐熱性に影響を及ぼし，高温条件下ではミカンコミバエ幼虫が馴化し，耐熱性の高い個体が現れると考えられた。蒸熱処理等，熱による殺虫試験を実施する場合は，野外のミバエ発育温度も考慮する必要があると考えられた。