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Short Communication

Heat tolerance comparison among different hour-ages in egg and first instar larva period of *Bactrocera dorsalis* (Diptera: Tephritidae) to vapor heat treatment

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Abstract: Eggs (early-, middle-, and late-age) and first instar larvae (early- and late-age) of the Oriental fruit fly *Bactrocera dorsalis* (Hendel) infesting mangoes were treated using a vapor heat treatment machine to compare heat tolerance among different hour-ages in each developmental stage. Each stage had a different heat tolerance with different hour-ages; late-age eggs were more heat tolerant than the other-age eggs, and early-age first instar larvae were more heat tolerant than late-age first instar larvae. These results suggest that late-age eggs and early-age first instar larvae should be used in a series of mortality tests of *B. dorsalis* to develop a vapor heat treatment schedule.

Key Words: *Bactrocera dorsalis,* heat tolerance, vapor heat treatment, different hour-age

Introduction

Some countries ban imports of fruits and vegetables which may host some important fruit fly species from countries where they exist. However, in accordance with Article VII (2) (a) of the International Plant Protection Convention (FAO, 1997), the import ban shall be lifted for specific commodities if plant quarantine measures such as thermal treatment (e.g. vapor heat treatment, hot water treatment and cold treatment) with scientific justification are developed. In developing a plant quarantine treatment schedule, a series of insect mortality tests is conducted. Firstly, a heat tolerance comparison test is done to determine the most heat-tolerant developmental stage which can infest the commodity, and then a treatment schedule is developed using the most tolerant stage (Armstrong and Mangan, 2007; Corcoran *et al*., 1993; Heard *et al*., 1992; Heather and Hallman, 2008).

Vapor heat treatment is applied to tropical fruits such as mango, papaya, etc. as a plant quarantine measure against fruit flies (Armstrong and Mangan, 2007; Japan Fumigation Technology Association, 1996; USDA, 2016). In developing a vapor heat treatment schedule, all developmental stages which can infest fruits are used in mortality tests. On the other hand, many researchers have reported variations of heat tolerance depending on age within a certain stage after a comparison test by immersing only insects in hot water (Corcoran, 1993; Dohino *et al*., 2014; Foliaki and Armstrong, 1996; Heard *et al*., 1991; Jang, 1991; Kaneyuki *et al*., 2014; Kaneyuki *et al*., 2016; Moss and Jang, 1991; Sales *et al*., 1996; Shamsudin *et al*., 2009; Tanabe *et al*., 1994; Tora Veuti *et al*., 1996; Waddell *et al*., 1997). These results suggest that it is important to use the age that is tolerant to heat in the mortality test when developing a heat treatment schedule. However, variations of heat tolerance depending on hour-age within each developmental stage have not been thoroughly studied under the heating condition of exposing infested fruit to vapor heat treatment. This variation is more important because vapor heat treatment is applied to *B. dorsalis* host fruits in many countries, and variations of heat tolerance depending on age might be different between insects in fruit that is heated gradually from ambient temperature using vapor heat and insects immersed in hot water.

We compared heat tolerance among different hour-ages in each of the egg stage and first instar larva stage of *B. dorsalis* after exposing infested fruit to vapor heat treatment and compared our results with those of Dohino *et al*. (2014) and Kaneyuki *et al*. (2016) in which heat tolerance was compared among different hour-ages in each of the egg stage and first instar larva stage of *B. dorsalis* after immersing only the insects in hot water.

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Materials and Methods

Test 1. Heat tolerance comparison among different hour-age eggs of *B. dorsalis*

A laboratory colony of *B. dorsalis* reared at the Naha Plant Protection Station in Okinawa was used. The colony of *B. dorsalis* (Permit No. 63Y2152) was originally collected in Okinawa island before their eradication in 1986 (Yoshizawa, 1997). The rearing conditions were 27.0 ± 1.0℃, 60 ± 10% RH and a photoperiod of 14L:10D (L: 6:00-20:00). Flies were reared in cages (450×300×300 mm, 2,500 adults per cage) and given an artificial diet of autolysis yeast (Asahi Group Foods, Ltd., AY-65) and granulated sugar.

Tommy-Atkins (first replicate) and Kent (second and third replicates) varieties of mangoes, *Mangifera indica* L., from Brazil with a weight of 420 g ± 100 g were used. A preliminary examination revealed that *B. dorsalis* eggs hatched in mango fruit 30-32 hours after oviposition when the fruit inoculated with eggs was kept at 27.0℃. So, eggs were collected 3, 15, 21 and 27 hours before treatment to prepare fruit infested with eggs of age 3 hours (early-age), 15 hours, 21 hours (middle-age) and 27 hours (late-age), respectively.

Eggs were collected in a cylindrical polyethylene bottle (78 mm diameter and 135 mm height) with small oviposition holes (198 holes of 0.5 mm diameter per bottle). The inner surface of the bottle was moistened with lemon juice (Pokka Sapporo Food & Beverage Ltd., pokka lemon new pokka). This bottle was exposed to gravid female flies inside the rearing cage for 30 minutes to collect eggs. Collected eggs were counted up to 100. Two flaps were cut in the skin of a mango, and a ventilating hole was made on each flap by a cork borer. Then, 100 eggs on small gauze were inoculated on the pulp under one flap. Thus, 200 eggs were inoculated into each fruit. Inoculated fruits were kept at 27.0 ± 1.0℃ until treatment, in containers (Iwasaki Industry Inc., Lustroware keeper B-358N, 147×215×130 mm) which were closed with a lid having an opening covered with nylon mesh (Tokyo Screen Co., Ltd., bolting cloth nylon, mesh size 65 ㎛) for ventilation.

Fruits were treated with a vapor heat treatment machine (Sanshu Sangyo Co., Ltd., EHK-500AO) by program mode. The chamber temperature was set to hold at 30.0℃ for 15 minutes, increase gradually from 30.0 to 48.0℃ over 2 hours and hold at 48.0℃, and the humidity was set to hold at 95.0% RH for the duration of the treatment (Fig. 1). Before the treatment, the ventilating hole on fruit was covered with tape (Pip Co., Ltd., kinesiology stretch fit tape) to avoid direct heating of eggs. To measure the core temperature of fruit, a sensor probe (FTH Co., Ltd., FT-10VF-F, ϕ 3.2 100 mm Pt100 Ω) was inserted into the innermost pulp of each of three un-inoculated fruits. The temperature of each probe was recorded every one minute. Inoculated fruits were taken out from the vapor heat treatment machine when two of the three sensor fruits reached each target

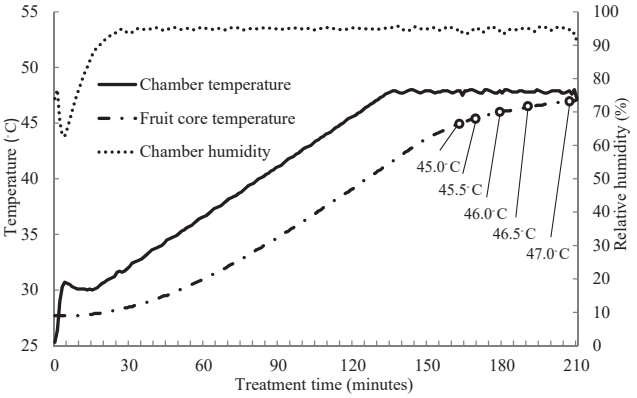

Fig. 1 Temperature and humidity of the chamber air in the vaper heat treatment machine and fruit core temperature during operation. (Replicate 2 of test 1)
Open circles mean time when inoculated fruits were unloaded from chamber.

temperature of 45.0, 45.5, 46.0, 46.5 and 47.0℃. After treatment, inoculated fruits were cooled by an electric fan for one hour, and then eggs on small gauze were withdrawn from fruits and put on moist filter paper (Advantec Ltd., qualitative filter paper No. 2 white 9 cm) in a glass petri dish. The petri dishes were placed in the keeping container at 27.0 ± 1.0℃. Untreated control fruits were kept at room temperature during vapor heat treatment and eggs on gauze were withdrawn from fruits and kept under the same conditions as the treated eggs. After four days of storage, treated and untreated eggs were checked and the number of hatched eggs was counted as survivors. Two fruits per replicate were used in the untreated control plot and treatment plot, and this test was replicated three times.

For data analysis, mortality data was corrected by mean survival rate in the control plot using Abbott's formula (Abbott, 1925) and the number of inoculated insects and survivors was subjected to Fisher's exact test at $\alpha = 0.05$ by application of the Holm procedure by using R 3.1.1 (R Development Core Team, 2014) and referring to Aoki (2010).

Test 2. Heat tolerance comparison among different hour-age first instar larvae of *B. dorsalis*

The same colony as Test 1 was used. Kent variety of mangoes from Brazil with a weight of 450 g ± 100 g were used.

Eggs were collected and inoculated into a fruit, and the inoculated fruit was kept by the same method as Test 1 except that eggs were collected from adults for 15-20 minutes and 140 eggs on small gauze were inoculated into fruit. When many eggs had hatched 31-34 hours after egg collection, the gauze was removed from fruit, and then the number of unhatched eggs and first instar larvae on the gauze was counted. After that, the number of inoculated insects was calculated by subtracting the number of unhatched eggs and remaining first instar larvae on the gauze from the number of inoculated eggs, so 100 to 120 first instar

larvae were inoculated into one fruit. Eggs were collected 35 and 46 hours before the treatment to prepare fruit infested with first instar larvae of age 35 hours (early-age) and 46 hours (late-age) at the time of starting treatment, respectively.

Treatment and cooling were operated in the same manner as Test 1 except for target temperatures of 44.0, 45.0, 45.5, 46.0, 46.5 and 46.8℃. After cooling, the tape covering the ventilation hole on fruit was removed and eight slits were made in the surface of the fruit by knife to remove extra juice. Then each fruit was put in the keeping container one by one. Untreated control fruits were kept at room temperature during vapor heat treatment and kept under the same conditions as the treated fruits. After 4 days storage, treated and untreated control fruits were dissected and the number of survivors was counted. Moving larvae were counted as survivors. Two fruits and one fruit per replicate were used in the untreated control plot and treatment plot, and this test was replicated three times.

For data analysis, all mortality data was corrected by mean survival rate in the control plot using Abbott's formula (Abbott, 1925) and the number of inoculated insects and survivors were subjected to Fisher's exact test at $\alpha = 0.05$ by using R 3.1.1 (R Development Core Team, 2014).

Results and Discussion

Test 1. Heat tolerance comparison among different hour-age eggs of *B. dorsalis*

The number of survivors and corrected mortality of different age eggs of *B. dorsalis* in mango after vapor heat treatment are shown in Tables 1, 2 and 3. As a result, the 27 hours age eggs had the lowest corrected mortality in the 47.0℃ plot through all replicates, although the natural mortality of the 27 hours age eggs was higher than that of the 21 hours age eggs in the first replicate and the other hour-age eggs in the second replicate. The most tolerant age to heat varied such as 15, 21 or 27 hours age in the 45.0, 45.5 and 46.0℃ plots, and 21 or 27 hours age in the 46.5℃ plot depending on the replicate. On the other hand, the 3 hours age eggs had the highest corrected mortality in the 45.0, 45.5 and 46.0℃ plots of all replicates and the 46.5℃ plot of the first and second replicates.

These results show that late-age eggs are the most tolerant to heat and early-age eggs are the least tolerant to heat when fruit infested with *B. dorsalis* eggs was exposed to vapor heat treatment. This is the same tendency as reported by Dohino *et al*. (2014) in which heat tolerance was compared among different hour-age eggs of *B. dorsalis* when only insects were immersed in hot water.

Table 1 Number of survivors and corrected mortality of different age eggs of *B. dorsalis* in mango after vapor heat treatment (Replicate 1)

Fruit core temperature	No. of fruits /each age	No. of inoculated insects /each age	Number of survivors (Corrected mortality)			
			3 hours age	15 hours age	21 hours age	27 hours age
Untreated control	2	400	325 a −	309 a −	359 b −	332 a −
45.0℃	2	400	24 a (92.6%)	343 b (0.0%)	327 b (8.9%)	328 b (1.2%)
45.5℃	2	400	13 a (96.0%)	314 b (0.0%)	332 b (7.5%)	316 b (4.8%)
46.0℃	2	400	1 a (99.7%)	330 b (0.0%)	331 b (7.8%)	312 b (6.0%)
46.5℃	2	400	1 a (99.7%)	172 c (44.3%)	274 d (23.7%)	42 b (87.3%)
47.0℃	2	400	3 a (99.1%)	3 a (99.0%)	3 a (99.2%)	5 a (98.5%)

Different letters show significant difference among different age eggs at same fruit core temperature (Fisher's exact test at α =0.05 by application of the Holm procedure).

Table 2 Number of survivors and corrected mortality of different age eggs of *B. dorsalis* in mango after vapor heat treatment (Replicate 2)

Fruit core temperature	No. of fruits /each age	No. of inoculated insects /each age	Number of survivors (Corrected mortality)			
			3 hours age	15 hours age	21 hours age	27 hours age
Untreated control	2	400	345 b −	338 b −	338 b −	313 a −
45.0℃	2	400	18 a (94.8%)	313 c (7.4%)	311 c (8.0%)	272 b (13.1%)
45.5℃	2	400	8 a (97.7%)	316 c (6.5%)	296 c (12.4%)	258 b (17.6%)
46.0℃	2	400	2 a (99.4%)	200 c (40.8%)	296 d (12.4%)	143 b (54.3%)
46.5℃	2	400	0 a (100.0%)	58 b (82.8%)	128 c (62.1%)	129 c (58.8%)
47.0℃	2	400	4 a (98.8%)	4 a (98.8%)	8 a (97.6%)	18 b (94.2%)

Different letters show significant difference among different age eggs at same fruit core temperature (Fisher's exact test at α =0.05 by application of the Holm procedure).

Table 3 Number of survivors and corrected mortality of different age eggs of *B. dorsalis* in mango after vapor heat treatment (Replicate 3)

Fruit core temperature	No. of fruits /each age	No. of inoculated insects /each age	Number of survivors (Corrected mortality)			
			3 hours age	15 hours age	21 hours age	27 hours age
Untreated control	2	400	364 a −	362 a −	365 a −	359 a −
45.0℃	2	400	21 a (94.2%)	334 b (7.7%)	358 b (1.9%)	348 b (3.1%)
45.5℃	2	400	0 a (100.0%)	273 b (24.6%)	365 d (0.0%)	333 c (7.2%)
46.0℃	2	400	2 a (99.5%)	103 b (71.5%)	305 c (16.4%)	366 d (0.0%)
46.5℃	2	400	0 a (100.0%)	0 a (100.0%)	71 b (80.5%)	219 c (39.0%)
47.0℃	2	400	0 a (100.0%)	0 a (100.0%)	0 a (100.0%)	1 a (99.7%)

Different letters show significant difference among different age eggs at same fruit core temperature (Fisher's exact test at α =0.05 by application of the Holm procedure).

Test 2. Heat tolerance comparison among different hour-age first instar larvae of *B. dorsalis*

The number of survivors and corrected mortality of different age first instar larvae of *B. dorsalis* in mango after vapor heat treatment are shown in Tables 4, 5 and 6. As a result, the 35 hours age first instar larvae had lower corrected mortality than the 46 hours age in all temperature plots in which survivors existed through all replicates, although the natural mortality of the 35 hours age first instar larvae was higher than that of the 46 hours age in the first replicate.

These results show that 35 hours age first instar larvae are more tolerant to heat than 46 hours age when fruit infested with *B. dorsalis* first instar larvae was exposed to vapor heat treatment. This tendency is different from that reported by Kaneyuki *et al*. (2016) in which heat tolerance was compared among different hour-age first instar larvae of *B. dorsalis* when only insects were immersed in hot water. In their study, 44 hours age first instar larvae were more tolerant to heat than 34 hours age. Two possible reasons are considered for the difference in heat tolerant tendency between Kaneyuki *et al*. (2016) and our study. As a first possible reason, the difference of heating method between both studies may have affected the heat tolerance tendency. Only insects were immersed in hot water at 45.0℃ for several exposure times in Kaneyuki *et al*. (2016). However, fruit infested with insects was heated gradually from ambient temperature to each target fruit core temperature of 44.0, 45.0, 45.5, 46.0, 46.5 and 46.8℃, and first instar larvae existing in the fruit surface might have been heated to a higher temperature than those in the fruit core in our study. In this case, our result is more important for selecting the hour-age of first instar larvae for the mortality test to develop a vapor heat treatment schedule, because the heating method in our study simulated the commercial-based vapor heat treatment applied as a phytosanitary measure. As another possible reason, the heat tolerance of first instar larvae may change drastically with age. If first instar larvae become more tolerant to heat from 34 hours age to 35 hours age and/or less tolerant from 44 hours age to 46 hours age, the different tendency between the two studies can be explained. More detailed research is needed to know the relationship between heat tolerance and age of first instar larvae.

Our results indicated that there were various heat tolerances among different hour-ages in the egg and first instar larva period when infested fruit was exposed to vapor heat treatment. Heating speed and the environment around insects are different between vapor heat treatment of infested fruit and hot water immersion of only insects which has been used in many studies to know the relationship between heat tolerance and age. If the heat tolerant tendency with age is different between these two methods, the results of mortality tests by vapor heat treatment are more important for selecting age for the mortality test to develop a vapor heat treatment schedule, because it is more similar to commercial-based vapor heat treatment.

Some countries use a vapor heat treatment schedule with a fruit core temperature of around 47.0℃ as a phytosanitary measure against *B. dorsalis*. Late-age eggs had the lowest corrected mortality in the 47.0℃ plot in our study. Therefore, it is suggested that late-age eggs should be used in mortality tests for *B. dorsalis* to develop a vapor heat treatment schedule with a fruit core temperature of around 47.0℃ against this fruit fly.

Heat tolerance is influenced by many factors in insect rearing methods for mortality tests such as rearing temperature and rearing density (Miyazaki and Dohino, 2000; Yamamoto *et al*., 2008). Our results indicate that hour-age in the same developmental stage is one of these factors, and this age factor is also important when developing a heat treatment schedule with phytosanitary safety.

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Table 4 Number of inoculated insects, survivors and corrected mortality of different age first instar larvae of *B. dorsalis* in mango after vapor heat treatment (Replicate 1)

Fruit core temperature	No. of fruits /each age	35 hours age				46 hours age			
		No. of inoculated insects	No. of survivors		Corrected mortality	No. of inoculated insects	No. of survivors		Corrected mortality
Untreated control	2	235	207	a	−	237	231	b	−
44.0℃	1	102	99	b	(0.0%)	102	74	a	(25.6%)
45.0℃	1	106	91	b	(2.5%)	106	11	a	(89.4%)
45.5℃	1	107	79	b	(16.2%)	108	0	a	(100.0%)
46.0℃	1	110	43	b	(55.6%)	110	0	a	(100.0%)
46.5℃	1	112	1	a	(99.0%)	111	0	a	(100.0%)
46.8℃	1	102	0	a	(100.0%)	101	0	a	(100.0%)

Different letters show significant difference among different age larvae at same fruit core temperature (Fisher's exact test at α =0.05).

Table 5 Number of inoculated insects, survivors and corrected mortality of different age first instar larvae of *B. dorsalis* in mango after vapor heat treatment (Replicate 2)

Fruit core temperature	No. of fruits /each age	35 hours age				46 hours age			
		No. of inoculated insects	No. of survivors		Corrected mortality	No. of inoculated insects	No. of survivors		Corrected mortality
Untreated control	2	212	207	a	−	214	206	a	−
44.0℃	1	102	96	b	(3.6%)	103	71	a	(28.4%)
45.0℃	1	106	99	b	(4.3%)	106	44	a	(56.9%)
45.5℃	1	104	71	b	(30.1%)	103	1	a	(99.0%)
46.0℃	1	108	32	b	(69.7%)	108	0	a	(100.0%)
46.5℃	1	109	0	a	(100.0%)	110	0	a	(100.0%)
46.8℃	1	107	0	a	(100.0%)	108	0	a	(100.0%)

Different letters show significant difference among different age larvae at same fruit core temperature (Fisher's exact test at α =0.05).

Table 6 Number of inoculated insects, survivors and corrected mortality of different age first instar larvae of *B. dorsalis* in mango after vapor heat treatment (Replicate 3)

Fruit core temperature	No. of fruits / each age	35 hours age				46 hours age			
		No. of inoculated insects	No. of survivors		Corrected mortality	No. of inoculated insects	No. of survivors		Corrected mortality
Untreated control	2	206	203	a	−	202	196	a	−
44.0℃	1	105	101	b	(2.4%)	104	74	a	(26.7%)
45.0℃	1	103	87	b	(14.3%)	103	19	a	(81.0%)
45.5℃	1	108	38	a	(64.3%)	108	32	a	(69.5%)
46.0℃	1	101	11	b	(88.9%)	101	0	a	(100.0%)
46.5℃	1	102	8	b	(92.0%)	102	0	a	(100.0%)
46.8℃	1	109	0	a	(100.0%)	109	0	a	(100.0%)

Different letters show significant difference among different age larvae at same fruit core temperature (Fisher's exact test at α =0.05).

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和文摘要

蒸熱処理によるミカンコミバエ卵及び1齢幼虫の同一齢期内における熱耐性の比較（英文）

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ミカンコミバエ卵の前期、中期及び後期、1齢幼虫の前期及び後期をそれぞれマンゴウ生果実に接種し蒸熱処理を行い、卵及び1齢幼虫期内の熱耐性を調査した。その結果、卵の中期及び後期は前期よりも、1齢幼虫の前期は後期よりも熱耐性が高かった。また、日本向け生果実の蒸熱処理でよく用いられる温度帯である47.0℃区では、卵後期の補正死亡率が最も低かった。これらの結果は、ミカンコミバエ卵及び1齢幼虫について熱感受性比較試験の試験設計を行う際に有用と考えられる。

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