

Vapor Heat Mortality Tests on the Eggs of Oriental Fruit Fly, *Bactrocera dorsalis*, Infesting Different Fruit Shape of Fresh Mango.

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Abstract: Three different mango varieties, 'Tommy Atkins' (oval/round shape, 431.5-575.7g), 'Nam Doc Mai' (flat/elongated shape, 379.1-444.5g), 'Carabao' (flat/elongated shape, 217.8-241.9g), infested with eggs of Oriental Fruit Fly, *Bactrocera dorsalis* (Hendel) were subjected to vapor heat treatment to study the relationship between mango variety and mortality. The innermost pulp of each variety was heated to 45.5 °C. The result showed that the heavier the fruit weight, the longer the run-up time and the higher the mortality. When the 'Nam Doc Mai' reached 45.5 °C, 'Tommy Atkins' was 42.8-44.2 °C. However, the mortality of eggs in 'Tommy Atkins' was higher than those in 'Nam Doc Mai'. We speculate that this was because heavy and oval 'Tommy Atkins' kept surplus heat longer than 'Nam Doc Mai'. We supposed that fruit shape affected the heat loss after treatment, but it could not be denied that fruit weight affected heat loss.

Then, nearly the same weight and different shaped mangoes, 'Nam Doc Mai' (flat/elongated shape, 429.1g-480.9g) and 'Kent' (oval/round shape, 427.8-480.9g) were heated to 45.0 °C. The result showed that increase and decrease in temperature of 'Nam Doc Mai' was faster and the mortality of *B. dorsalis* eggs in 'Nam Doc Mai' was lower than 'Kent'. These results indicated that the fruit shape was one of the factors which affect mortality in vapor heat treatment.

Key words: quarantine vapor heat treatment, *Bactrocera dorsalis*, shape

Introduction

All over the world, many countries maintain a strict watch on fruit flies because they cause serious damage to agricultural production. As a quarantine treatment against fruit flies, vapor heat treatment, hot water treatment and forced hot air treatment have been developed (Seo *et al.*, 1974; Sharp *et al.*, 1989; Mangan *et al.*, 1998).

Many of these treatments specify the target temperature and exposure time (Armstrong and Mangan 2007). Yoshinaga *et al.*, (2009) used 'Carabao' mangoes in different fruit sizes S, M and L (classified according to the fruit weight) infested with *B. dorsalis* eggs and studied the effects of fruit sizes on mortality with vapor heat treatment. Their result showed that the fruit weight affected the increase of innermost pulp temperature and was one of the factors which affected mortality. USDA treatment manual (2013, T102-a)

specifies dip time of mangoes in hot water treatment based on fruit weight and shape. Furthermore, the manual (T103-c-1) specifies the mangoes size (weight) in high temperature forced air treatment. In this study, we studied if the differences in mango weight and shape affected mortality with the vapor heat treatment.

Materials and Methods

1. Test insects

A laboratory colony of *B. dorsalis* maintained at the Research Division of Yokohama Plant Protection Station in Yokohama was used. The colony was originally 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. Eggs were obtained from gravid females by placing a polyethylene receptacle (8cm in diameter, 13cm in height) with small oviposition holes into the adult cage containing about 1,000 flies for one hour. The inner surface of the

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receptacle was moistened with orange juice. In the following test 1), 28 hour-old eggs were used and test 2), 26 hour-old eggs were used.

2. Test fruit

1) Mortality test by mango variety

Vapor heat treatment was conducted on three different mango varieties infested with eggs of *B. dorsalis* to clarify the effect of the difference in cultivated variety on mortality.

For this experiment, 'Carabao' from the Philippines (flat/elongated shape, 217.8-241.9g), 'Nam Doc Mai' from Thailand (flat/elongated shape, 379.1-444.5g) and 'Tommy Atkins' from Brazil (oval/round shape, 431.5-575.7g) were selected. These varieties are different in weight and shape.

2) Mortality test by mango shape

'Kent' from Mexico (oval/round shape, 427.8-480.9g) and 'Nam Doc Mai' from Thailand (flat/elongated shape, 429.1g-480.9g) were selected to clarify the effect of fruit shape on mortality. These two varieties have almost the same weight but are different in shape.

3. Infesting test fruit

1) Mortality test by mango variety

One hundred fifty eggs were counted on black filter paper using a fine brush under the microscope. A □-shaped flap was cut in the skin of mango and a small amount of flesh was removed. The black filter paper carrying 150 eggs was inserted under the flap. The black filter paper was removed from the fruit after egg infestation and the flap was lowered and covered with surgical tape to prevent desiccation of eggs. Infested fruits were stored at 26 ± 1 °C until vapor heat treatment started.

2) Mortality test by mango shape

Two flaps were cut in the skin of the mango and one hundred eggs on the black filter paper were inserted under each flap and infested to the fruit in the same way as described above.

One of the black filter papers with eggs was covered with gauze so that the filter paper with eggs could be removed from mango pulp when the innermost pulp temperature reached the target temperature. Another of the black filter paper with eggs was inoculated to the pulp so that the eggs were exposed to the surplus heat after the innermost pulp reached the target temperature. The flap was covered with surgical tape after infestation

and infested fruits were stored at 26 ± 1 °C until vapor heat treatment started.

4. Vapor heat treatment and assessment

1) Mortality test by mango varieties

A vapor heat treatment machine (Sanshu Sangyo Co. Ltd., Model EHK-1000), was used for the experiment. Three varieties infested with *B. dorsalis* eggs were placed inside the vapor heat treatment chamber, set at 49 °C and relative humidity 95%, and heated simultaneously. During the vapor heat treatment, the temperature of the innermost and outermost pulp of each variety was recorded by the minute with a sensor (CHINO Co. Ltd., Pt100). After the vapor heat treatment, wireless sensor probes (T&D Co. Ltd., Model RTR-52) were used and measured both temperatures (innermost and outermost pulp) of the other treated fruit. These sensors were corrected at 45.5 °C before vapor heat treatment

When two of the three sensors which were inserted in the innermost pulp of each variety ('Carabao', 'Nam Doc Mai' and 'Tommy Atkins') reached 45.5 °C , the six infested fruit of each mango variety were removed from the chamber. The experimental section in which the three mango varieties were heated until the 'Carabao' reached 45.5 °C , was defined as experimental section A. Similarly, we defined the experimental section, in which the 'Nam Doc Mai' and 'Tommy Atkins' reached 45.5 °C , as experimental sections B and C, respectively. Six infested fruit of each variety were used as control fruit. After treatment, the tested fruit were stored at 26 ± 1 °C for 5 days and the control fruit were stored for 4 days. The fruit were cut open and the survivors were counted. The weight of tested fruit and sensor fruit were shown in **Table.2**. Tests were replicated twice.

2) Mortality test by mango shape

A vapor heat treatment machine (FTH Co. Ltd., Model VHC-10) was used for this experiment. The chamber was set at 49 °C and 95% RH. The temperature of the innermost and outermost pulp was recorded with the same sensor probes as test1). During vapor heat treatment, the temperature of the mangoes was recorded every 1 minute and after the treatment, that was recorded every 30 seconds. These sensors were calibrated at 45.0 °C before vapor heat treatment.

When two of the three sensors, which were inserted in the innermost pulp of 'Nam Doc Mai' and 'Kent' , reached 45.0 °C , the infested fruit were removed from the chamber. One of the two black filter papers containing 100 eggs inoculated to the fruit was transferred to

another fruit which had been stored at $26 \pm 1^\circ\text{C}$ so that we could determine the mortality of the eggs at the instant of 45.0°C . The other black filter paper (100 eggs) was kept in the fruit so that we could determine the effect of the surplus heat on mortality.

Three fruit of each variety were used as the control fruit. These untreated fruit were infested in the same way as noted above. When the treatment finished, one of the two black filter papers (100 eggs x 2) was removed from the untreated fruit and transferred to the other fruit which had been stored at $26 \pm 1^\circ\text{C}$.

After the treatment, these fruit with eggs were stored at $26 \pm 1^\circ\text{C}$ for 5 days and control fruit were stored for 4 days. Then, fruit were cut open and the survivors counted. This experiment was replicated twice. Corrected mortality was calculated from ABBOTT (1925) in test 1) and 2).

5. Data analysis

The mortality in test 1) and test 2) was assessed

at number of survivors and corrected mortality was calculated from ABBOTT (1925).

The number of survivors from each experiment was subjected to the chi-square test ($P < 0.05$).

Results

1) Mortality test by mango variety

The time required to reach 45.5°C was in ascending order, 56-58 min. ('Carabao'), then 74-75 min. ('Nam Doc Mai'), and finally 84-94 min. ('Tommy Atkins') (**Table 1**). The corrected mortality was 58.4-64.0% ('Carabao'), 98.8% ('Nam Doc Mai') and 100% ('Tommy Atkins'). Significant difference was observed in corrected mortality among 3 mango varieties in experimental section A and B in each replication (chi-square test, $P < 0.05$). **Fig.1.** shows the increase in temperature until the innermost pulp of each mangoreached 45.5°C and **Fig.2.** shows the decrease in temperatures after the treatment.

Table 1 Comparison of the mortality of *B. dorsalis* eggs in 'Carabao', 'Nam Doc Mai' and 'Tommy Atkins' by vapor heat treatment

Replication	Experimental section	Heat up time (min.) ^{*1}	No. of treated fruits	'Carabao'		'Nam Doc Mai'		'Tommy Atkins'	
				Corrected mortality(%) ^{*2}	Innermost pulp temp.	Corrected mortality(%)	Innermost pulp temp.	Corrected mortality(%)	Innermost pulp temp.
1	A('Carabao' at 45.5°C)	56	6	58.4a ^{*3}	45.5°C	59.1a	41.8°C	69.9b	38.5°C
	B('Nam Doc Mai' at 45.5°C)	74	6	100a	47.5°C	98.8b	45.5°C	100a	42.8°C
	C('Tommy Atkins' at 45.5°C)	94	6	100	48.2°C	100	47.3°C	100	45.5°C
2	A('Carabao' at 45.5°C)	58	6	64.0a	45.5°C	31.2b	42.1°C	30.7b	40.4°C
	B('Nam Doc Mai' at 45.5°C)	75	6	100a	47.3°C	98.8b	45.5°C	100a	44.2°C
	C('Tommy Atkins' at 45.5°C)	84	6	100	47.7°C	100	46.5°C	100	45.5°C

^{*1}The time required to reach 45.5°C at innermost pulp of mangoes

^{*2}Corrected by the method of ABBOTT(1925)

^{*3}Values in a line followed by the same letter don't differ significantly(Ryan's multiple-range test for proportions after the chi-squared test, $P > 0.05$)

Table 2 The number of 'Carabao', 'Nam Doc Mai' and 'Tommy Atkins' tested and their weight

Replication	Use	No. of treated fruit of each varieties	Average weight $\pm$ SD(g)		
			'Carabao'	'Nam Doc Mai'	'Tommy Atkins'
1	test fruit	18	228.3 ± 6.8	427.8 ± 11.2	557.0 ± 10.1
	control	6	228.6 ± 7.1	428.6 ± 9.9	560.1 ± 11.3
	sensor fruit	3	227.6 ± 0.5	432.2 ± 0.3	553.3 ± 0.6
2	test fruit	18	230.8 ± 7.4	413.5 ± 27.6	443.4 ± 10.9
	control	6	230.4 ± 7.6	401.9 ± 15.1	445.7 ± 14.4
	sensor fruit	3	229.0 ± 0.5	396.5 ± 1.9	442.3 ± 0.3

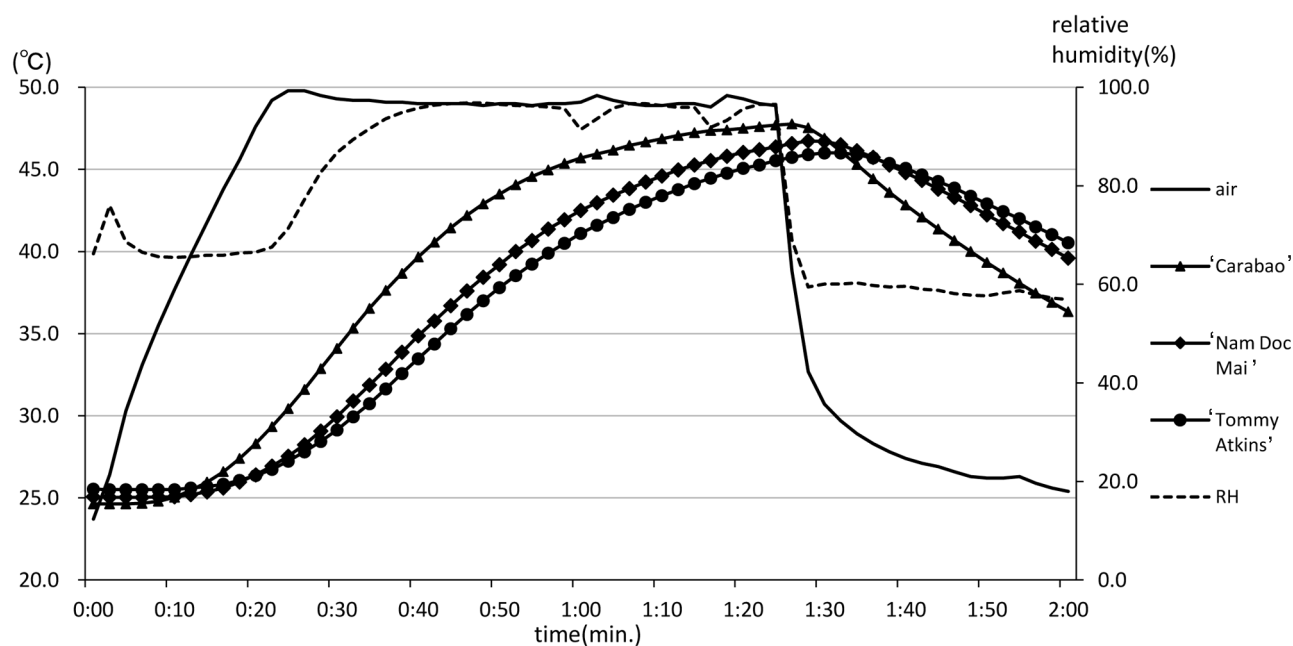


Fig. 1. The temperature of innermost pulp of three mango varieties and relative humidity during vapor heat treatment.

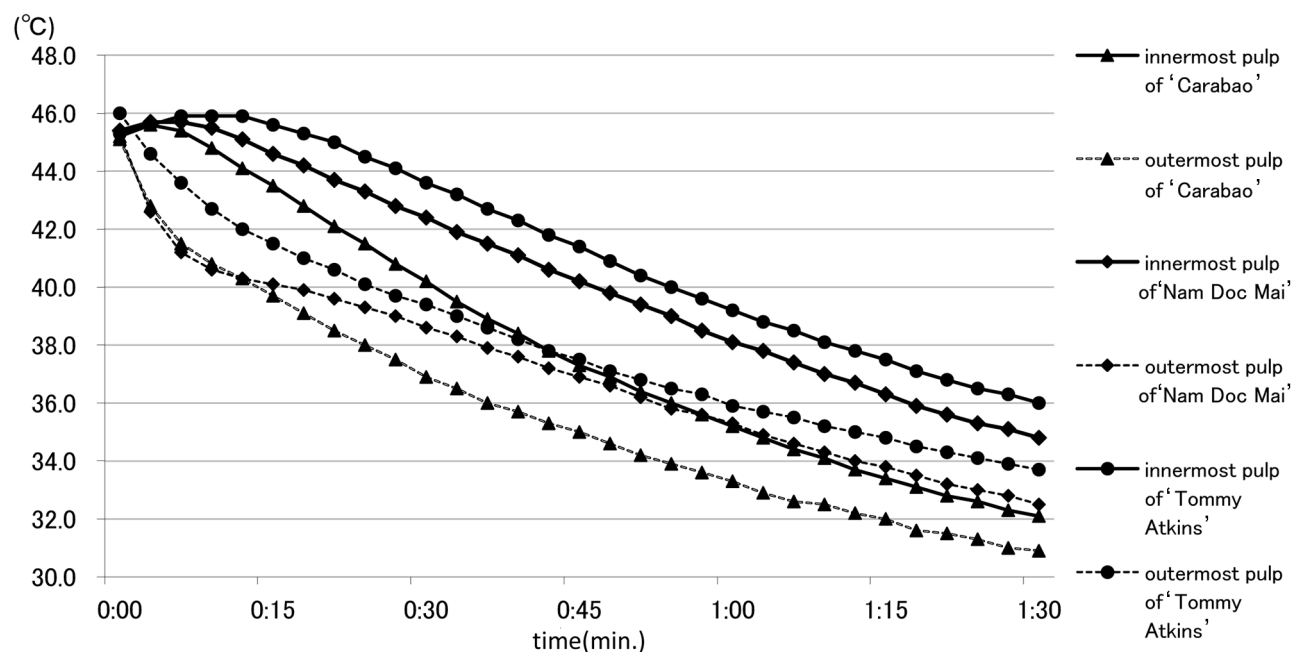


Fig. 2. The temperature of innermost and outermost pulp of three mango varieties after vapor heat treatment.

2) Mortality test by mango shape

'Nam Doc Mai' required 67-74 min. to reach 45.0 °C and 'Kent' required more time (75-83 min.). 'Kent' showed higher corrected mortality than 'Nam Doc Mai' at the instant of 45.0 °C ('Nam Doc Mai' was 45.3-67.9% and 'Kent' was 93.7-94.6%)(Table 3). There was a statistically-significant difference in the number of

survivors between 'Nam Doc Mai' and 'Kent' (chi-square test, $P < 0.05$). In the experimental section exposed to surplus heat after the innermost pulp reached 45.0 °C, 'Kent' showed higher mortality than 'Nam Doc Mai' and there was a significant difference between these two varieties (chi-square test, $P < 0.05$). Furthermore, there was a significant difference in each replication between

the experimental section exposed to surplus heat and not exposed to surplus heat in each variety except 'Kent' in replication 1 (chi-square-test, $P < 0.05$).

The temperature of innermost pulp of 'Kent' took longer to reach 45.0 °C than 'Nam Doc Mai' but the rate of temperature increase of outermost pulp was similar

in these two mango varieties (**Fig.3.**). On the other hand, in the process of decrease in temperature after treatment, both the temperature of the innermost and outermost pulp of 'Kent' decreased slightly slower than 'Nam Doc Mai' (**Fig. 4.**).

Table 3 Comparison of mortality of *B. dorsalis* eggs in 'Nam Doc Mai' and 'Kent' by vapor heat treatment

Replication	Varieties	Weight(g) mean $\pm$ SD	No. of treated fruit	Heat up time (min.) ^{*1}	eggs removed from treated fruit at 45.0°C			eggs kept in the treated fruit after 45.0°C		
					No. of survivors/fruit		Corrected mortality(%)	No. of survivors/fruit		Corrected mortality(%) ^{*2}
					treated (non-surplus heat)	control		treated (surplus heat)	control	
1	'Nam Doc Mai'	430.4 $\pm$ 1.7	3	67	46.3 $\pm$ 22.4	84.7 $\pm$ 2.6	45.3	10.3 $\pm$ 6.6	86.3 $\pm$ 1.3	88.0
	'Kent'	430.2 $\pm$ 1.9	3	75	5.7 $\pm$ 7.3	89.7 $\pm$ 2.1	93.7	5.3 $\pm$ 3.7	87.0 $\pm$ 2.9	93.9
2	'Nam Doc Mai'	470.4 $\pm$ 6.3	5	74	29.0 $\pm$ 22.2	90.3 $\pm$ 1.9	67.9	9.2 $\pm$ 10.8	89.3 $\pm$ 0.5	89.7
	'Kent'	470.8 $\pm$ 6.4	5	83	4.8 $\pm$ 5.9	89.0 $\pm$ 2.5	94.6	1.6 $\pm$ 2.7	89.0 $\pm$ 0.8	98.2

^{*1}The time required to reach 45.0°C at innermost pulp of mangoes

^{*2}Corrected by the method of ABBOTT(1925)

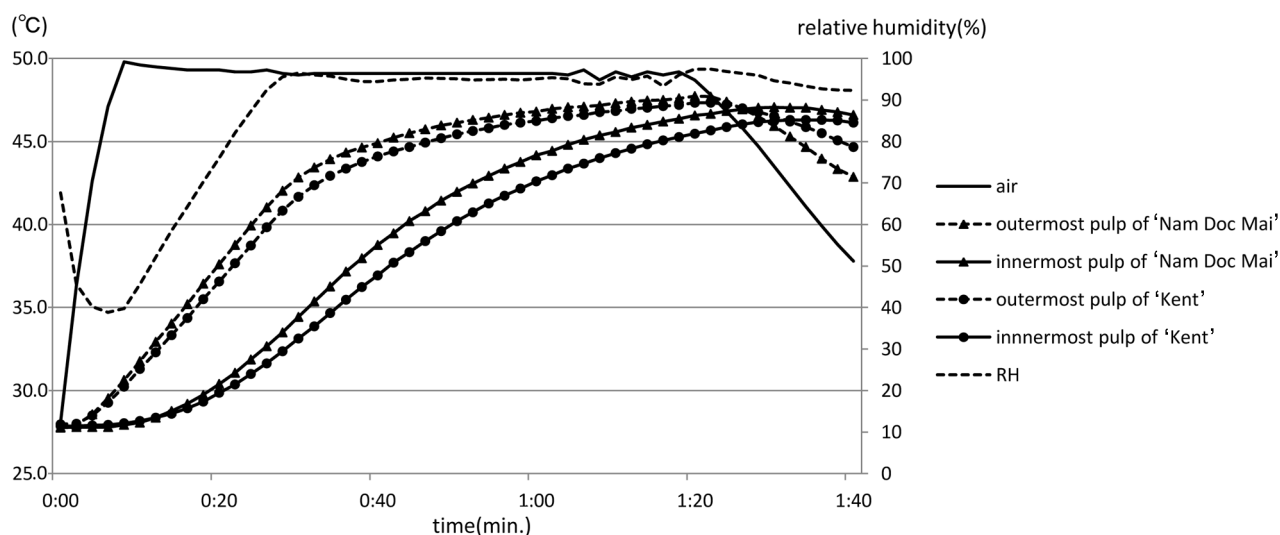


Fig. 3. The temperature of innermost and outermost pulp of 'Nam Doc Mai' and 'Kent' and relative humidity in chamber during vapor heat treatment.

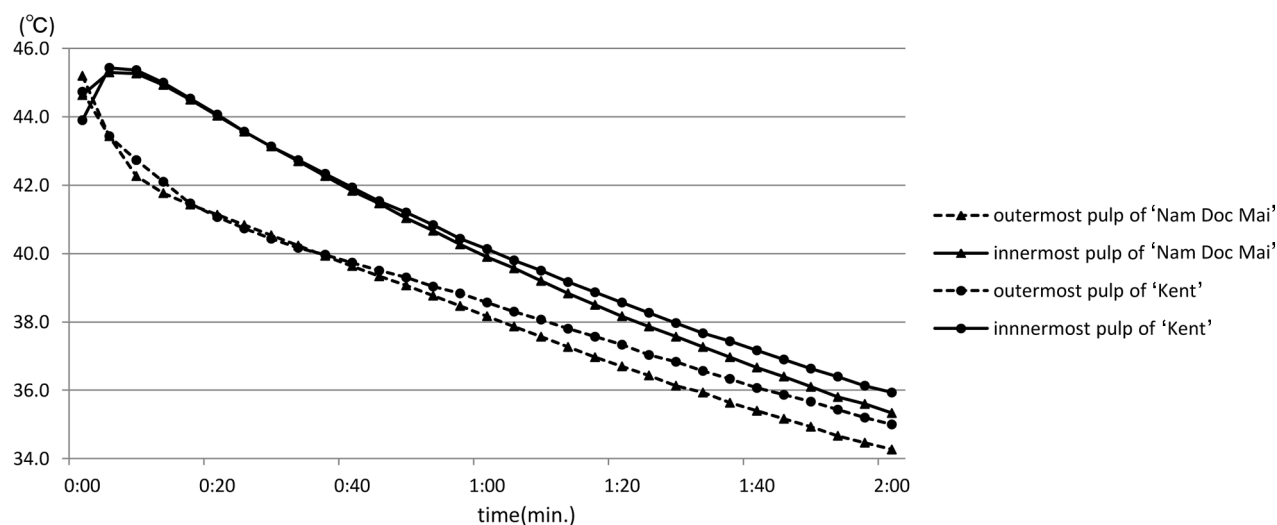


Fig. 4. The temperature of innermost and outermost pulp of 'Nam Doc Mai' and 'Kent' after vapor heat treatment.

Discussion

Our results of vapor heat treatment conducted with three mango varieties, which had differences in weight and shape showed that heavier mango varieties needed longer exposure time to reach 45.5 °C of innermost pulp temperature and provided a trend of higher mortality of *B. dorsalis* eggs (Table 1). Our results fit well with those of Yoshinaga *et al.*, (2009) who conducted vapor heat treatment with 'Carabao' mangoes classified into three types (S, M and L) according to weight.

However, the corrected mortality of *B. dorsalis* in 'Nam Doc Mai' was 98.8% but that of 'Tommy Atkins' was 100% although the temperature of the innermost pulp of 'Tommy Atkins' was lower than 'Nam Doc Mai' (Table 1 and 2, experimental section B of replication 1 and 2). We suspected that the surplus heat after vapor heat treatment affected the mortality. Fig. 2. shows that the temperatures of innermost pulp and outermost pulp of each variety after the innermost pulp reached 45.5°C. 'Tommy Atkins', which is heavier and oval in shape, increased after the treatment and decreased most slowly among the three varieties.

When these three varieties were treated for the same exposure time and removed from the chamber, the temperature of the 'Tommy Atkins', which was heavy and oval, was the lowest. However, the temperature of 'Tommy Atkins' increased and it is suggested that the surplus heat of 'Tommy Atkins' was higher than 'Nam Doc Mai' and the corrected mortality became higher (Table 1 and 2, replication 1). In the replication 2, the difference in mortality between 'Nam Doc Mai' and 'Tommy Atkins' became narrow due to reduction of difference in weight and surplus heat. We thought that the shape of the fruit affected the surplus heat as well as the weight of the fruit.

We used two mango varieties, 'Kent' and 'Nam Doc Mai' which were almost the same in weight and different in shape, for vapor heat treatment. The results showed that run-up time to 45.0°C for 'Kent', which was oval/round shape, was longer than for 'Nam Doc Mai' and the decrease in temperature for 'Kent' after treatment took longer than for 'Nam Doc Mai' (Fig. 3 and 4).

Fields (1992) reported that development of stored-product insects stopped at 35 °C and above, and they died in hours at 45 °C. So we suspected that temperature over 40.0 °C had some influence on mortality of *B. dorsalis* inoculated under the skin of fruit.

During vapor heat treatment in replication 1 of test 2), 'Nam Doc Mai' was exposed to vapor heat at 40.0 °C and above for 64min. inside the chamber and the outermost pulp of 'Nam Doc Mai' kept over 40.0°C for 43 min.

On the other hand, 'Kent' was exposed to vapor heat at 40.0 °C and above for 71 min. and the outermost pulp of 'Kent' kept over 40.0 °C for 48 min. 'Kent' was kept over 40.0 °C longer than 'Nam Doc Mai' in replication 2 as well as replication 1. We thought that the difference in exposure time and exposure to higher temperature caused the difference in mortality (Table 3).

'Kent' required more time to reach 45.0 °C than 'Nam Doc Mai' and the mortality of 'Kent' was over 90% when it reached 45.0 °C and the surplus heat contributed to the mortality a little. On the other hand, the mortality of 'Nam Doc Mai' was dependent on the presence or absence of surplus heat and it was thought that the surplus heat after treatment quite affected the mortality.

Kawai *et al.*, (2012) and Shellie *et al.*, (2002) reported that the cooling method (air cooling or hydro-cooling) affected mortality after vapor heat treatment and hot water treatment, respectively. Both results showed that surplus heat after treatment increased the mortality and fit well with our results for 'Kent' and 'Nam Doc Mai'.

There was no explanation of the cooling method in USDA hot water treatment (1998, T102-a), but it does state that dip time is valid if the fruit is not hydrocooled within 30 min. of removal from the hot water immersion tank (2013, T102-a).

It was thought that surplus heat after heat treatment affected the mortality and it was one of the important factors in quarantine treatment.

Our results from two experiments indicated that the difference in fruit shape affected the increase and decrease of fruit temperature in addition to the fruit weight, and that fruit shape affected mortality.

Factors other than fruit weight and shape might affect increase and decrease of temperature of innermost pulp. Yoshinaga *et al.* (2009) reported that the temperature of innermost pulp of 'Tommy Atkins' (410.5-518.5g) took about 2 min. longer to reach 45.0°C than that of 'Kensington Pride' (413.2-514.5g) and the mortality of 'Tommy Atkins' was higher. They also reported that there were significant differences in firmness, sugar content, pH and thickness between these two varieties. Among the 4 factors, thickness may have provided the most significant difference in temperature increase/decrease of innermost pulp. Then, the thickness could

be considered one of the factors which constituted the fruit shape. We would like to refer to our unpublished data concerning the other 3 factors.

The results of the vapor heat treatment test conducted with 'Kent' differing in firmness (hard/soft), showed no significant difference in the rate of increase of innermost pulp temperature and mortality of *B. dorsalis* eggs (Omura *et al.*, unpublished data).

Furthermore, the following two test results indicated that sugar content and pH had little effect on the mortality in vapor heat treatment (Dohino *et al.*, unpublished data);

1) Eggs of *B. dorsalis* were subjected to vapor heat treatment and then grown in 3 mango varieties or an artificial diet.

2) Third instar larvae of *B. dorsalis* grown in 3 mango varieties or fed an artificial diet were subjected to vapor heat treatment. In these tests, there were significant differences in sugar content and pH among mango varieties and/or artificial diet. However, the results showed no significant difference in mortality of survivors. Further studies will be needed to determine the effect of fruit weight and shape on heat treatment in plant quarantine.

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和 文 摘 要

蒸熱処理における果実形状の違いによる殺虫効果の比較（英文）

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重量及び形状の異なる3品種のマンゴウ生果実、トミートキンス種(卵型、431.5-575.7g)、ナンドクマイ種(扁平、379.1-444.5g)、カラバオ種(扁平、217.8-241.9g)にミカンコミバエの卵を寄生させて蒸熱処理を行い、品種と殺虫率の関係を調査した。各品種の果実中心温度が45.5℃に達するまで同時に蒸熱処理を行い、殺虫率及び果実中心温度の比較を行った。果実重量が大きい品種ほど45.5℃到達までの時間が長くなり、殺虫率が高まることが確認できた。しかし、ナンドクマイ種が45.5℃に達したとき、それよりも果実重量の大きいトミートキンス種では42.8℃-44.2℃と低かったにもかかわらず、殺虫率はナンドクマイ種よりも高かった。等しい蒸熱処理時間でも、重量が大きく卵型のトミートキンスは処理終了後に果肉部が

保有する余熱がナンドクマイよりも大きいため、殺虫率が高くなったと推察された。また処理後の放熱については果実形状が関係すると考えられたが、果実重量が影響した可能性も否定できなかった。

そこで、重量が同じで形状の異なるマンゴウ生果実2品種、ナンドクマイ種(扁平、429.1-480.9g)、及びケント種(卵型、427.8-480.9g)を使い果実中心温度が45.0℃になるまで蒸熱処理を行った。ケント種のほうが、ナンドクマイ種に比べ温度上昇が遅く、また、処理後の温度低下も遅く、殺虫率が高いことが確認できた。このことから、果実形状は殺虫率に影響する要因となることが示唆された。

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