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Response of Mexican Fruit Fly, *Anastrepha ludens* (Diptera : Tephritidae) Immatures to Low Temperature

Toshiyuki DOHINO, Seiki MASAKI and Kohji YAMAMURA*

Research Division, Yokohama Plant Protection Station
Shin-yamashita 1-16-10, Naka-ku, Yokohama 231-0801, Japan
*National Institute of Agro-Environmental Sciences
Kannondai 3-1-1, Tsukuba 305-8604, Japan

Abstract: Naked eggs (1, 2, 3 and 4-day-old) and larvae (1st-2nd and 3rd instars) of Mexican fruit fly, *Anastrepha ludens* (Loew), were exposed to low temperature (1°C). Cold tolerance was age-dependent and the estimated treatment period to provide 99% mortality (LT_{99}) at 1°C was 13.6days, 15.8days, 24.4days and 14.4days for 1, 2, 3 and 4-day-old eggs, respectively. Mature larvae (3rd instars) were more tolerant than younger larvae (1st-2nd instars). The LT_{99} of mature larvae was 14.8 days and 12.1 days for their pupation and adult eclosion, respectively. The value of LT_{99} estimated by probit analysis indicated that the 3-day-old egg was the most tolerant stage among the egg and larval stages treated.
Key words: *Anastrepha ludens*, low temperature, cold tolerance

Introduction

Mexican fruit fly, *Anastrepha ludens* (Loew), is pest of tropical fruits and vegetables in Central America as well as in South America. *A. ludens* is not established in Japan, but *Anastrepha* flies such as South American fruit fly *A. fraterculus* (Wiedemann), West Indian fruit fly *A. obliqua* (Macquart), dark fruit fly, *A. serpentina* (Wiedemann) and Caribbean fruit fly, *A. suspensa* (Loew) as well as *A. ludens* have been intercepted at Japanese airports in 1996-1998 as larvae in various fruits (Yokohama Plant Protection Station, MAFF, 1997, 1998, 1999). They are potential economic pests for Japanese horticulture because of their wide host range and their high fertility (SWANSON and BARANOWSKI, 1972 ; WEEMS, 1963, 1966).

Thermal treatments like hot water dipping, hot-air and low-temperature storage are effective procedures to control *Anastrepha* flies (BURDITT and MCALISTER, 1982 ; BENSCHOTER, 1983 ; SHARP and CHEW, 1987 ; SHARP *et al.*, 1989ab ; SHARP, 1993). In fact, these thermal treatments are accepted quarantine procedures for commercial of agricultural commodities by the USDA Animal and Plant Health Inspection Service (USDA-APHIS, 1998). The USDA-APHIS-Plant Protection Quarantine Treatment Manual gives schedules for cold treatments based on both unpublished work done in the 1930s and substituting work done by the citrus industry (BURDITT and MCALISTER, 1982 ; BENSCHOTER, 1983, 1984). In the treatment manual, the schedule for disinfestation of *A. ludens* is more severe than for other species of *Anastrepha* flies, Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) and Queensland fruit fly *Bactrocera tryoni* (Froggatt) (USDA-APHIS, 1998). TANABE *et al.* (1994) determined the heat tolerance of immature stages (egg, young larvae and mature larvae) of *A. ludens* to hot water at 46°C. In this study, we investigated cold-tolerance of the immature stages to 1°C.

Materials and Methods

1. Test insect

Test insect were obtained from a laboratory colony of *A. ludens* and this colony was founded with insects originating in Mexico in 1988 (Import Permit No.63Y756). All experiments were done at Yokohama Plant Protection Station, Research Division, Physical and Chemical Control Laboratory in 1994-1995. Flies were reared at 25±1°C and 70±10% RH under a photoperiod of 16L : 8D and provided artificial diet (phytone® peptone : yeast extract : sucrose=1 : 1 : 5) and water.

Eggs were collected using an oviposition receptacle reported in TANABE *et al.* (1994). The receptacle was placed in an adult cage (29×44×30 cm) holding 400-500 flies at a sex ratio of 1 : 1. Flies had eclosed 3-4 weeks previously. They were allowed to oviposit through the receptacle for 24 hours. Eggs were collected with a fine brush and placed on black filter paper moistened with 3 ml of phosphate-buffered saline (PBS) including 0.1% w/v of sorbic acid and 0.1% w/v of methyl p-hydroxy benzoate, in a Petri dish. Eggs in the dishes were then held in an incubator set at 25°C in the dark for 0, 1, 2 and 3 days before the commencement of cold storage treatment, corresponding to the target egg stages, 1, 2, 3 and 4-day-old eggs. Under these conditions, the mean egg duration was 5.3 days.

For the collection of larvae, mangoes produced in the Philippines were exposed to flies in an adult cage for 2 days. Infested mangoes were removed from the cage and held in a polypropylene container (18 cm in diameter, 14 cm in height) under the rearing condition described above. Younger larvae (1st instar : 2nd instar=48.8% : 51.2%) and mature larvae (3rd instar) were collected by dissecting the infested fruits 8 days and 18 days after cage infestation, respectively.

2. Cold treatment and post-treatment

Approximately 100-200 individuals of each developmental stage were placed on black filter paper moistened with 3 ml PBS including sorbic acid and methl p-hydroxy benzoate, in a Petri dish. The dishes were covered with Parafilm 'M'® to prevent desiccation and stored in a cold chamber (Yamato Scientific Co., Ltd., IN61) set at 1°C for 3, 5, 7, 10 and 15 days.

After each exposure period, the dishes were removed from cold chamber. Treated eggs were held in an incubator at 25°C for 10 days and then the number of hatched eggs and unhatched eggs were counted. As treated younger larvae, 120 larvae were removed from the dish with fine brush and inoculated to a mango in which a flap was cut in the skin and a small amount of flesh was removed. The flap was then replaced. Infested fruit was placed in a polypropylene container with a lid and held under the rearing conditions for 9-10 days to permit surviving larvae to mature, leave the fruit and pupate. Survivors were placed on moist sand for their pupation. The number of pupae and emerged adults were counted. Treated mature larvae were placed on moist sand to allow pupation and the number of pupae and emerged adults were counted.

Treatment for each developmental stage and each exposure-period of cold storage was replicated 3 times. Controls were set up in each replicate and conducted in a similar

manner without cold storage.

3. Analysis of mortality data

Lethal exposure time for 99% mortality (LT_{99}), and upper and lower 95% confidence limits were estimated by probit analysis. The procedure PROBIT of SAS was used for this calculation (SAS Institute, 1989). Pearson χ^2 statistics was much larger than the corresponding degree of freedom in our data. Hence, the heterogeneity factor was used to calculate the confidence limit.

Results and Discussion

The susceptibility of naked eggs (1, 2, 3 and 4-day-old) to cold treatment at 1°C are shown in Table 1. Cold tolerance was age-dependent and the hatchability of 3-day-old eggs was higher than that of other egg stages. The probit analysis indicated that the estimated treatment period for LT_{99} at 1°C was 13.6 days, 15.8 days, 24.4 days and 14.4 days for 1, 2, 3 and 4-day-old eggs, respectively (Table 2). Therefore, 3-day-old eggs were considered to be the most tolerant egg stage. The LT_{99} of 3-day-old eggs was significantly larger than that of 1-day-old eggs and that of 4-day-old eggs as indicated by their confidence limits.

Mature larvae were more tolerant to cold than younger larvae because the percentage of pupation and adult emergence of treated mature larvae were higher than that of treated younger larvae (Table 3). However, the percentage of adult emergence from the pupae which survived from younger larvae was higher than that of survived mature larvae, in other words, many mature larvae survived and pupated but most of them failed to emergence as adults. The LT_{99} value of mature larvae was 14.8 days and 12.1 days, while the LT_{99} value of younger larvae was 7.2 days and 6.9 days for their pupation and adult

Table 1. Hatchability of aged eggs of *A. ludens* following cold treatment at 1°C

Egg age	0 d	3 d	5 d	7 d	10 d	15 d
1-day-old	70.0±4.4%*	50.4±8.8	43.7± 9.8	22.7± 1.7	1.8±0.8	0
2-day-old	61.6±6.7%	67.6±7.9	39.2±12.4	45.6±10.0	0.7±0.8	0
3-day-old	70.3±1.6%	63.4±6.9	60.8± 8.3	54.1±10.7	40.4±2.5	2.8±0.9
4-day-old	76.7±3.7%	59.7±6.8	32.2±10.5	19.0± 6.5	10.8±2.5	0.9±0.8

*Mean±SD

Table 2. Statistics for probit analysis: cold treatment (1°C) of egg stages of *A. ludens*

Egg age	χ^2	df	LT_{99}	(95%CL)
1-day-old	82.33	16	13.60 days	(12.08-15.75)
2-day-old	317.15	16	15.84	(12.75-22.10)
3-day-old	166.28	16	24.35	(20.28-31.43)
4-day-old	56.47	16	14.40	(13.00-16.31)

Data from three replicates were analyzed.

Table 3. Survival to pupation and adult emergence of *A. ludens* younger larvae and mature larvae following cold treatment at 1°C

Larval stage	0 d	3 d	5 d	7 d	10 d	15 d
Pupation						
1st-2nd instar	65.5±13.9%*	27.8±4.3	5.0±9.8	0.5±0.5	0.3±0.5	0
3rd instar	98.3± 0.6%	91.7±2.1	89.7±2.1	68.7±4.5	20.3±7.5	0
Adult emergence						
1st-2nd instar	58.9±13.1%	21.7±5.0	5.0±0.8	0	0.3±0.5	0
3rd instar	87.3± 1.2%	74.0±5.3	56.0±6.9	23.0±5.2	1.0±0.0	0

*Mean±SD.

Table 4. Statistics for probit analysis: cold treatment (1°C) of larval stages of *A. ludens*

Larval stage	χ^2	df	LT_{99}	(95%CL)
Inhibition of pupation				
1st-2nd instar	51.83	16	7.23 days	(6.36- 8.47)
3rd instar	52.17	16	14.80	(13.61-16.43)
Inhibition of adult emergence				
1st-2nd instar	56.47	16	6.92 days	(5.98- 8.32)
3rd instar	47.76	16	12.13	(11.02-13.63)

Data from three replicate were analyzed.

emergence of each developmental stage, respectively (Table 4). Significant differences in the inhibition of pupation and adult emergence between younger larvae and mature larvae were observed based on non-overlap of the LT_{99} values at the 95% confidence limits.

The value of LT_{99} to 1°C indicated that the 3-day-old egg was the most tolerant stage among the egg and larval stages (Tables 2 and 4). The cold tolerance of the egg stage of *A. ludens* changed as well as the heat tolerance of eggs (TANABE *et al.*, 1994). MOSS and JANG (1991) reported the thermotolerance of egg of *Ceratitis capitata* to hot water at 43°C varied with egg age at treatment. In some *Bactrocera* species, 60% of its embryogenesis was the most tolerant egg stage to hot water at 45°C (R. CORCORAN, unpublished data). In *Bactrocera dorsalis* egg, there were two peaks of the tolerance at 21-hr-old egg and 27-30-hr-old egg in which the hot water treatment at 43°C with the egg stage between 3 and 30-hr-old at 3 hr intervals (MIYAZAKI, personal communication). Our finding that egg age is an important factor determining tolerance to cold or heat treatment should be considered in the design of disinfestation method in protocol for lifting import ban and in the design of thermal death models (MOSS and JANG, 1991).

To develop low cost disinfestation systems for fruit, especially in heat treatment, recently, hot water dipping treatments have been conducted with naked egg or larvae of fruit fly and the thermal tolerance of egg and larval stage have been compared (JANG, 1986, 1991 ; SHARP and CHEW, 1987 ; WADDELL *et al.*, 1997). In this study, we tried to determine the most cold-tolerant immature stage of *A. ludens* conducted with low-temperature storage using the naked egg and larvae. From our experiments, 3-day-old egg was

assumed as the most cold-tolerant stage at 1°C. Further studies as comparison of the cold tolerance between immature stages in commodities and naked immatures, will be needed to verify if our method is available for determination of the most tolerant stage and for development of low-temperature storage for quarantine treatment.

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和文摘要

メキシコミバエ *Anastrepha ludens* (Loew) (Diptera: Tephritidae) の卵及び幼虫の低温殺虫試験

土肥野利幸・正木 征樹・山村 光司*

横浜植物防疫所調査研究部

*農業環境技術研究所環境生物部

メキシコミバエ *Anastrepha ludens* (Loew) の卵 (1日齢, 2日齢, 3日齢及び4日齢卵), 1-2齢幼虫及び3齢幼虫の1°Cにおける低温感受性を調査した。

マンゴウ生果実及び人工飼料で累代飼育中のメキシコミバエ (農林水産省指令63横植第756号で輸入許可) の各発育ステージを, プラスチックシャーレ内のリン酸緩衝液で湿らせた濾紙上に放飼し, 1°Cで3, 5, 7, 10及び15日間低温処理して, その後の孵化率, 又は蛹化率及び羽化率を調査した。

卵では日齢により異なった低温感受性を示し, 胚発育の過程で低温耐性は変化することが明らかになった。プロビット解析により, メキシコミバエ卵を1°Cにおいて99%殺虫するのに要する処理日数 (LT_{99}) を求めたところ, 1日齢では13.6日間, 2日齢では15.8日間, 3日齢では24.4日間, 4日齢卵では14.4日間の低温処理が必要と算出された。

幼虫では, 1-2齢幼虫よりも3齢幼虫の方が1°Cにおける低温耐性が高かった。3齢幼虫の蛹化率及び羽化率に関し, その LT_{99} を求めたところ, 14.8日間及び12.1日間と算出された。

以上の結果から, 卵期及び幼虫期を通して, 1°Cに対して最も耐性のあるステージは3日齢卵であると考えられた。加熱処理に加えて, 低温処理においても胚発育の過程で耐性が変化することが明らかになった。植物検疫措置としての低温殺虫技術を開発するにあたっては, 同じ発育ステージにおいても耐性が変化することを考慮する必要があると考えられた。