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Short Communication

Vapor Heat Mortality Tests on the Eggs of the Oriental Fruit Fly, *Bactrocera dorsalis*, Infesting Different Sizes and Varieties of Fresh Mango

Masakuni YOSHINAGA*, Seiki MASAKI** and Toshiyuki DOHINO

Research Division, Yokohama Plant Protection Station,
1–16–10 Shin-Yamashita, Naka-ku, Yokohama 231–0801, Japan.

Abstract: A series of vapor heat treatment tests was conducted on mango fruits infested with eggs of the Oriental fruit fly, *Bactrocera dorsalis* (Hendel), to clarify the effect of the fruit size and cultivated variety factor on the mortality of the *B. dorsalis* eggs.

In the mortality test by fruit size, Carabao mangoes in three different sizes (S, M and L) infested with the eggs were heated to raise the fruit pulp temperature of each size to 44.0, 45.0, 45.5, 46.0, 46.5 and 47.0°C inside the vapor heat treatment chamber set at 49.0°C and relative humidity 95%. Then the mortality of the eggs among the different fruit sizes was compared. The results showed that mortality at each target temperature differed among the sizes: the larger the fruit size, the higher the mortality. Larger fruits had slower increases in pulp temperature, which meant longer exposure to high temperatures. This was thought to be the cause of the higher mortality.

In the mortality test by variety, Kensington Pride and Tommy Atkins mangoes were used. Vapor heat treatment was applied under the same conditions as above. In order to eliminate the size effect on mortality, mango fruits of nearly the same weight were selected for the test. Probit analysis showed that eggs in the Tommy Atkins variety were significantly more likely to be disinfested (LT_{90}, LT_{95}), revealing that variety affects mortality. However, 100% mortality was obtained at 46.5°C or higher temperatures for both varieties. It was thought that the effect of variety was reduced under severe conditions close to complete-kill conditions. The two varieties of fruit had nearly the same rate of temperature increase, suggesting the possibility that some differing factor affecting mortality may exist between varieties, other than the rate of temperature increase.

Key words: plant quarantine treatment, vapor heat, *Bactrocera dorsalis*, mango varieties, fruit sizes

Introduction

The Oriental fruit fly, *Bactrocera dorsalis* (Hendel), and the melon fly, *Bactrocera cucurbitae* (Coquillett), were present in the southwestern islands of Japan and caused serious damage to the area's agricultural production. However, *B. dorsalis* was eradicated through the male annihilation method in 1986 after an investment of 18 years and 5 billion yen, and *B. cucurbitae* was eradicated through the sterile insect release method in 1993 after more than 20.4 billion yen was spent over 22 years (YOSHIZAWA, 1997). With such a history, Japan keeps a strict watch on Tephritid fruit flies of agricultural importance. The Japanese Plant Protection Act prohibits the import of host plants of these fruit flies from the countries where these fruit flies exist. 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 that can completely kill these fruit flies is established by the exporting country and treatment conditions are satisfied.

As quarantine treatment, heat treatments such as hot-water immersion, vapor heat, and high-temperature forced air treatment have been developed and applied for disinfestations of these fruit flies from many commodities (SHARP *et al.*, 1989; SEO *et al.*, 1974; MANGAN *et al.*, 1998). However, factors other than target temperature and exposure time at target temperature that may affect the mortality effectiveness of these heat treatments are not sufficiently known, making it difficult to use these techniques in effective ways.

In the present study, we used mangoes infested with *B. dorsalis* eggs and studied the effects of different fruit sizes and varieties on the mortality effectiveness of vapor heat treatment. We report the results of this research herein.

* Naha Plant Protection Station.

** Niigata Sub-station, Yokohama Plant Protection Station

Materials and Methods

1. Test insects

A laboratory colony of *B. dorsalis* maintained at the Research Center of the Yokohama Plant Protection Station in Yokohama was used. The colony was originally introduced from Thailand in 2005 (Import Permit No. 17Y566). Flies were kept at 26±1°C, 65±10% RH, and a photoperiod of 13L:11D, and given an artificial diet and water.

2. Test fruit

a. Mortality test by mango size:

For this experiment, Carabao mangoes from the Philippines, which had conditional import permission, were used. The weight of individual fruits in the sizes S, M and L was about 200 g, 250 g and 300 g, respectively.

b. Mortality test by mango variety:

For this experiment, Kensington Pride mangoes from Australia and Tommy Atkins mangoes from Brazil were used because these two varieties have same shape, size, and weight. The weight of Kensington Pride and Tommy Atkins fruits was 413.2–514.5 g and 410.5–518.5 g, respectively. Sugar content (Brix %), hardness (=firmness), acidity (pH) and fruit pulp thickness of the two cultivars are shown in Table 1.

Table 1. Summary profile of Kensington Pride and Tommy Atkins mangoes

Variable	Kensington Pride	Tommy Atkins
Sugar content (Brix %)	12.15±1.14[a]	10.35±1.39[b]
Hardness/firmness (kg)	0.645±0.11[a]	1.235±0.37[b]
Acidity (pH)	5.089±0.20[a]	4.621±0.19[b]
Thickness (cm)	2.81±0.15[a]	3.44±0.15[b]

Investigation of each item was conducted with 10 fruits of each variety.
Thickness means the pulp thickness from seed to surface.
Values in the same line followed by a different letter are significantly different (Tukey HSD test, $P<0.05$).

3. Infesting fruit

For infestation, the artificial inoculation method was adopted. Eggs were obtained from gravid females (age >21 d) by placing a polyethylene receptacle (7 cm in diameter, 13 cm in height) with small oviposition holes into the adult cage (30×30×45 cm) containing about 1,000 flies for a period of 2 hours. The inner surface of the receptacle was moistened with orange juice. The collected eggs were placed on wet filter paper and kept at 26°C for 24 hours. After 24 hours, 100 eggs were counted onto black filter paper (1×1 cm) using a fine brush under a microscope. A ⊐-shaped flap was cut in the skin of the mango, and a small amount of fruit pulp was removed. The black filter paper carrying 100 eggs was inserted under the flap. The flap was lowered and covered with tape to prevent desiccation of the eggs.

4. Vapor heat treatment, storage after treatment, and assessment

A vapor heat treatment machine form Sanshu Sangyo Co. Ltd., Model EHK-1000D (1 m^3 chamber capacity), was used for the experiment.

The mangoes infested with *B. dorsalis* eggs in a stainless-steel tray (30×50×7 cm) were placed inside the vapor heat treatment chamber, set at 49.0°C and relative humidity 95%, and heated to fruit-core temperature of 44.0, 45.0, 45.5, 46.0, 46.5, and 47.0°C. In each test plot, 2–3 mangoes infested 100–400 eggs per fruit were used. Information about the treatment schedules, test fruits, and test insects are summarized in Tables 2 and 3. Sensor probes (Pt 100 Ω) were inserted into uninfested fruits to monitor the fruit core temperature. For this purpose, three uninfested fruits in each size or in each variety were also placed inside the chamber. When two of three sensors attained the target temperature, the fruits in each test were removed from the treatment chamber and cooled at 25°C for 2 hours. The tape was removed from the fruits, and the fruits were placed in a plastic box (30×22×10 cm).

After the treatment, the fruits in the plastic box were stored at 26±1°C, 65±10% RH for incubation. The control

Table 2. Treatment schedule, test fruits, and test insects for fruit size test

Target temperature (°C)	Number of Carabao mango fruits (number of *B. dorsalis* eggs)[1]		
	S	M	L
	200 g	250 g	300 g
44.0	9 (1,500)	9 (1,500)	9 (1,500)
45.0	9 (1,500)	9 (1,500)	9 (1,500)
45.5	9 (1,500)	9 (1,500)	9 (1,500)
46.0	9 (1,500)	9 (1,500)	9 (1,500)
46.5	9 (1,500)	9 (1,500)	9 (1,500)
47.0	9 (1,500)	9 (1,500)	9 (1,500)
Control	9 (1,500)	9 (1,500)	9 (1,500)
	9 (–)[2]	9 (–)[2]	9 (–)[2]
Total	72 (10,500)	72 (10,500)	72 (10,500)

[1] Total number of fruits and eggs of 3 replications.
[2] Fruits for sensor probe.

Table 3. Treatment schedule, test fruits, and test insects for fruit cultivar test

Target temperature (°C)	Number of fruits (number of *B. dorsalis* eggs)[1]	
	Kensington Pride	Tommy Atkins
	413.2–514.5 g	410.5–518.5 g
44.0	7 (1,800)	7 (1,800)
45.0	7 (1,800)	7 (1,800)
45.5	7 (1,800)	7 (1,800)
46.0	7 (1,800)	7 (1,800)
46.5	7 (1,800)	7 (1,800)
47.0	7 (1,800)	7 (1,800)
Control	7 (1,800)	7 (1,800)
	9 (–)[2]	9 (–)[2]
Total	58 (12,600)	58 (12,600)

[1] Total number of fruits and eggs of 3 replications.
[2] Fruits for sensor probe.

fruits were also stored in the same conditions. Five days after the treatment, the fruits were cut open and the survivors were counted.

All experiments were replicated 3 times.

5. Data Analysis

Data from these experiments were subjected to the Tukey-HSD test and Probit analysis to compare the heat response of the infesting eggs to different fruit sizes or different varieties using the computer programs JMP4 (Statistical Discovery Software, SAS Institute Inc., 2000) and PoloPlus (LeOra Software, 2003).

Results

1. Mortality test by mango size:

The results are shown in Tables 4 and 5. As shown in Table 4, egg mortality in larger fruits tended to be higher than those in smaller ones at the same target temperature. Probit analysis also showed that the temperature to obtain the same mortality rate tends to be lower for larger fruits. For LT_{50}, a significant difference was recognized between L-size and S-size (Table 5). The time required to reach target temperature was longer for larger fruits (elapsed times

Table 4. Time-mortality relationship of S-, M-, and L-size Carabao mangoes infested with *B. dorsalis* eggs by vapor heat treatment

Target temperature (°C)	Run-up time (min)[1]			Corrected mortality (%)[2]		
	S	M	L	S	M	L
44.0	49.0 ± 2.6^{a}	53.0 ± 2.0^{ab}	59.7 ± 2.5^{b}	5.0 ± 15.3	10.0 ± 12.7	26.9 ± 16.5
45.0	53.7 ± 2.9^{a}	58.0 ± 3.0^{ab}	66.0 ± 2.0^{b}	9.0 ± 9.2	36.9 ± 20.3	36.5 ± 19.5
45.5	57.0 ± 3.5^{a}	61.3 ± 2.5^{ab}	70.0 ± 2.0^{b}	35.5 ± 25.1	39.8 ± 20.3	56.8 ± 9.0
46.0	60.3 ± 2.5^{a}	66.0 ± 3.6^{ab}	74.3 ± 2.1^{b}	35.2 ± 21.0	40.8 ± 4.9	71.8 ± 18.4
46.5	65.3 ± 3.2^{a}	71.7 ± 4.0^{ab}	79.7 ± 2.5^{b}	62.6 ± 38.1	79.0 ± 9.4	96.7 ± 2.6
47.0	72.0 ± 4.4^{a}	77.7 ± 4.5^{ab}	84.7 ± 3.2^{b}	88.4 ± 15.1	99.1 ± 1.6	100

[1] Run-up time (mean ± SD) indicates the required period for fruit pulp temperature to rise from room temperature at 25°C to each target temperature by vapor heat treatment when chamber temperature and humidity were set at 49.0°C and 95%RH, respectively. Values in the same line followed by a different letter are significantly different (Tukey HSD test, $P < 0.05$).
[2] Corrected mortality (mean ± SD) was calculated from ABBOTT (1925).

Table 5. Comparison of lethal pulp temperature for 50%, 90%, and 95% mortality of *B. dorsalis* eggs infesting S, M, and L sizes of Carabao mangoes subjected to vapor heat treatment

Fruit size	LT_{50} (95% CL)	LT_{90} (95% CL)	LT_{95} (95% CL)
S	46.50 (46.15–47.10)	47.87 (47.22–49.60)	48.26 (47.49–50.37)
M	45.56 (44.65–46.31)	47.26 (46.44–51.15)	47.75 (46.76–52.83)
L	45.21 (44.05–45.87)	46.65 (45.95–49.71)	47.07 (46.24–51.14)

Estimated LT_{50}, LT_{90} and LT_{95} for each fruit size with their associated 95% confidence limits were calculated by Probit analysis.

from 25°C to 47°C and temperature increase rates were: S: 72 min., 0.306°C/min.; M: 77.7 min., 0.282°C/min.; and L: 84.7 min., 0.260°C/min.) (see Table 4). This means that exposure to high temperatures was longer for the eggs in the larger fruits; this was thought to explain the higher mortality rate.

The mortality of *B. dorsalis* eggs in untreated control fruits was 48.7±17.6% for S, 51.9±18.5% for M and 50.5±17.6% for L, and there was no significant difference in insect development among fruit sizes.

2. Mortality test by mango variety:

The results are shown in Tables 6 and 7 and Fig. 1. The egg mortality in Tommy Atkins mangoes was consistently higher than those in Kensington Pride (Table 6) at the same target temperature. Probit analysis showed that the temperatures required to attain LT_{90} and LT_{95} in the former variety were also significantly lower than those in the latter (Table 7). These results indicated that the heat responses of the eggs in mango fruits were different between the two mango varieties. On the other hand, the run-up periods to the target fruit temperatures of the two varieties did not show so much difference, although the Tommy Atkins variety showed a slightly longer period than Kensington Pride (heating rate from 25°C to 47°C was 0.209°C/min. for Kensington Pride and 0.201°C/min. for Tommy Atkins).

In spite of the results described above, the temperature conditions that provide 100% mortality were 46.5°C or higher in both varieties, and at this point there was no difference between the two varieties (Table 6).

The mortality of *B. dorsalis* eggs in untreated controls was 58.3±18.6% for Kensington Pride and 58.4±21.1% for Tommy Atkins, and there was no significant difference in insect development between the varieties.

Discussion

The results of our experiment with three different sizes of Carabao mango showed that larger fruits with a slower heating rate provided higher mortality for *B. dorsalis* eggs than smaller fruits with quicker heating rates at the same target temperature (pulp temperature) during vapor heat treatment. As for the effect of heating rates on insect mortality, different researchers have different opinions. NEVEN (1998) insists that a slower heating rate enhances acclimation

Table 6. Time-mortality relationship of Kensington Pride and Tommy Atkins mango varieties infested with *B. dorsalis* eggs by vapor heat treatment

Target temperature (°C)	Run-up time (min)[1]		Corrected mortality (%)[2]	
	Kensington Pride	Tommy Atkins	Kensington Pride	Tommy Atkins
44.0	72.7 ± 5.5	74.3 ± 7.0	26.2 ± 30.8	53.3 ± 22.0
45.0	80.3 ± 5.0	82.7 ± 8.5	60.0 ± 29.8	83.0 ± 24.4
45.5	86.0 ± 5.6	88.3 ± 9.0	90.2 ± 8.7	93.2 ± 9.6
46.0	92.7 ± 6.1	95.7 ± 9.1	85.6 ± 12.5	99.1 ± 1.6
46.5	100.3 ± 6.7	102.7 ± 9.7	100	100
47.0	107.7 ± 6.8	110.3 ± 9.3	100	100

[1] Run-up time (mean ± SD) indicates the required period for fruit pulp temperature to rise from room temperature at 25°C to each target temperature by vapor heat treatment when chamber temperature and humidity were set at 49.0°C and 95%RH, respectively.
[2] Corrected mortality (mean ± SD) was calculated from ABBOTT (1925).

Table 7. Comparison of lethal pulp temperature for 50%, 90%, and 95% mortality of *B. dorsalis* eggs infesting Kensington Pride and Tommy Atkins mango varieties subjected to vapor heat treatment

Mango variety	LT_{50} (95% CL)	LT_{90} (95% CL)	LT_{95} (95% CL)
Kensington Pride	44.74 (43.23–45.26)	45.86 (45.35–46.64)	46.18 (45.70–47.32)
Tommy Atkins	44.01 (43.74–44.20)	44.95 (44.79–45.14)	45.22 (45.05–45.45)

Estimated LT_{50}, LT_{90} and LT_{95} for each variety with their associated 95% confidence limits were calculated by Probit analysis.

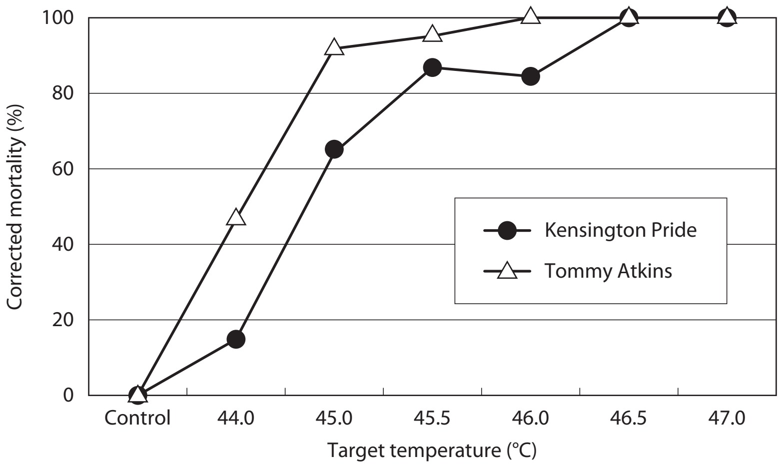

Fig. 1. Target temperature–mortality relationship of Kensington Pride and Tommy Atkins mango varieties infested with *B. dorsalis* eggs by vapor heat treatment.

of the treated pest and makes the pest difficult to kill. Other researchers claim that a slow heating rate provides longer exposure of pests to high temperature and increases pest mortality (WANG *et al.*, 2002). The results we obtained fit well with the latter opinion.

Smaller fruits take a shorter time to reach a target temperature, and the mortality rate at that point is lower. So, if the fruits to be treated are all smaller than the fruits used in the experiment that established a complete-kill treatment standard, then there is the possibility that the time required to reach the target temperature is shorter than that of the standard, allowing some pest individuals to survive. In light of such circumstances, it is thought to be important to set up a minimum treatment time from the beginning to the end of treatment.

So far, there have been no reports on investigations into the effect of the variety of treated fruit on the mortality effectiveness of vapor heat treatment. This study showed that a difference in mango variety had an effect on the mortality rate of eggs infesting fruit. As for the cause of the difference among varieties, there is the possibility that the slightly longer treatment time for the Tommy Atkins variety had an impact. However, this was a slight difference. Therefore, there is a strong possibility that factors other than treatment time may be involved. This point should be resolved in future.

It was also shown that the difference in mortality between the two varieties as described above did not directly connect to the difference in treatment conditions for 100% mortality. That is, 100% mortality was obtained only in treatment areas at 46.5°C or higher in both varieties. In vapor heat treatment, it was thought that the effect of variety on mortality was reduced under severe treatment conditions close to the complete-kill conditions.

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和 文 摘 要

サイズ又は品種の異なるマンゴウ生果実に寄生させたミカンコミバエ*Bactrocera dorsalis*卵の蒸熱殺虫試験

吉永真訓*，正木征樹**，土肥野利幸
横浜植物防疫所調査研究部

ミカンコミバエ*Bactrocera dorsalis*卵をサイズ又は品種の異なるマンゴウ生果実に寄生させて蒸熱処理を行い、サイズ又は品種の違いが殺虫効果に及ぼす影響について調査した。

1）殺虫効果に及ぼすサイズの影響を調べるため、カラバオ種マンゴウのS、M、Lサイズにミカンコミバエの卵を寄生させ、果実中心温度が44.0、45.0、45.5、46.0、46.5、47.0℃になるまで蒸熱処理（庫内温度49.0℃、相対湿度95％設定）し、殺虫率をサイズ間で比較した。その結果、各目標温度に達した時点での殺虫率はサイズによって異なり、大きいサイズの果実ほど殺虫率は高くなることが示された。大きい果実ほど中心部の温度上昇は緩やかで、その分だけ高温にさらされる時間が長くなることから、このことが原因したものと考えられた。

2）次に、殺虫効果に及ぼす品種の影響を調べるため、2品種マンゴウ（ケンジントン種、トミーアトキンス種）にミカンコミバエ卵を寄生させ、前述の条件で蒸熱処理して、得られた殺虫率を品種間で比較した。処理に際しては品種間でサイズに差が出ないように配慮した。プロビット解析の結果、トミーアトキンス種に寄生させた卵が有意に殺虫されやすいことが示され（LT_{90}、LT_{95}）、品種の違いが殺虫率に影響していることが明らかとなった。ただ100％殺虫が得られた処理条件はいずれの品種でも46.5℃以上と変わらず、完全殺虫条件に近い厳しい処理条件下では品種の違いの影響は少なくなるものと考えられた。なお、2品種間で果実の温度上昇速度はほとんど違わず、殺虫効果を左右する要因として、温度上昇速度以外の要因が品種間で存在する可能性が示唆された。

* 現在、那覇植物防疫事務所
** 現在、横浜植物防疫所新潟支所