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Thermal Death of Immature Stages of Mexican Fruit Fly, *Anastrepha ludens* LOEW (Diptera : Tephritidae)

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Abstract: Naked eggs and larvae of Mexican fruit fly, *Anastrepha ludens* LOEW were immersed into hot water at 46.0°C. Eggs raised its tolerance with age. The most tolerant stage was the third instar larvae of pre-pupation. Heat tolerance of the third instar larvae was enhanced by conditioning at 37.0°C for 30 minutes prior to hot water immersion. The conditioning stimulated pupation but not adult emergence, and a restore period of 10~30 minutes at room temperature was required to induce elevated tolerance of the larvae.
Key words: Insecta, *Anastrepha ludens*, immature stage, hot water immersion, heat shock

Introduction

Mexican fruit fly, *Anastrepha ludens*, is not established in Japan, but is a possible economic pest for Japanese horticulture because of the wide range of host plants and the fly's high fertility (FLITTERS and MESSENGER, 1965; OHTO, *et al.*, 1991; SHARP, *et al.*, 1989a; WEEMS, 1963). *Anastrepha* have been intercepted at airports as larvae in various fruits, which in most cases were tropical fruit in traveller's hand baggage. Hot water dipping or hot air treatment disinfests immature stages from some genera of *Anastrepha* without injuries to fruit (SHARP, 1993; SHARP and CHEW, 1987; SHARP, *et al.*, 1988; SHARP, *et al.*, 1989a; SHARP, *et al.*, 1989b). Thermal treatment is an effective procedure to control fruit flies. However, since few data have been reported on details of the thermal properties of *A. ludens*, their heat tolerance should be revealed in order to establish thermal treatment as a valid quarantine procedure. In quarantine treatment schedules, the immature stages infesting fruits would be exposed to gradually rising temperatures (HAYES and YOUNG, 1989; HANSEN, 1992). When various organisms receive heat shock at moderate temperatures, they acquire tolerance against lethal temperatures by synthesizing a specific set of heat shock proteins (ALAHIOTIS, 1982; DEAN and ATKINSON, 1983; LINDQUIST and CRAIG, 1988). We examined naked immature *A. ludens* in hot water immersion at 46.0°C to compare the thermal tolerance among the various stages. Larvae conditioned at 37.0°C prior to hot water immersion were also examined.

Materials and methods

Fruit fly

Test insects were obtained from a laboratory colony of Mexican fruit fly *Anastrepha ludens* of Mexican origin (Import Permit No. 63 Y756). Flies were reared at 25°C±1°C and

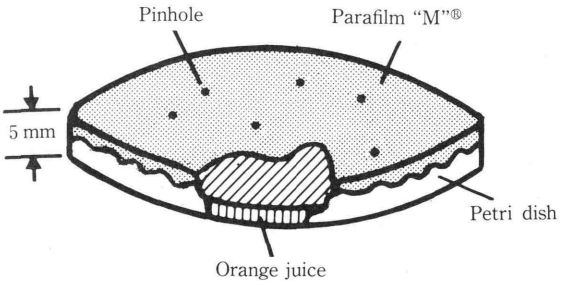

Fig. 1. Egging receptacle

65～85% r.h., under a photoperiod of 16L : 8D and given a water and artificial diet (phytone® peptone : yeast extract : sucrose=1 : 1 : 5). Eggs were collected by exposing egging receptacles to flies for 8 hours (Fig. 1), and then held on filter paper moistened with phosphate-buffered saline (PBS) including 0.1% of sorbic acid and 0.1% of methl p-hydroxy benzoate at 25.0°C±0.5°C in the dark until they reached the adequate embryonic age. Under these conditions, the mean egg duration was 5.3 days.

Full-ripe mango fruits produced in the Philippines were exposed to flies to obtain larvae. Young larvae were collected by dissecting fruits 6～8 days after oviposition for 2 days. Age composition of these larvae was 1st : 2nd : 3rd=3 : 47 : 1. Third instar larvae, immediately prior to pupation, were collected from infested fruits 14～19 days after oviposition for 3～4 days.

Hot water immersion

Eggs on a strip of filter paper (3×20 mm) were suspended in 5 ml of PBS in test tubes, which were heated in a water bath (Model BF-21, Yamato Scientific) at 46.0°C prior to treatment. The temperature of hot water was measured with a standard thermometer (Model No. 2, Toyo Keiryoki). After treatment, the eggs were transferred from the test tubes onto filter paper moistened with PBS, and placed in Petri dishes. The Petri dishes were covered with Parafilm "M"® to prevent desiccation, held at 25°C under the dark and examined everyday for hatchability.

One hundred larvae in a glass tube with nylon gauze were immersed into hot water at 46.0°C. Immediately after treatment, the larvae were cooled by dipping into water which as at a temperature of 20°C for one minute. Young larvae were inoculated into new fruit, reared under the rearing condition and transferred from fruit onto moist sand 8～10 days after treatment. Treated third instar lavae were cooled and transferred onto moist sand after treatment. All tests were replicated three times.

Conditioning

One hundred of 3rd instar larvae, which were put into a glass tube with nylon gauze, were conditioned by immersion into water at 37.0°C for 30 minutes, and immersed in hot water at 46.0°C for 8 minutes after intervals of 0～30 minutes. During the interval period, larvae were held on a Petri dish at 20°C. After treatment, larvae were cooled and

transferred onto moist sand. Each test was replicated three times.

Results and discussion

The eggs had elevated thermal tolerance as a function of age (Table 1). Two-day-old eggs, however, showed high susceptibility and a remarkable hatching delay. An embryologic event, which results in alteration of thermal response, might occur 2 days after oviposition.

The third instar larvae were the most tolerant against hot water immersion (Table 2 and 3). In both young and mature larvae, the sex seems not to have been related to

Table 1. Hatchability of aged eggs immersed into hot water at 46.0°C

Age	Treatment time (min)	Tested* eggs	Hatch*	Egg** duration (day)	Hatchability (%)	Corrected*** mortality (%)
1-day-old	0	372	284	5.3	76.2± 5.8	–
	6	430	34	7.2	7.8± 2.2	89.8
2-day-old	0	365	246	5.3	68.8±15.4	–
	6	519	6	8.0	1.3± 2.3	98.1
3-day-old	0	404	266	5.2	65.8± 6.8	–
	6	406	135	6.9	32.4± 4.0	50.8
4-day-old	0	391	257	5.3	65.2±12.1	–
	4	351	144	5.4	41.3± 2.1	36.7
	6	401	121	7.7	37.4± 6.0	42.6
	8	347	35	7.0	10.3± 4.6	84.2
	10	384	6	8.3	1.5± 1.6	97.7

*; The amount of three replications.

**; Egg duration $=\frac{\Sigma\{(\text{egg duration})i \times (\text{viable eggs})i\}}{\text{the amount of viable eggs}}$

***; ABBOTT (1925)

Table 2. Pupation and adult emergence of young larvae immersed into hot water at 46.0°C*

Treatment Time (min)	Survivors**			Pupation (%)	Adult emergence (%)
	3rd instar larva	Pupa	Adult (♂ : ♀)		
0	217	209	158 (71 : 87)	69.7±9.1	52.7±6.4
4	56	54	43 (21 : 22)	18.2±2.0	14.3±1.5
6	10	10	7 (5 : 2)	3.3±1.2	2.3±2.1
8	1	1	0	0.3±0.6	0.0

*; Three hundred larvae (100 larvae×3 replications) were used for each level.

**; The amount of three replications.

Table 3. Pupation and adult emergence of third instar larvae immersed into hot water at 46.0°C*

Treatment Time (min)	Survivors**		Pupation (%)	Adult emergence (%)
	Pupa	Adult (♂ : ♀)		
0	296	248 (128 : 120)	98.7±2.3	82.7± 1.2
4	269	146 (62 : 84)	89.7±4.7	48.7±10.5
6	272	135 (68 : 67)	90.6±2.1	45.0± 8.9
8	144	23 (9 : 14)	48.0±2.0	7.7± 2.5
10	27	1 (1 : 0)	9.0±5.3	0.3± 0.6
12	8	0	2.7±1.5	0.0
14	0	–	0.0	–

*; Three hundred larvae (100 larvae×3 replications) were used for each level.
**; The amount of three replications.

thermal tolerance.

Probit analysis for time-mortality relationship in pupation of third instar larvae provided regression lines consisting of at least two components. The first component is represented by an equation of $y=-0.3844\log x+3.9017$ in 4~6 minutes of treatment time, which resulted in no marked effect of tremtment on pupation. However, adult emergence of the larvae treated for 4~6 minutes was diminished in half of the control group. The second component is $y=11.7806\log x-5.5695$ in 6~14 minutes of treatment time, in which the slope is similar to that in the regression equation of adult emergence. Therefore, different factors in short term and in long term treatment might determine mortality. Although the second component provides 17.2 minutes of treatment time corresponding to probit 9, treated larvae were eradicated within 14 minutes of treatment time, which was significantly shorter than the calculated value (95% confidence limit). This suggests that the third component exists in the critical phase near 100% of mortality.

Table 4. Effects of heat shock and hot water immersion on pupation and adult emergence of third instar larvae*

Treatment	Survivors**		Pupation (%)	Adult emergence (%)
	Pupa	Adult (♂ : ♀)		
37°C • 30 min	290	202 (95 : 107)	96.7±3.1 a	67.3±3.2 a
46°C • 8 min	144	23 (9 : 14)	48.0±2.0 b	7.7±2.5 b
37°C • 30 min+46°C • 8 min	139	4 (3 : 1)	46.3±3.1 b	1.3±0.6 c
37°C • 30 min+20°C • 10 min+46°C • 8 min	156	11 (8 : 3)	52.0±5.0 c	3.7±1.5 b
37°C • 30 min+20°C • 20 min+46°C • 8 min	179	15 (9 : 6)	59.7±8.1 c	5.0±3.0 b
37°C • 30 min+20°C • 30 min+46°C • 8 min	162	12 (4 : 8)	54.0±5.6 c	4.0±2.6 b

*; Three hundred larvae (100 larvae×3 replications) were used in each group.
**; The amount of three replications.
Letters indicate a significant difference ($P<0.05$, DUNCAN's new multiple range test) (HARTER, 1960).

Hot water immersion resulted in delayed pupation or inhibition of maturity. Abnormal pupae were obtained from all lots of treated larvae. They retained larval form, turned dark brown or black, and could not emerge. The abnormal pupal development was similarly observed in larvae submersed in water for 16 hours or longer (TASCHENBERG, *et al.*, 1974). Limited oxygen supply might cause the abnormal pupae. Most likely the elevated temperature would aggravate oxygen deficiency by accelerating the respiration of larvae.

Third instar larvae conditioned at 37.0°C for 30 minutes showed elevated tolerance in pupation against hot water immersion at 46.0°C for 8 minutes (Table 4). Similar phenomenon is observed in third instar larvae of *Ceratitis capitata* (ALAHIOTIS, *et al.*, 1982), although *C. capitata* showed a more drastic shift of thermal tolerance. In the present study, acquisition of elevated tolerance required intervals of 10~30 minutes at room temperature. The delay of heat shock response is due to the accumulation of mRNA of heat shock proteins during recovery from heat shock (CARRETERO, M.T., *et al.*, 1991 ; PETERSEN, *et al.*, 1990). The conditioning did not raise the survival rate in adult emergence after treatment. The exact relationship between heat shock and long term survival in *A. ludens* is not clear.

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和 文 摘 要

メキシコミバエ *Anastrepha ludens* Loew (Diptera : Tephritidae) の卵および幼虫の熱感受性について

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メキシコミバエ *Anastrepha ludens* の卵および幼虫を温湯浸漬(46.0°C)して熱感受性を調べた。

1. 卵の感受性は発育とともに低下し，孵化直前(産卵後4日目)に最も低くなった。

2. 幼虫のうちで感受性が最も低かったのは，蛹化直前の3齢幼虫だった。

3. 温湯浸漬の前に30分間37.0°Cで条件付けしたとき，3齢幼虫の蛹化率が高くなった。しかし，羽化率には条件付けの影響は認められなかった。

4. 条件付けされた3齢幼虫が耐熱性を高めるには，条件付けと温湯浸漬との間に常温で10～30分間の間隔が必要であった。