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Original Paper

Factors affecting on mortality of *Bactrocera dorsalis* (Diptera: Tephritidae) eggs in different mango varieties subjected to vapor heat treatment

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Abstract: Vapor heat treatment schedules are often regulated by innermost pulp temperature and holding time. The purpose of this study was to research the essential factors required to keep phytosanitary safety when plural mango varieties are treated with one treatment schedule. Heavy and light mango varieties were subjected to respective vapor heat treatments with different chamber temperature which were controlled so that both mango varieties reached the same innermost pulp temperature at the same exposure time. The results showed that the chamber temperature affected the mortality of *Bactrocera dorsalis* (Hendel) eggs. Furthermore, heavy and light mango varieties with *B. dorsalis* eggs were subjected to vapor heat treatment at the same time. The light one did not show 100% mortality at the same innermost pulp temperature and same holding time as the heavy one showed 100% mortality, because the light one reached the target temperature sooner than the heavy one. However, the light one showed 100% mortality at the same total treatment time as the heavy one. It is concluded that for phytosanitary safety, the vapor heat treatment schedule should keep not only the innermost pulp temperature and holding time but also the chamber temperature and total treatment time.

Key words: vapor heat treatment, mango, heating rate, total treatment time, *Bactrocera dorsalis*

Introduction

Vapor heat treatment (VHT) is one of the physical treatments against fruit flies as a plant quarantine measure, and is applied to post-harvest fruits and vegetables in particular. The treatment is commonly used for tropical fruits such as dragon fruits, papaya and mangoes. The VHT schedule is mainly regulated by innermost pulp temperature and holding time (Armstrong and Mangan, 2007). Some VHT schedules are regulated by the heating rate of innermost pulp temperature in addition to target temperature and holding time (USDA Treatment Manual T106-d-1 (mango from the Philippines), T106-e (yellow pitaya from Colombia) (USDA-APHIS 2016).

The purpose of this study was to investigate the essential factors when plural mango varieties were treated with one VHT schedule while retaining phytosanitary safety. Effects of chamber temperature, holding time and total treatment time on vapor heat mortality of *Bactrocera dorsalis* eggs were investigated.

Materials and Methods

1. Test insects

A laboratory colony of *B. dorsalis* maintained at the Research Division of the Yokohama Plant Protection Station was used for Test 1 and Test 2. The colony was originally from Thailand in 2005 (Permit No. 17Y566). Flies were kept at 26 ± 1℃, 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 (8 cm in diameter, 13 cm in height) with small oviposition holes into the adult cage containing about 2,000 flies for one hour. The inner surface of the receptacle was moistened with orange juice. Eggs (24 hours old) were subjected to VHT.

2. Infesting test fruit

Test 1) Effects of chamber temperature

The 'Carabao' variety of mangoes from the Philippines (flat/elongated shape, 239.3–246.4 g) and 'Kent' variety of mangoes from Mexico (oval/round shape, 475.2–493.0 g) were subjected to

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VHT. These varieties are different in weight and shape. The weight and shape of these fruits are shown in **Table 1**.

These fruits were kept at 26 ± 1℃, 65 ± 10% RH for a day before inoculation. One hundred eggs were put on black filter paper and counted under a microscope using a brush. One flap was cut in the skin of a mango and 100 eggs were inoculated into the fruit. The black filter paper was removed from the fruit and the flap was lowered and covered with surgical tape to prevent desiccation of eggs. Infested fruits were stored at 26 ± 1℃ until VHT started.

Test 2) Effects of holding time and total treatment time

The 'Nam Doc Mai' variety of mangoes from Thailand (flat/elongated shape, 275.9–321.6 g) and 'Kent' from Mexico (oval/round shape, 445.1–511.9 g) were used. The shape and weight of these fruits are shown in **Table 2**. The infested fruits were prepared in the same manner as Test 1 except 200 eggs were inoculated to each fruit (100 eggs × two flaps).

3. Vapor heat treatment and assessment

The definitions of terms in this report are as follows.

Target temperature: The innermost pulp temperature at which the holding time is started to count

Approach time: The time required for the innermost pulp temperature to reach the target temperature

Holding time: The time for which the innermost pulp temperature was kept at the target temperature or more

Total treatment time: The time from the beginning to the end of VHT

Chamber temperature: The chamber temperature transition

Table 1 Average weight of 'Carabao' and 'Kent' subjected to vapor heat treatment (Fix and Program mode)

Fix mode				
Variety	Weight (g)*	Shape (sensor fruits only)		
		Length (mm)*	Width (mm)*	Thickness (mm)*
'Carabao'	243.1 ± 3.6	112.8 ± 5.5	66.0 ± 4.6	55.6 ± 4.3
'Kent'	484.4 ± 8.2	113.4 ± 2.1	95.9 ± 1.6	84.1 ± 1.3
Program mode				
Variety [Program No.]	Weight (g)*	Shape (sensor fruits only)		
		Length (mm)*	Width (mm)*	Thickness (mm)*
'Carabao' [PRG1]	243.4 ± 5.7	112.7 ± 2.9	70.0 ± 0.8	58.3 ± 1.9
'Carabao' [PRG2]	245.8 ± 3.1	114.0 ± 4.1	68.0 ± 1.4	57.7 ± 0.5
'Carabao' [PRG3]	244.4 ± 2.9	116.7 ± 5.0	67.7 ± 0.5	56.7 ± 1.9

*Average ± standard deviation

Length: The length of the fruit was taken along the axis through the stalk attachment and the furthest point.
Width: The width was taken at the broadest part perpendicular to the length.
Thickness: The thickness was taken at the longest part from the plane that contains Length and Width.

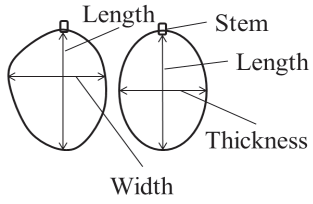

Table 2 Weight and shape of 'Nam Doc Mai' and 'Kent'

Variety	Weight (g)*	Shape (sensor fruits only)		
		Length (mm)*	Width (mm)*	Thickness (mm)*
'Nam Doc Mai'	303.7 ± 17.9	141.3 ± 8.2	70.1 ± 4.4	61.0 ± 6.4
'Kent'	475.1 ± 11.6	117.2 ± 2.2	95.2 ± 4.9	80.9 ± 1.6

Weight of treated fruits and shape of sensor fruits are measured.
*Average ± standard deviation

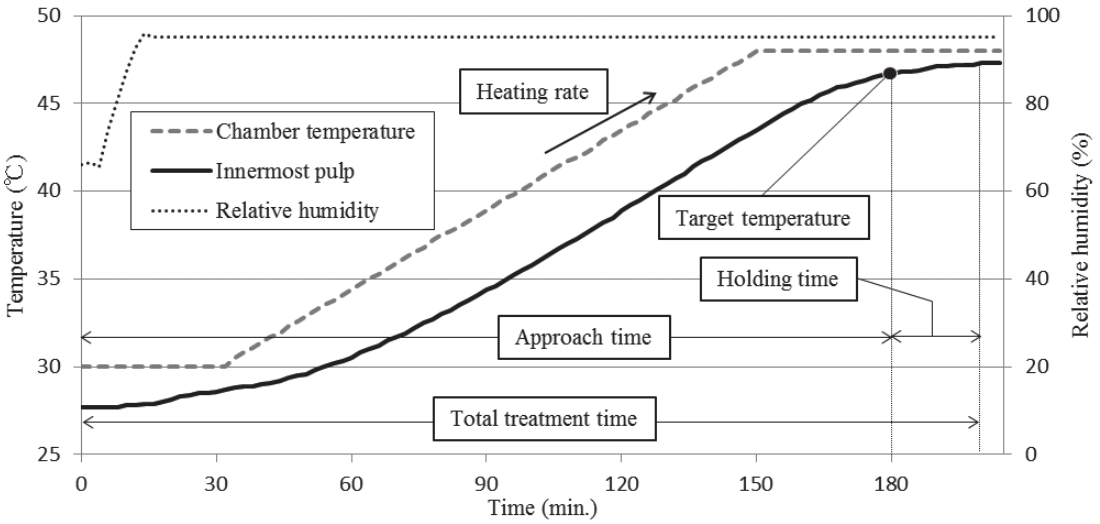

Fig. 1 An example of the temperature transition of chamber and innermost pulp during VHT.

during VHT, including the heating rate (the increase of chamber temperature for a certain time)

Examples of innermost pulp temperature and chamber temperature during VHT are shown in **Fig. 1.**

Test 1) Effects of chamber temperature

At first, 'Carabao' and 'Kent' were subjected to the same VHT to investigate the difference of approach time and mortality at the respective target temperature ('Carabao': 44, 45, 46, 47 and 48℃, 'Kent': 42, 43, 44, 45 and 46℃).

The following devices were used for this test: VHT machine (FTH Co., Ltd., VHC-10TM), temperature recorder (Chino Co., Ltd., LE5100), sensors (Chino Co., Ltd., Pt100), standard thermometer (TOA Keiki Mfg Co., Ltd. Model no. 1) and water bath (Yamato Scientific Co., Ltd., BA500).

The VHT program was set so that the chamber temperature reached 49℃ promptly and was kept at 49℃ and relative humidity was set at 95% (Fix mode).

The temperatures of the innermost and outermost pulp of each variety were recorded every 30 seconds with the temperature recorder and sensors. These sensors were calibrated at 45.0℃ with a standard thermometer and water bath before VHT.

Five infested fruits of each mango variety were unloaded from the chamber when two of the three sensors which were inserted into the innermost pulp of each variety reached the target temperature. Five control fruits of each mango variety were kept at 26 ± 1℃ during VHT.

After VHT, treated fruits were kept at 26 ± 1℃ and 65 ± 10% RH for 5 days and control fruits were kept at the same conditions for 4 days. The fruit were dissected and the number of larvae was counted. The corrected mortality was calculated by Abbott (1925). Tests were replicated three times.

Next, VHT with program mode was conducted so that the innermost pulp temperature transition of 'Carabao' corresponded to that of 'Kent' treated in Fix mode (49℃, 95% RH) as described above.

The details of the program are shown below.

PRG1: Chamber temperature was set to reach 49℃ taking 60 min., then kept at 49℃.

PRG2: Chamber temperature was set to reach 40℃ taking 30 min., then set to reach 49℃ taking 40 min., then kept at 49℃.

PRG3: Chamber temperature was set to reach 40℃ taking 20 min., then set to reach 49℃ taking 50 min., then kept at 49℃.

Five infested fruits were unloaded from the chamber when two of three sensors measuring the innermost pulp temperature (sensor fruits) reached the target temperature (42, 43, 44, 45, 46, 47 and 48℃).

The ways of measuring temperature, handling of fruits after VHT and assessing mortality were the same as described above. Tests were replicated three times.

Test 2) Effects of holding time and total treatment time

The VHT machine and experimental devices were the same as Test 1.

The VHT program was set so that the chamber temperature reached 46.5℃ promptly, and then was kept at 46.5℃ and relative humidity was kept at 95% (Fix mode).

Three sensors were inserted into each variety ('Nam Doc Mai' and 'Kent') of fruit to measure the innermost pulp temperature during VHT and the moment two of them reached 45.5℃ was considered to be the beginning of the holding time of each variety. Test fruits were unloaded from the VHT chamber just after holding times of 0, 10, 20, 30 and 40 min. Three infested fruits were included in each test plot.

The innermost pulp temperature of 'Nam Doc Mai' rose more quickly than that of 'Kent' and the approach time of 'Nam Doc

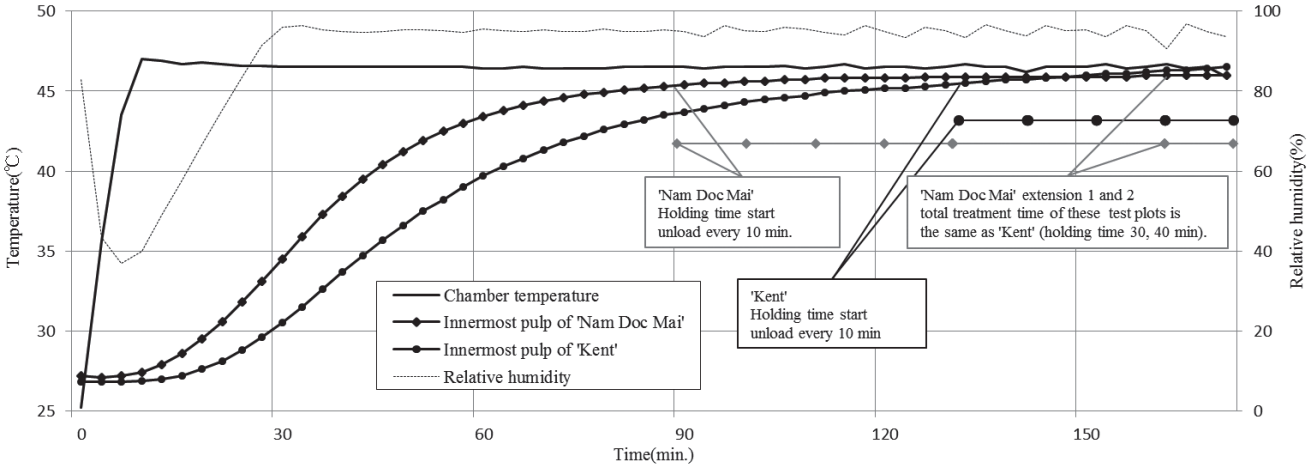

Fig. 2 Innermost pulp temperature transitions of 'Nam Doc Mai' and 'Kent'.

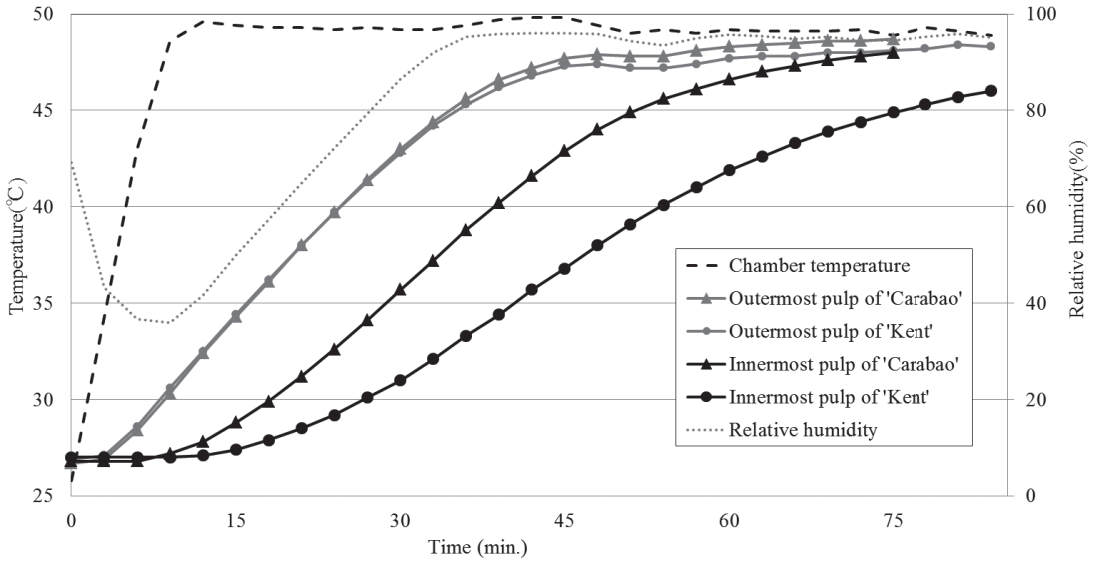

Fig. 3 Temperature transition of chamber temperature and two mango varieties 'Carabao' and 'Kent' during vapor heat treatment (Fix mode).

Mai' was shorter than that of 'Kent'. Therefore, the total treatment time of 'Nam Doc Mai' became shorter than that of 'Kent' at the same holding time plot.

To research the mortalities in 'Kent' and 'Nam Doc Mai' at the same total treatment time, two test plots (extension 1 and 2) for 'Nam Doc Mai' were prepared. The total treatment times in extension 1 and 2 of 'Nam Doc Mai' were the same as those of the 30 min and 40 min holding time plots for 'Kent' respectively (**Fig. 2**).

The way of storing treated and control fruits after VHT and mortality assessment was the same as test 1. This test was replicated three times.

Results

Test 1) Effects of chamber temperature

The temperature transition of 'Kent' and 'Carabao' during VHT (Fix mode 49℃, 95% RH) is shown in **Fig. 3**. Comparing the approach time and corrected mortality at the same target temperature, the approach time of 'Kent' was longer and corrected mortality tended to be higher than 'Carabao' (**Table 3**)

VHT for 'Carabao' with program mode was conducted so that the temperature transition of 'Carabao' corresponded to 'Kent' with Fix mode. This test was replicated three times.

The program was arranged for each replication and some of the test sections corresponded to Kent's approach time (**Fig. 4**).

The approach time of 'Carabao' at the target temperatures of 43, 44 and 45℃ in PRG1 and PRG3 corresponded to 'Kent' at the same target temperature in Fix mode. The approach time of 'Carabao' at the target temperature of 46℃ in PRG2 and at 42℃ in PRG3 corresponded to 'Kent' at the same target temperature in Fix mode. The corrected mortality of 'Carabao' was lower than that of 'Kent' at the same target temperature in the corresponding test plots (**Table 4**, boldface).

Table 3 Number of survivors and corrected mortalityof *Bactrocera dorsalis* eggs in 'Carabao' and 'Kent' after vapor heat treatment (chamber temp. 49° C, 95% RH, Fix mode)

Innermost pulp temperature	'Carabao'				'Kent'			
	Approach time (min.)*	Number of test insects	Number of survivors	Corrected mortality (%)	Approach time (min.)*	Number of test insects	Number of survivors	Corrected mortality (%)
42° C	-	-	-	-	58.3 (56–60)	1,500	414	68.4
43° C	-	-	-	-	63.0 (61–65)	1,500	243	81.5
44° C	46.7 (46–48)	1,500	757	42.0	67.3 (65–69)	1,500	37	97.2
45° C	50.3 (50–51)	1,500	520	60.2	74.0 (72–76)	1,500	6	99.5
46° C	55.3 (55–56)	1,500	127	90.3	82.0 (80–84)	1,500	0	100
47° C	62.0 (61–63)	1,500	0	100	-	-	-	-
48° C	73.3 (72–74)	1,500	0	100	-	-	-	-
Control	-	1,500	1,305	-	-	1,500	1,310	-

*Figures in the upper row of approach time indicate the average time of three replicates and figures in the lower row of approach time indicate the minimum and maximum times of three replicates.

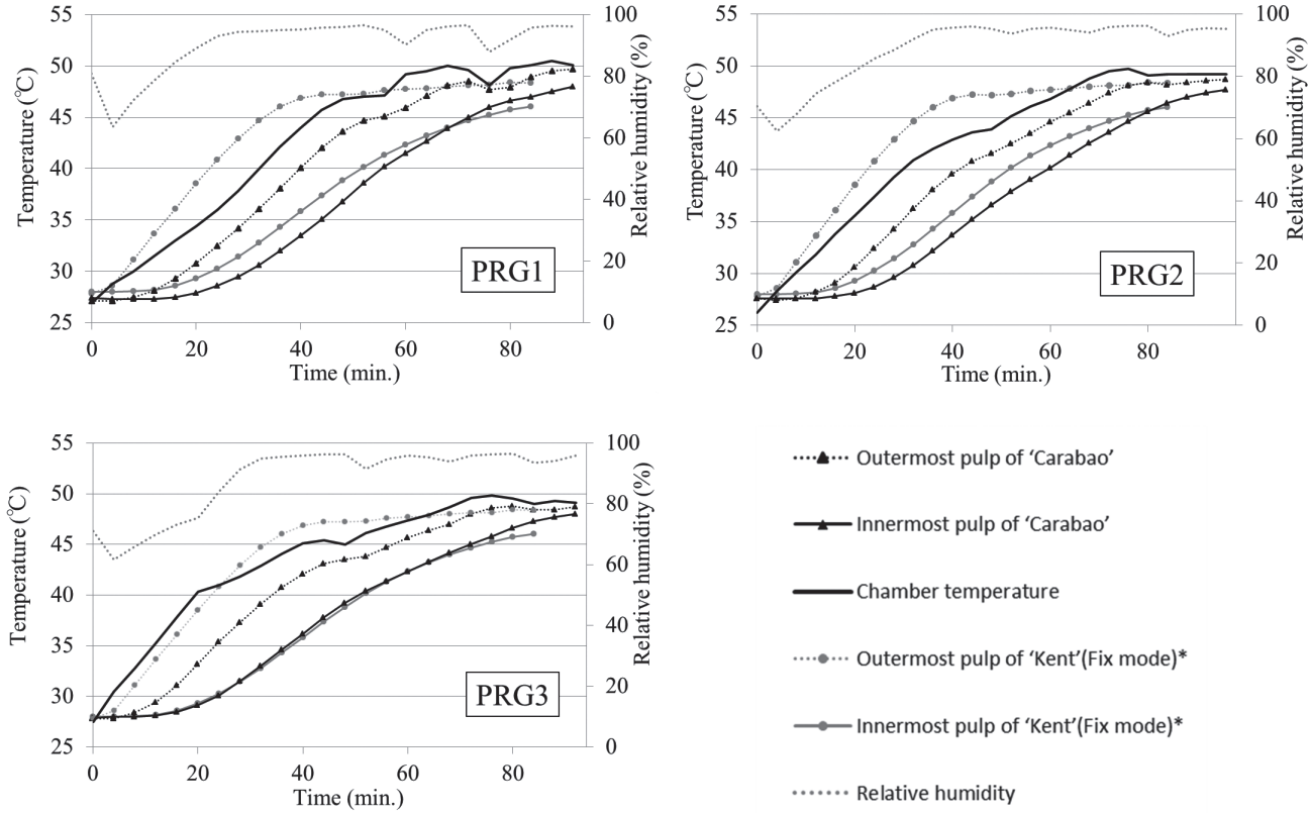

Fig. 4 Innermost and outermost pulp temperature transition of 'Carabao' during vapor heat treatment with Program mode (PRG1-3). *The innermost and outermost pulp temperature transitions of 'Kent' during vapor heat treatment (Fix mode) are shown in **Fig. 3** for comparison. These two varieties are not subjected to vapor heat treatment at the same time.

Table 4 Number of survivors and corrected mortality of *Bactrocera dorsalis* eggs in 'Carabao' treated after vapor heat treatment (Program mode:PRG1-3)

Innermost pulp temperature	'Carabao'									'Kent'[*1]	
	PRG1			PRG2			PRG3			Fix mode	
	Approach time (min)	Number of survivors	Corrected mortality (%)	Approach time (min)	Number of survivors	Corrected mortality (%)	Approach time (min)	Number of survivors	Corrected mortality (%)	Approach time (min)	Corrected mortality (%)
42° C	61	384	12.9	66	402	8.2	**58**	**417**	**1.9**	58.3	68.4
43° C	**65**	**323**	**26.8**	69	324	26.0	**63**	**390**	**8.2**	63.0	81.5
44° C	**68**	**279**	**36.7**	73	314	28.3	**67**	**404**	**4.9**	67.3	97.2
45° C	**72**	**140**	**68.3**	77	267	39.0	**72**	**302**	**28.9**	74.0	99.5
46° C	76	141	68.0	**81**	**97**	**77.9**	77	140	67.1	82.0	100
47° C	83	0	100	88	14	96.8	82	6	98.6	-	-
48° C	91	0	100	99	0	100	92	0	100	-	-
Control	-	441	-	-	438	-	-	425	-	-	-

Figures in this table are the total amount of five infested fruits.
[*1]Approach time and corrected mortality of 'Kent' treated with Fix mode are shown for comparison. These figures are the same as in **Table 3**.
Boldface indicates approach time coincidence[*2] with 'Kent' in **Table 3**.
[*2]Coincidence indicates that the approach times of 'Carabao' with the three programs fall within the range between the minimum and maximum approach times of 'Kent' with Fix mode vapor heat treatment (three replicates).

Table 5 Number of survivors and corrected mortalityof *Bactrocera dorsalis* eggs in 'Nam Doc Mai' and 'Kent' at 45.5° C in each holding time

Holding time	'Nam Doc Mai'				'Kent'			
	Total treatment time (min.)[*]	Number of test insects	Number of survivors	Corrected mortality (%)	Total treatment time (min.)[*]	Number of test insects	Number of survivors	Corrected mortality (%)
0 min.	90.7 (90–92)	1,800	1,205	21.1	129.3 (128–131)	1,800	675	56.8
10 min.	100.7 (100–102)	1,800	902	41.0	139.3 (138–141)	1,800	249	84.1
20 min.	110.7 (110–112)	1,800	388	74.6	149.3 (148–151)	1,800	82	94.8
30 min.	120.7 (120–122)	1,800	132	91.4	159.3 (158–161)	1,800	3	99.8
40 min.	130.7 (130–132)	1,800	12	99.2	169.3 (168–171)	1,800	0	100
Extension 1	159.3 (158–161)	1,800	0	100	–	–	–	–
Extension 2	169.3 (168–171)	1,800	0	100	–	–	–	–
Control	–	1,800	1,528	–	–	1,800	1,564	–

Figures in this table are the total amount of three replicates.
[*] Figures in the upper row of Total treatment time indicate the average time of three replicates and figures in the lower row of total treatment time indicate the minimum and maximum times of three replicates.

Test 2) Effects of holding time and total treatment time

The approach time and corrected mortality of *B. dorsalis* eggs are shown in **Table 5**.

'Nam Doc Mai' took 90–92 min. and 'Kent' took 128–131 min. to reach 45.5℃. The corrected mortality was 100% in 'Kent' with the 40 min. holding time section but there were some survivors in the same section (holding time: 40 min.) of 'Nam Doc Mai'.

Discussion

Test 1) Effects of chamber temperature

The results of Fix-mode VHT with 'Carabao' and 'Kent' showed that the corrected mortality of 'Kent' was higher than that of 'Carabao' at the same target temperature (44, 45 and 46℃) (**Table 3**). The weight of 'Kent' was heavier than that of 'Carabao' and it

was thought that a heavier weight led to a longer approach time. This result corresponds to those of Yamamoto et al. (2011) using 'Irwin' and 'Keitt' varieties of mangoes and Yoshinaga et al. (2009) using 'Carabao' of different sizes.

On the other hand, the corrected mortality was higher in 'Kent' treated with Fix mode than 'Carabao' treated with slow heating rate Program mode at the same target temperature, even though both of their approach times were almost the same. The reason for this may be the difference of chamber temperature. The outermost pulp temperature was higher in 'Kent' than in 'Carabao' at the same target temperature because 'Carabao' was lighter than 'Kent' and the heating rate of chamber temperature had to be set slower for 'Carabao' than for 'Kent'. Therefore, eggs in 'Kent' were exposed to a higher temperature for a longer time than in 'Carabao'.

The results of this test showed that the mortality was different between two mango varieties depending on chamber temperature transition even under the same approach time and the same target temperature.

VHT schedules are often regulated by two factors, target temperature and holding time. Some VHT schedules are regulated by the heating rate of innermost pulp temperature in addition to target temperature and holding time (USDA Treatment Manual T106-d-1 (mango from the Philippines), T106-e (yellow pitaya from Colombia) (USDA-APHIS 2016).

The results of the present study indicate that chamber temperature is also important.

Test 2) Effects of holding time and total treatment time

Although 'Nam Doc Mai' and 'Kent' were treated with the same conditions of VHT (target temperature: 45.5℃, holding time: 40 min), the corrected mortality was 100% in 'Kent' but was not 100% in 'Nam Doc Mai'.

This result indicated that a treatment schedule may not be able to kill *B. dorsalis* eggs at a sufficient confidence level even when the target temperature and holding time are regulated, when the treatment schedule for a heavy mango variety is applied to light mango varieties.

On the other hand, the corrected mortality of 'Nam Doc Mai' was 100% when the total treatment time of extension 1 and 2 was the same as the holding time plot of 30 min. and 40 min. of 'Kent'.

This result in Test 2 indicates that the total treatment time should be added to the treatment schedule consisting of target temperature and holding time when the treatment schedule developed with a heavy mango variety is applied to light mango varieties.

The result of Test 1 indicates that chamber temperature affects fruit fly mortality. Therefore, considering the results of Test 1 and Test 2, the four factors of fruit core temperature, holding time, chamber temperature and total treatment time should be regulated to ensure mortality at a sufficient confidence level when the treatment schedule developed with a heavy mango variety is applied to light mango varieties.

Considering operation in commercial treatment, it is difficult to accomplish a quick heating rate in a large-scale commercial VHT machine loaded with many fruits. Further research on the relationship between chamber temperature and mortality is needed.

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和文摘要

蒸熱処理におけるマンゴウ果実中のミカンコミバエ卵 *Bactrocera dorsalis* の殺虫に影響を与える要因（英文）

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重量及び形状の異なるマンゴウを同一の目標果実中心温度及びホールディングタイムで蒸熱処理した場合に、殺虫率に影響を与える要因を明らかにするため以下の調査を行った。

試験 1. 目標果実中心温度到達までの所要時間が等しくなるよう、庫内温度を調整して蒸熱処理をした場合の殺虫率の調査。供試果実はケント種（重量約 480g、卵形）及びカラバオ種（重量約 240g、扁平）。

試験 2. 庫内温度は一定で、ホールディングタイムが異なる場合の加熱総処理時間及び殺虫率の調査。供試果実はケント種（重量約 480g、卵形）及びナンドクマイ種（重量約 300g、扁平）。ナンドクマイ種の方が果実中心温度の上昇が速く、加熱総処理時間が短くなるため、ケント種のホールディングタイム 40 分処理が終了するまで、ナンドクマイ種のホールディングタイムを延長した処理区を設定した。

試験 1 の結果、カラバオ種はケント種よりも殺虫率が低い傾向が見られた。このため、庫内温度は殺虫率に影響することが示唆された。

試験 2 の結果、ホールディングタイムが同じ 40 分の試験区であっても、加熱総処理時間の短いナンドクマイ種では生存虫が確認されたが、ケント種では生存虫は確認されなかった。一方、ナンドクマイ種のホールディングタイムを延長した処理区では生存虫は確認されなった。

以上の結果から、庫内温度、加熱総処理時間は殺虫効果に影響することが明らかとなった。このため、複数品種のマンゴウを同一の消毒基準で処理する場合には果実中心温度、ホールディングタイムに加え、庫内温度、加熱総処理時間を規定すれば 100% 殺虫効果が得られる可能性が示唆された。

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