



No. AC 0913/2578

Department of Agriculture  
Chatuchak Bangkok 10900  
THAILAND

23 May B.E. 2562 (2019)

Plant Health Contact Point of the European Commission  
Directorate General Health and Food Safety,  
Unit D2-Multilateral International Relations,  
Rue Froissart 101, B-1049 Brussels.

Dear Sir,

Reference is made to the Notification G/SPS/N/EU/290 on the Commission Implementing Decision amending Annexes I to V to Council Directive 2000/29/EC on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community which will be effective in September 2019.

Two commodities i.e. pummelo (*Citrus maxima*) and mango (*Mangifera indica*) which are currently exported from Thailand may be affected by the above mentioned decision. To prevent trade disruption, Department of Agriculture (DOA) would like to officially propose the following measures to mitigate the risk posed by fruit flies.

For pummelo, experiments were conducted in Thailand to determine the host status of pummelo of 'Thong Dee' cultivar to the oriental fruit fly (OFF), *Bactrocera dorsalis*. Data collected from field studies indicated that commercial grade pummelo is not a suitable field host of the OFF. Natural infestation was not found. In addition, laboratory forced-infestations were carried out by exposing intact and punctured pummelos to gravid female for oviposition. It was found that test fruits with punctures through the flesh were susceptible to fruit fly infestation. However, the data demonstrated that the intact and punctured fruits with 2 mm. depth were resistant to fruit fly infestation. No fruit fly infestation was found on all test fruits. Based on these results, pummelo grown commercially in Thailand is a conditional non-host to the OFF. We attached, herewith, the research study of title "Nonhost Status of Commercial Pummelo to the Oriental Fruit Fly (Diptera : Tephritidae) in Thailand" for your consideration (Attachment 1). Please kindly be informed that this research was

/ submitted to...

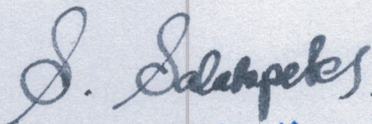


submitted to the Australian Department of Agriculture, Fisheries and Forestry (DAFF) in May 2007. DAFF already accepted that pummelo is a conditional non-host to the OFF. Currently, no phytosanitary measure against the OFF is required for the exportation of pummelo from Thailand to Australia.

For mango, the hot water immersion was developed as a quarantine treatment for mangoes. We would like to proposed hot water immersion at the innermost fruit pulp temperature at 46 °C for 10 minutes as a post-harvest disinfection treatment to disinfest mangoes of eggs and larvae of fruit flies. The research paper of title "Hot Water Immersion Treatment of Nam Dorkmai Mango Infested with Oriental Fruit Fly, *Bactrocera dorsalis* (Hendel) for Export" (Attachment 2) is attached herewith for your consideration.

We would very much appreciate it if you could kindly consider our proposals and let us know whether or not the proposed phytosanitary measures are acceptable at your earliest convenience.

Yours sincerely,



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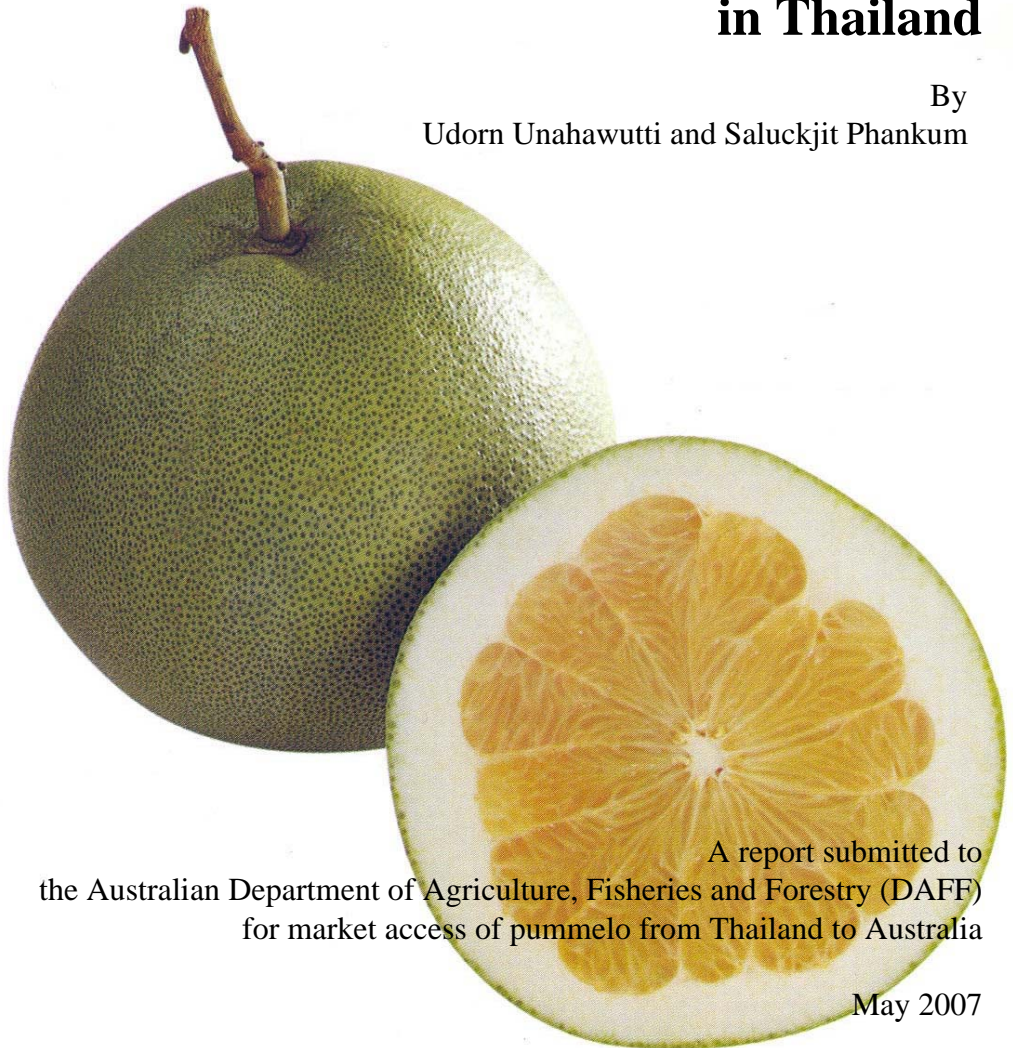
Department of Agriculture  
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Technical Document

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By

Udorn Unahawutti and Saluckjit Phankum



A report submitted to  
the Australian Department of Agriculture, Fisheries and Forestry (DAFF)  
for market access of pummelo from Thailand to Australia

May 2007



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A report submitted to  
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for market access of mangosteens from Thailand to Australia

May 2007

# Nonhost Status of Commercial Pummelo to the Oriental Fruit Fly (Diptera : Tephritidae) in Thailand

By

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## ABSTRACT

Pummelo, *Citrus maxima* (Burman) Merr., of 'Thong Dee' cultivar was investigated for its possibility to be infested by the oriental fruit fly (OFF), *Bactrocera dorsalis* (Hendel). Experiments were conducted in the field and laboratory. Field studies were carried out by randomly sampling of fruits during harvesting period from commercial orchards in major producing areas to determine natural infestation. Artificial forced-infestation studies were done in laboratory to show nonhost status of pummelo to the OFF. No natural infestation was observed on the samples. Laboratory studies revealed that pummelo was resistant to fruit fly infestation except under certain conditions. After laboratory cage infestation, eggs were found to be deposited only in the toxic flavedo (exocarp) and nontoxic albedo (mesocarp) regions. However, the formation of cavity with hardening of the outer periphery was sealed off eggs causing eggs and larvae from suffocation and starvation. In addition, resistance of pummelo to attack by the OFF was attributed to toxic oil in the flavedo and to the thick and tough albedo. It could be said that the only essential condition for the OFF to successfully infest pummelo was that the peel surface was damaged so deep enough to allow female flies to insert the ovipositor and forced their eggs into the pulp. Based on these results, pummelo grown commercially in Thailand should not be considered as a host of the OFF.

## INTRODUCTION

The origin of the pummelo, *Citrus maxima* (Burman) Merr., is uncertain. There is little doubt that the species is indigenous in Malaysia. It has spread to Indo-China, southern China and the southernmost part of Japan and westwards to India, the Mediterranean and tropical America. However, it remains a

fruit of the Orient; neither in India nor further west has it become popular. The best match of cultivars, environmental niches and growing skills appears to be found in Thailand (Anonymous, 2005). Pummelo is a medium-sized tree with very large leaves, flowers and fruits. The enormous, usually thick-skinned fruits (diameter up to 30 cm or more) are borne on drooping branches. The fruit fresh varies from yellow to red. Pummelo is often confused with grapefruit but the juice sacs or vesicles of pummelo are not fused together as in all other *Citrus* species. The leathery membrane of the fruit segment is easily peeled away and the flesh removed and eaten. Pummelo fruit and its components are showed in Figure 1

Pummelo is one of the most economic horticultural fruits of Thailand. The principal production areas are in the central, southern and northern parts of the country (Figure 2). In 2003, about 17,000 hectares were devoted to pummelo of 'Thoang Dee' cultivar cultivation with a total production of about 109,000 tones (Figure 3). While most of the pummelo produced are consumed locally, Thailand has been exporting pummelo to several overseas markets. The major markets included China, Hong Kong, Malaysia, Singapore, Brunei, European countries and Middle East countries.

Many fruit flies species have been reported to attack fruits of *Citrus* spp. However, research studies in many countries revealed that fruits of certain *Citrus* spp. and under certain conditions are not considered as host of fruit flies. Spitler *et al.* (1984) found that 'Eureka' and Lisbon lemons were virtually immune to attack by the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), and they concluded that lemons should not be considered a host for this fruit fly species. Hennessey *et al.* (1992) found no Caribbean fruit fly, *Anastrepha suspensa* (Loew), infestations in more than 100,000 'Tahiti' limes (*Citrus* x 'Tahiti') collected from 184 different groves on 60 harvest dates over a year-long period. These data led Hennessey *et al.* (1992) to conclude that this lime cultivar should be exempted from post-harvest disinfestation requirements for the Caribbean fruit fly. Aluja *et al.* (2003) studied nonhost status of *Citrus sinensis* Cultivar Valencia and *C. paradise* Cultivar Ruby Red to Mexican *Anastrepha fraterculus* (Wiedemann). The results of laboratory and field behavioral studies, added to consistently negative infestation reports by Mexican *A. fraterculus* of citrus collected in the field and recent morphological and molecular evidence proofed that Mexican *A. fraterculus* not only differ from South American *A. fraterculus* in their ability to infest citrus, but most likely also represent a different taxonomic entity. They suggested that *A. fraterculus* should not be regarded as a pest of citrus in Mexico and 'Valencia' oranges and 'Ruby Red' grapefruit should be removed from host listings referring to Mexican *A. fraterculus*.

In addition to citrus, mangosteen was one of target fruits for research investigation. Mangosteen has been reported as a host of several fruit fly species.

Recent studies in Thailand indicated that commercial mangosteen was resistant to the infestation of the oriental fruit fly (OFF) [*Bactrocera dorsalis* (Hendel)] (Unahawutti and Oongthonglang, 2003) and carambola fruit fly (*Bactrocera carambolae* Drew and Handcock) (Unahawutti *et al.*, 2004). Laboratory forced-infestation studies showed that the female flies could not be forced to oviposit through the undamaged mangosteen surface. The only essential condition for the OFF to successfully infest mangosteen was that the rind surface was damaged so deep enough to allow female flies to insert the ovipositor extending beyond the rind portion. The only likelihood of secondary infestation by the OFF was followed rind damage including; physical cracks or mechanical injury. Resistance of mangosteen to the OFF infestation was due to its hard rind surface, tough and thick skin and latex secretion. Based on these studies, mangosteen from Thailand was permitted to import into Australia (Anonymous, 2004) and New Zealand (Anonymous, 2006) without post-harvest disinfestation requirement for fruit flies.

Resistance to insect infestation is due to biophysical and/or biochemical attributes. Pummelo has been reported as a host of fruit flies. However, detailed information on fruit fly infestation was not available. Casual observations and poorly documented report occasionally have led to quarantine requirements of some fruits (Couey, 1983). It is obvious that thick peel and toxic oil gland of pummelo could be barriers to fruit fly infestation. Therefore, the status of pummelo as a host of the OFF should be thoroughly investigated. If pummelo is virtually immune to attack by the OFF, it will allow pummelo to enter marketing channels without quarantine treatment. The purpose of this research was to determine whether or not pummelo is susceptible to infestation by the OFF.

## MATERIALS AND METHODS

### Source of the Fruit Fly

The oriental fruit fly (OFF) used in the experiment was originated from mangoes, *Mangifera indica* Linn. and was collected at Amphur Phakchong, Nakornrasima province. The populations of the OFF were propagated through mass rearing technique at Plant Quarantine Research Group, Plant Protection Research and Development Office, Department of Agriculture, Chatuchak, Bangkok.

### Fruit Fly Mass Rearing Technique

The colonies of the OFF were held in 2 constant temperature and humidity rooms (3.5 x 4.6 x 2.3 m) (Figure 4) maintained at  $26 \pm 1$  ° C,  $65 \pm 5$  % RH and a light-dark cycle of L : D 12 : 12. The photophase occurred from

6:00 AM to 6:00 PM. The lighting system of rearing room was provided by 20 fluorescent lamps affixed to ceiling above the cages and 40 lamps on the wall. (Figure 5)

Adult fly: About 20,000 adult flies were housed in each 16 mesh-wire screening cage (65.5 x 69 x 77 cm). Adult flies were fed on the artificial diet consisting of a mixture of 10 parts sugar, 1 part enzymatic protein hydrolysate (Amber series 100), and 1 part yeast extract by weight. The diet was presented to the flies in a shallow plastic dish on the cage floor. Drinking water was supplied by placing plastic bottle (16 cm diameter x 7.5 cm high) having perforated lid on filter paper on the screened top of the cage. The filter paper was moistened by absorbing water through 3 holes (1 mm) punched on the bottle lid.

Adult flies were held in the cage for 6 week. After that the remaining flies were destroyed, and the cage was cleaned and prepared for new emerging flies. About 5 cages with a population of approximately 20,000 flies per cage were continuously maintained through the study.

Egg collecting: The perforated polyethylene container was used as egg receptacle. This container was 17 cm long and taper from 7 cm to 5.5 cm in diameter. Eggs were deposited through 0.4 mm holes punched through the side of the container. To provide the ovipositional stimulus and to prevent the egg from desiccating, the inside of the egg receptacle used for the OFF was moistened with a solution of orange juice diluted with water.

Eggs were collected once a week from 10:00 to 12:00 AM. starting 15 days after adult emergence and usually seeded on the larval diet in the same day. Egg collecting was done by placing egg receptacles inside the cage. The eggs inside containers were washed under running water into a fine-mesh cloth from which they were transferred into beaker. Eggs were kept under water until they were seeded on the larval diet. Periodic checks on egg hatch were made by placing samples with a fine carmel's-hair brush on moist blotting paper held in Petri dishes and recording the number of hatched eggs.

Larval diet: Larval diet base on corn flour was used for rearing the OFF (Watanabe *et al.*, 1973). The formulation now used at the laboratory in preparing the media is as follows.

Brewer's yeast	5	g
Butyl p-hydroxybenzoate	0.15	g
Sugar	5	g
Corn flour (20 mesh)	50	g
HCl (Conc.)	0.2	ml



Toilet tissue	3	g
Distilled water	85	g

The prepared diet was placed on shallow plastic tray (23 x 32 x 5 cm). Each tray was filled up to a depth of 2 cm (900 g of diet). The eggs were seeded on the top of 2 toilet paper strips (5.5 x 11 cm) placed across the surface of the larval diet. Each tray received about 0.4 ml of the OFF egg. Eggs were smeared on the larval diet by using a fine camel's-hair brush. The diet tray was sealed by the addition of second inverted tray to maintain the high humidity necessary for larval hatching. Subsequently, the diet was held in rearing room to wait for pupation.

Collection of mature larvae: The OFF larvae became mature in 6 days after eggs were seeded and began to leave diet. At the end of larval period, the cover was removed from diet tray. The diet trays were placed in pupal collecting boxes (43 x 74 x 23 cm) containing saw dust (20 mesh) to prevent crawling of larvae and to encourage pupation. The maturing larvae left the diet by crawling and popping off the sides of the stacked larval diet trays and fell into collecting boxes.

Pupal handling: Pupae were held in saw dust. Two days before the expected emergence, the pupae were separated from the saw dust by sieving through a 20-mesh screen sieve. Pupae were held in plastic tray (23 x 32 x 5 cm) and placed inside new cage until eclosion. Periodic checks on pupal weight, emerging rate and sex ratio were made by randomly sampling 3 samples of 100 pupae. The samples were held in plastic containers until adult emergence.

### Natural Infestation

To determine whether or not natural infestation was present, samples of pummelo of 'Thong Dee' cultivar were randomly collected during harvesting period from the orchards in Nakornpathum, Chumporn and Chiang Rai provinces. The samples were transported to laboratory in Bangkok. The samples were placed in plastic containers (36 x 54 x 15 cm). The plastic containers were covered with fine mesh clothes. All fruits were held in room at 25-27 °C for 10 days. At the end of holding period, each fruit was opened with a knife. The peel, albedo and pulp were visually inspected for immature stages of the OFF before disposal.

### Laboratory Cage Infestation

Laboratory studies were conducted with pummelo of 'Thong Dee' cultivar. Cage infestation studies were carried out by exposing pummelo fruits to gravid females for oviposition. Adults of both sexes were held at least 2 weeks posteclosion to assure sexual maturity and gravidity of females when used. Each

infestation cage was 16 mesh-wire screening cage (50.5 x 35.6 x 35.2 cm). and contained approximately 2,000 adult fruit flies with male and female ratio of 1:1 (Figure 6). Before exposure to female flies, the fruit surface was puncture using entomological pins of size # 5. Test fruits were investigated under 2 simulated conditions of undamaged and damaged fruits (1). Undamaged fruit: 10 shallow pinholes of 2-3 mm. deep were made into the fruit to allow easier access for ovipositing fruit flies. The holes did not reach to the flesh (endocarp). (2). Damaged fruit: 10 deep pinholes were made into the fruit. The holes penetrated through into the flesh.

Each condition test consisted of 10 replicates of 10 fruits. Test fruits were individually placed in an infestation cage in fruit fly rearing room for 24 h (Figure 7). After exposure period, fruits were removed from infestation cages and placed in plastic containers (36 x 54 x 15 cm) covered with fine mesh cloth. All test fruits were held in room at 25-27 ° C, 70-80 % RH for 10 days, then dissected and visually examined for immature stages of the OFF. In all of our trials, survival of larvae was used as the criterion for successful infestation.

## RESULTS AND DISCUSSION

### Natural Infestation

A total of 350 fruits were randomly collected from orchards in 3 provinces. No OFF infestations were detected from any samples. Therefore, there was no natural infestation. Back and Pemberton (1918 a) reported that the *C. capitata* cannot develop in lemons unless the fruit has been damaged while still on the tree. Quayle (1914, 1929) reported that lemons in the Mediterranean countries were not attacked by *C. capitata* until the fruit is overripe or partially decayed. Ovipositor wounds were rarely found in Italian lemons grown commercially. Back and Pemberton (1918 a), however, readily observed oviposition wounds in lemons grown in Hawaii every month of the year. In Italy, however, Quayle (1914, 1919) seldom found wounds in lemons. These investigators attributed the case in finding oviposition punctures in Hawaiian lemons to differences in the ecology of *C. capitata* populations between Italy and Hawaii.

In natural infestation study, pummelo fruits were randomly collected from the trees during harvesting period. The survey was done in orchards which commercially export fruits to many countries such as China and European countries. Since natural infestation was not found, it is possibly that pummelo grown commercially were unlikely to be naturally infested with fruit flies.



### Laboratory Cage Infestation

In laboratory forced infestation, exposures were made in enclosed cages under high fly densities, which may represent a worse-case condition. Examination of some fruits right after the end of forced infestation period showed that many clusters of eggs were found spread over the area which was exposed to fruit flies in the cages (Figure 8). The female flies were able to insert ovipositor directly through the peel (exocarp). However, it was observed that eggs were confined only in the flavedo (exocarp) and albedo (mesocarp) regions (Figure 9). This demonstrated the toughness of the albedo region which could prevent eggs to be forced into the endocarp region. Generally, the flavedo and albedo regions of pummelo had a thickness of approximately 1 mm. and 10 mm., respectively. The ovipositor of the OFF was about 3 mm. The thickness of albedo region is far over the length of ovipositor and the toughness prevented female fruit flies from directly depositing eggs beyond this region into fruit pulp.

The data in Table 1 demonstrated that fruits with punctures of 2 mm depth on the fruit surface were resistant to fruit fly infestation. No fruit fly infestation was found on all test fruits. The close examination of albedo region under stereo-microscope revealed that a formation of hard tissue was developed surrounding egg clusters (Figure 10-12). Eggs were confined in a cavity and probably died due to suffocation and starvation. The hardening of the outer periphery of the wound encapsulated the eggs and was a major factor in egg suffocation. In addition, the mortality of eggs as well as newly hatch larvae in pummelo may be attributed to the toxic oil in the flavedo region of the peel. No trace of larval feeding was found outside the cavity and fruit pulp. It indicated that the first instar larvae were not able to penetrate the hard tissue and were observed exiting the ovipositing punctures and crawling on the fruit surface. Since there was no moisture or food source on fruit surface, therefore, all larvae eventually died due to desiccation or starvation. From this study, it showed that the toughness (impenetrability) and thickness of albedo provided an effective natural barrier to fruit fly infestation.

Quayle (1914) and Back and Pemberton (1915) reported that the failure of *C. capitata* eggs to hatch in citrus may be due to suffocation, the formation of a sealed-off egg cavity, the oil in the flavedo, or some other injurious substance in the rind. Back and Pemberton (1918 b) attributed the great mortality of eggs as well as any newly hatched larvae in citrus to the thick rind. The oil cells located in the flavedo were an additional source of egg failure. Greany *et al.* (1983) found that oils in the flavedo of lemons were toxic to another Tephritidae, contributing to making lemons immune.

Greany *et al.* (1983) reported that resistance of citrus fruit to attack by the *A. suspensa* was attributed to allelopathic essential oils in the flavedo region of the peel. Hatchability of eggs laid between peel oil glands was significantly greater than that of eggs laid into glands. Mortality occurred principally in the 1<sup>st</sup> larval instar, and most larvae died before reaching the albedo region. Grapefruit were more susceptible to larval development than oranges, and lemons were virtually immune to successful attack by this fly. Fruit resistance was correlated with (1) flavedo thickness (2) a high concentration of linalool in relation to limonene in the peel oil and (3) the absolute amount of oil per unit area of peel.

Table 2 showed that all fruits with 10 punctures through the flesh were susceptible to fruit fly infestation. The infestation studies on pummelo showed that female flies oviposited in the track pin punctures. The female flies inserted ovipositor through the punctures and eggs were forced deep inside the fruit pulp (Figure 13). Larvae hatching from eggs deposited inside the punctures survived by feeding on fruit pulp (Figure 14). It was noticed that no trace of feeding was found on albedo portion. The results of postharvest exposure studies suggested that pummelo was susceptible to fruit fly infestation if the fruit surface was damaged so deep enough that allowed female flies to deposit eggs on the pulp.

## CONCLUSION

Host status in Thailand of commercially cultivated pummelo ‘Thong Dee’ cultivar to the OFF was determined. No natural infestation was detected on pummelo fruits collected in commercial orchards from 3 major producing regions. Laboratory forced infestation studies showed that pummelo fruits were resistant to fruit fly infestation. Eggs were deposited only within flavedo and albedo regions. However, eggs and newly hatched larvae eventually died due to suffocation and starvation. Pummelo was virtually immune to successful attack by this fruit fly due to (1) formation of a sealed-off egg cavity (2) toxic oil or injurious substance in the flavedo region and (3) the thickness and toughness of albedo region.

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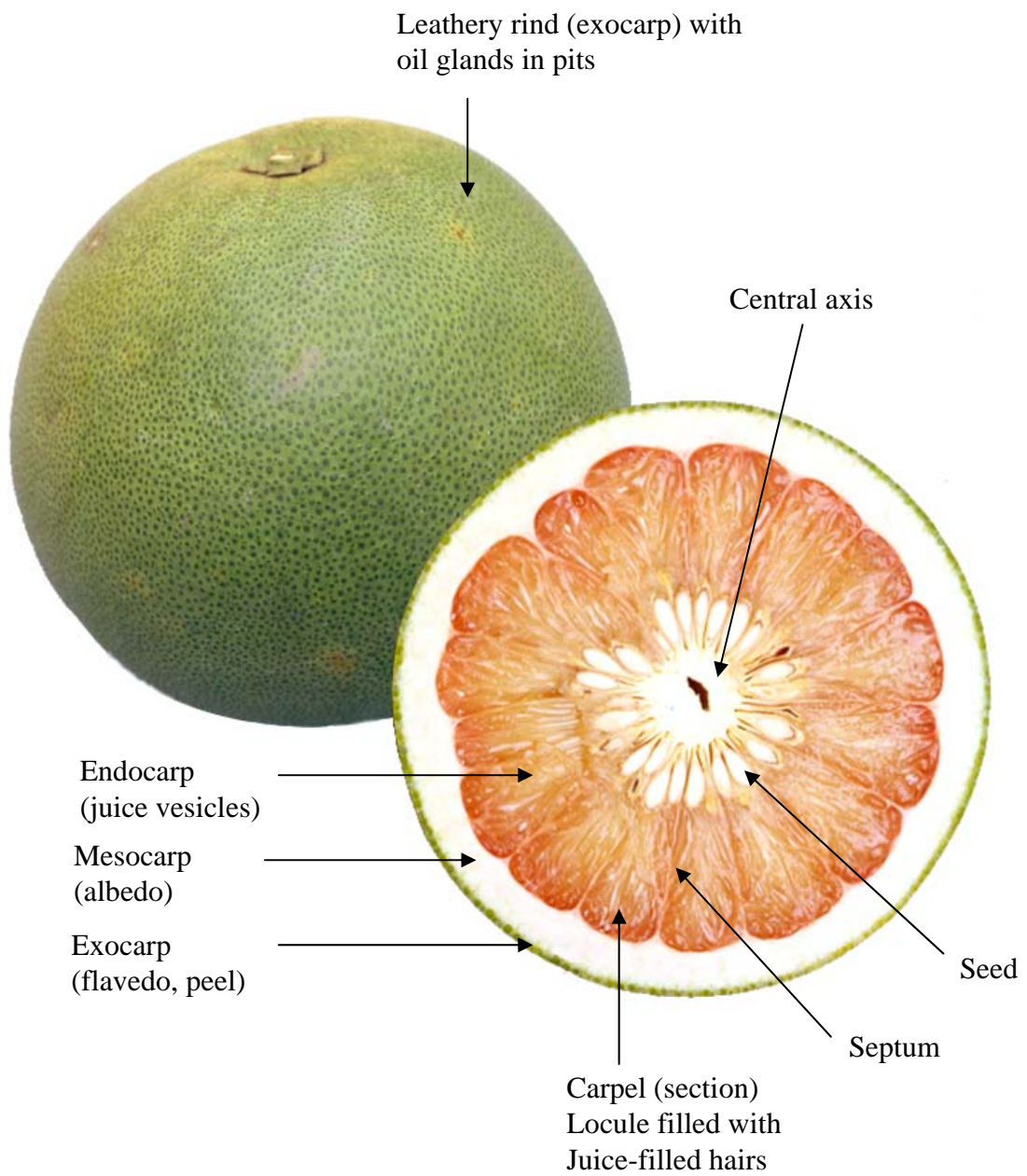
**Table 1.** Results of forced-infestation studies with the oriental fruit fly, *Bactocera dorsalis*, on undamaged pummelo.

Lot No.	Number of test fruits	No. infested fruits
1	10	0
2	10	0
3	10	0
4	10	0
5	10	0
6	10	0
7	10	0
8	10	0
9	10	0
10	10	0
Total	100	0

**Table 2.** Results of forced-infestation studies with the oriental fruit fly, *Bactocera dorsalis*, on damaged mangosteens with 10 punctures on surface through pulp.

Lot No.	Number of test fruits	No. infested fruits
1	10	10
2	10	10
3	10	10
4	10	10
5	10	10
6	10	10
7	10	10
8	10	10
9	10	10
10	10	10
Total	100	100



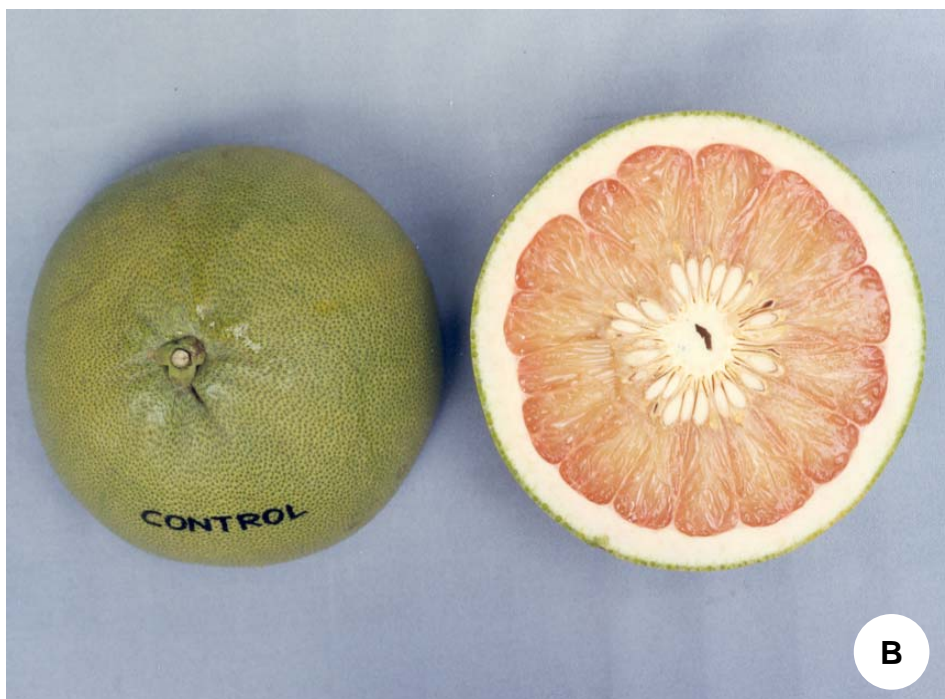


**Figure 1.** Pummelo fruit and its components.



**Figure 2.** Pummelo plantation in Chiang Rai province which is the major producing area in northern region.





**Figure 3.** Pummelo of “Thong Dee” cultivar. The edible part (endocarp) is pink.



**Figure 4.** Fruit fly rearing rooms at Plant Quarantine Research Group, Plant Protection Research and Development Office, Bangkok.



**Figure 5.** The environmental conditions of rearing rooms were maintained at  $26 \pm 1$  °C,  $65 \pm 5$  % RH and a light-dark cycle of L : D 12:12.

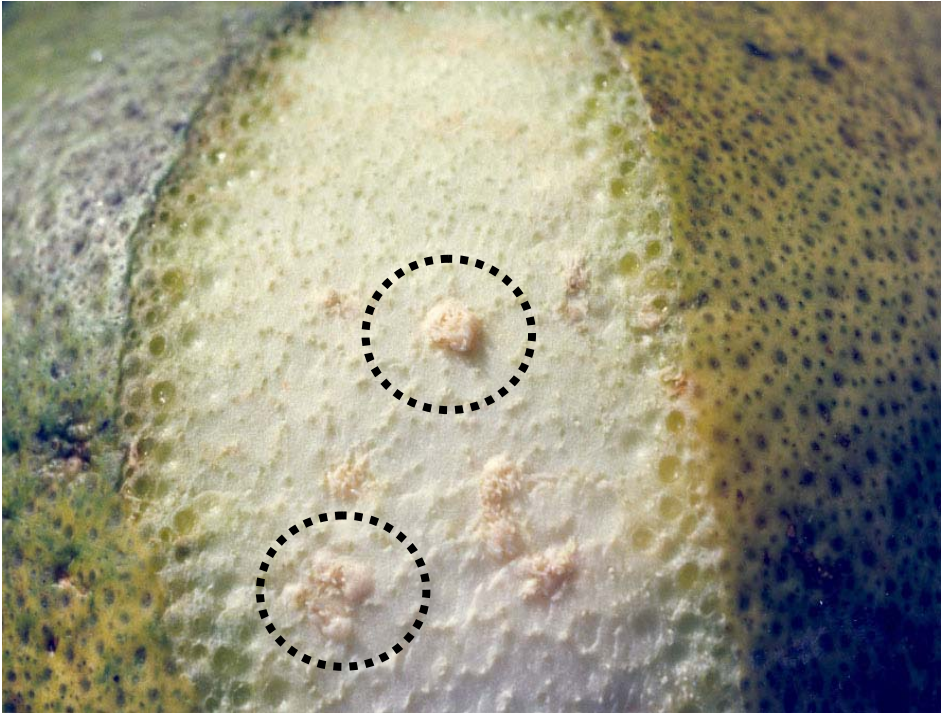




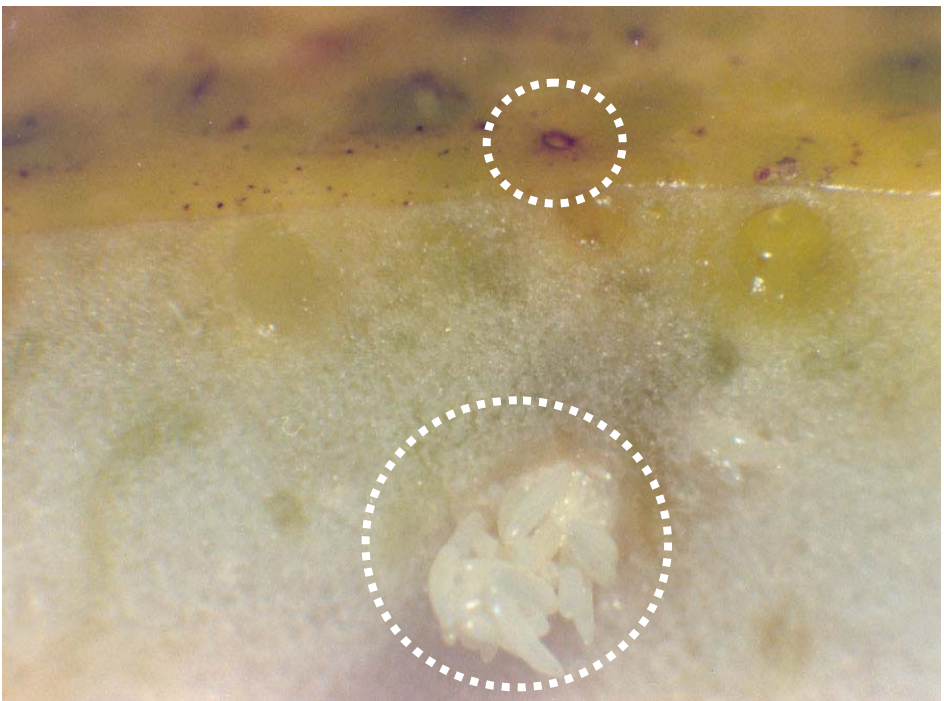
**Figure 6.** Laboratory forced infestation with *Bactrocera dorsalis* on pummelo fruits was done in aluminum insect cages 50.5 x 36.6 x 36.2 cm.



**Figure 7.** Pummelo fruits were exposed for 24 hr. to gravid fruit flies of *Bactrocera dorsalis* for oviposition.

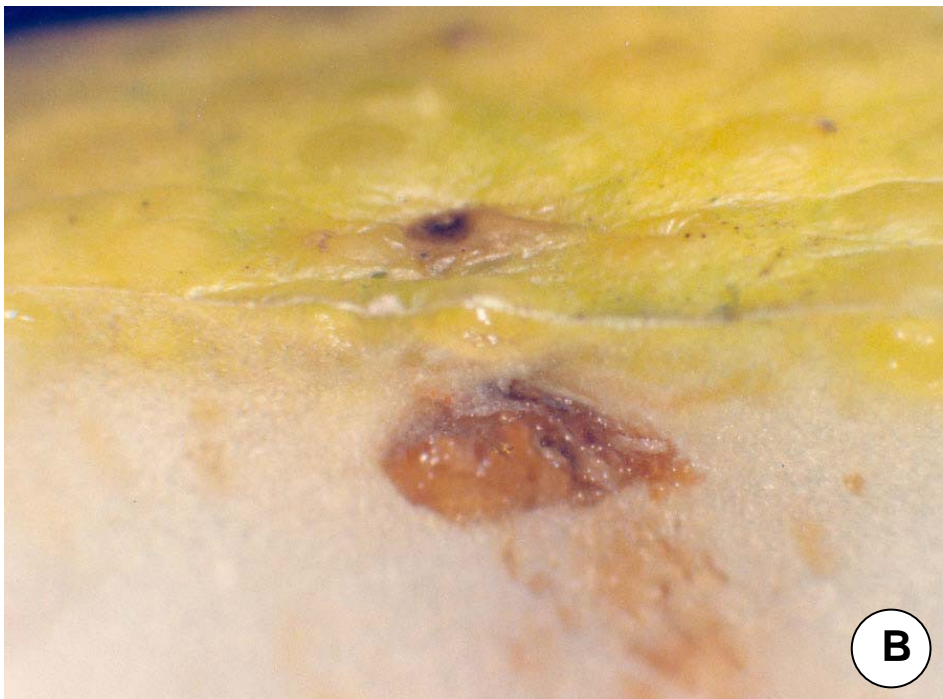
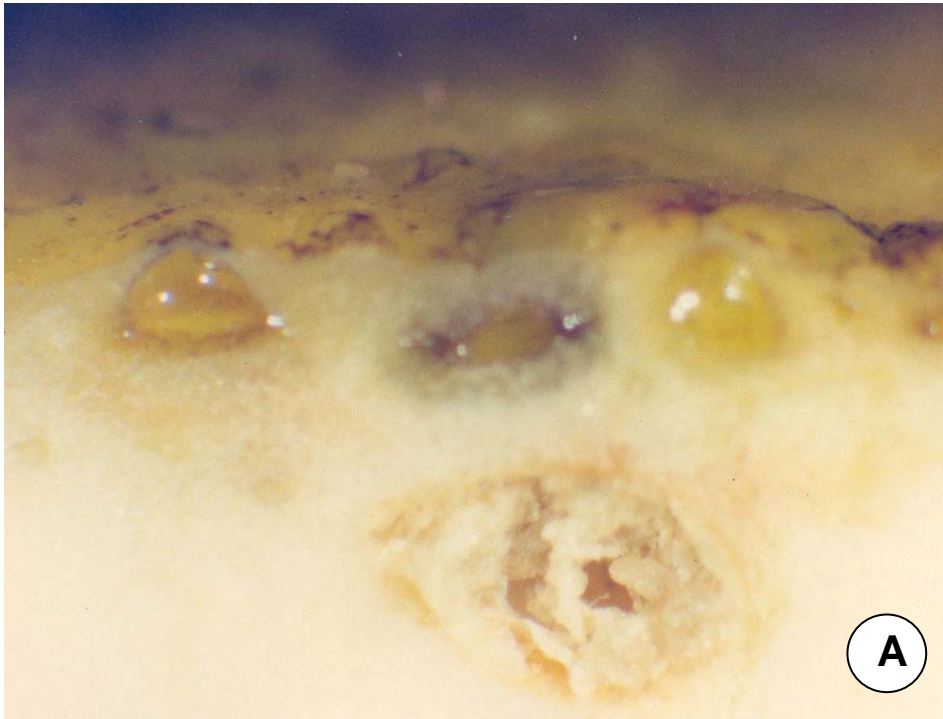


**Figure 8.** Gravid female fruit flies were found ovipositing eggs in the favedo and albedo regions.

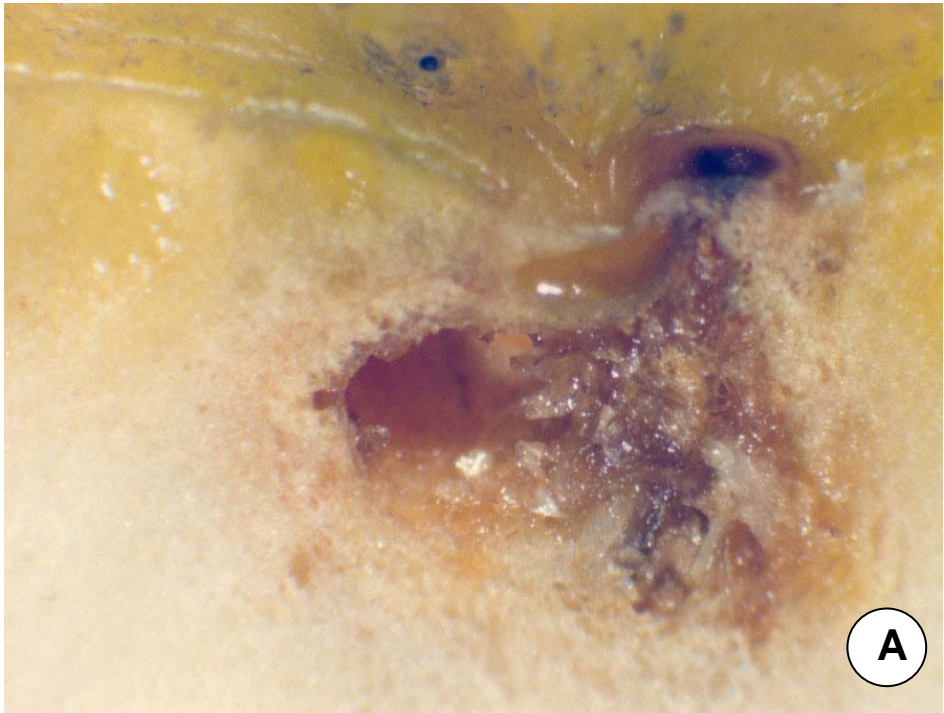


**Figure 9.** Eggs were forced through track pin punctures into the albedo region.



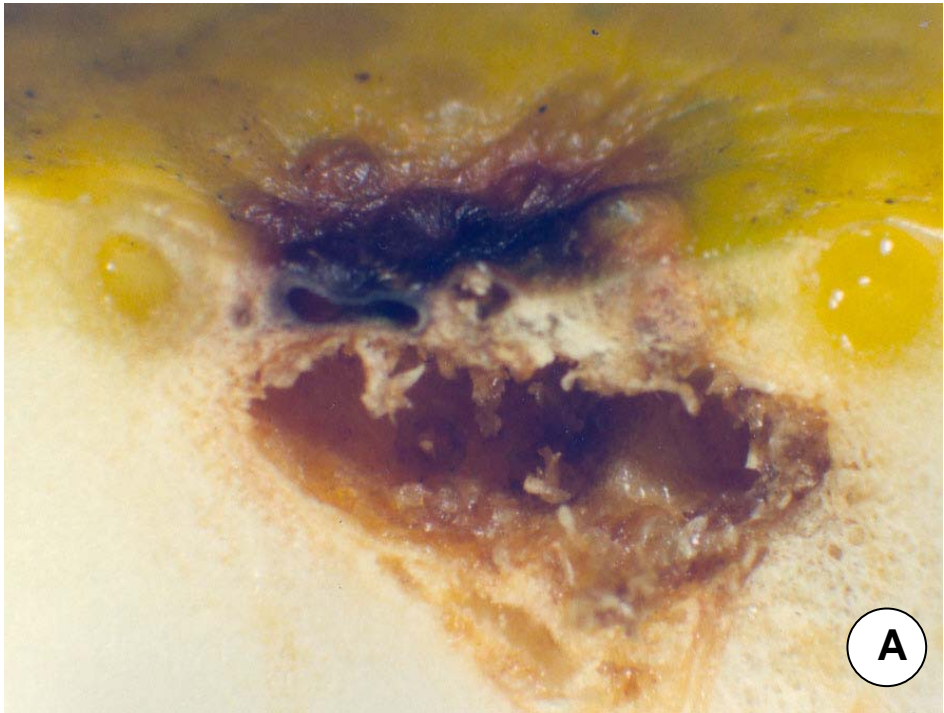


**Figure 10.** Formation of a seal-off cavity. The hardening of the outer periphery of the wound encapsulated the eggs.

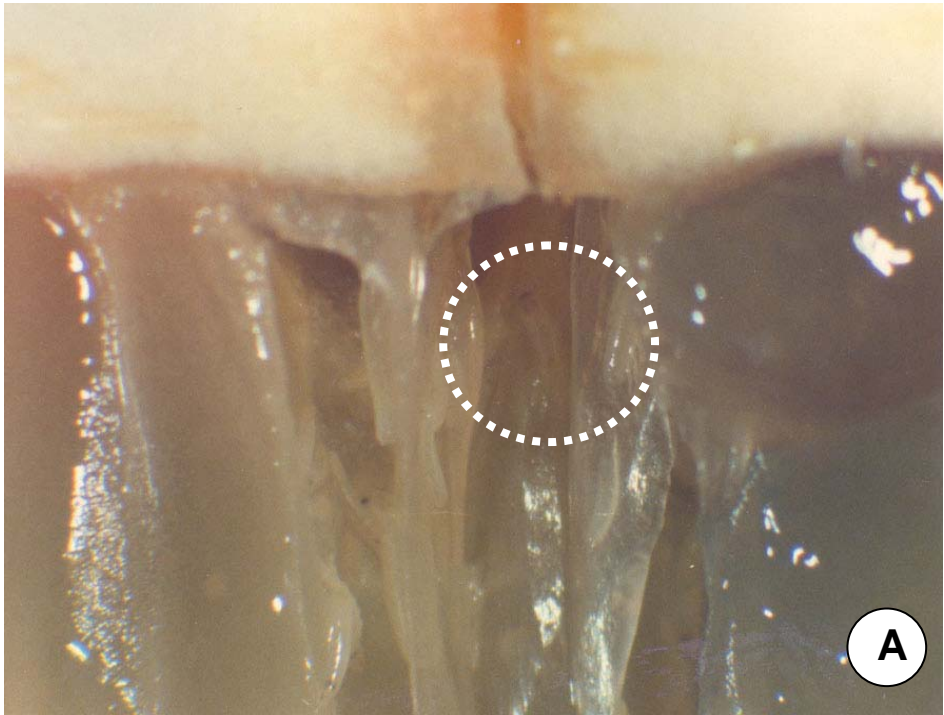


**Figure 11.** Formation of a sealed-off egg cavity in albedo region.

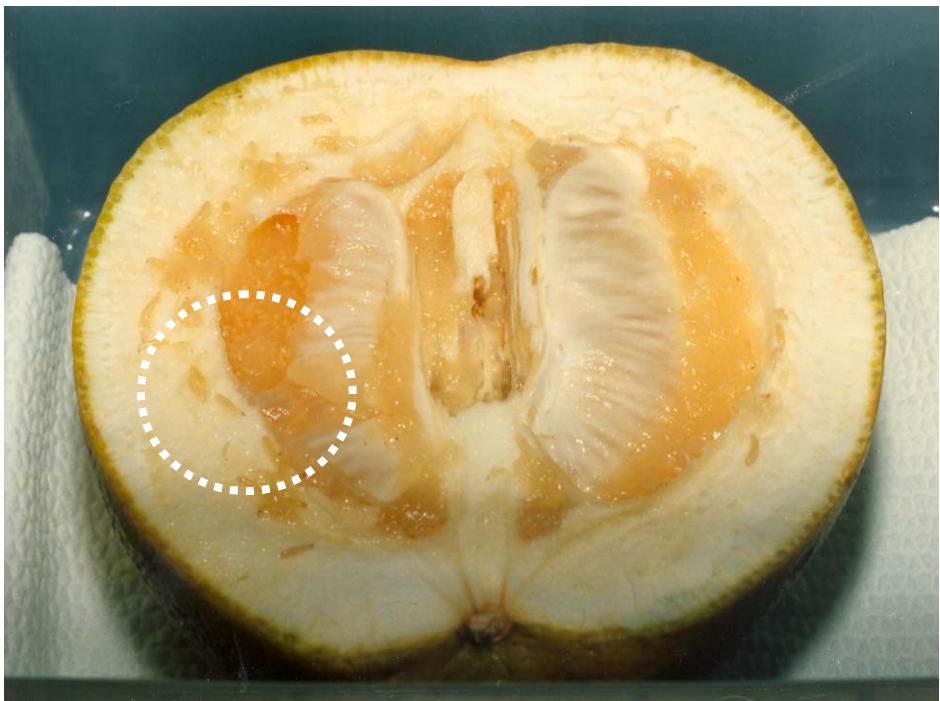




**Figure 12.** Eggs and newly hatched larvae eventually died due to suffocation and starvation.



**Figure 13.** Gravid female fruit flies could oviposit eggs through the holes and forced eggs deep into the flesh.



**Figure 14.** Larvae damaged inside punctured pummelo.



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# Hot Water Immersion Treatment of Nam Dorkmai Mango Infested with Oriental Fruit Fly, *Bactrocera dorsalis* (Hendel) for Export

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## Abstract

Hot water immersion treatment is a post-harvest treatment for fruit flies disinfestation and widely used as quarantine treatment in many countries, particularly in Latin America. The study was conducted at V.S. Freshco Company Limited, Crop Processing Research and Development Group, Postharvest and Processing Research and Development Division and Pest Management Group, Plant Protection Research and Development Office from October 2013 until September 2018. The studies determined the optimum innermost temperature and exposure period to control egg and 1<sup>st</sup> instar larvae Oriental fruit fly, *Bactrocera dorsalis* (Hendel) in Nam Dorkmai mangoes. The results showed that the treatment at the innermost of mango at 46°C for 10 minutes is effective against both stages above and had no impact on the quality of the fruit.

Keywords: Hot water immersion treatment, Mango, *Bactrocera dorsalis* (Hendel)

## Introduction

Mango is one of economic crops in Thailand. Among the cultivated mangoes, Nam Dorkmai variety is one of the best quality that grown for yellow eating both for domestic and export markets (Department of Agriculture, 1988). It is recognized as Queen of Thai mangoes with beautifully golden yellow pulp and the sweet taste. In 2015, the mango plants that bearing fruits was 779,198 acres and produced 2,582,495 tons of fruit (Anonymous, 2011). According to the report from the Export Plant Quarantine Service Group (Office Agricultural Regulation), Thailand exported 98,140 tons of mangoes (accompanied with Phytosanitary Certificate) worth 1,694.43 million bahts and 113,187 tons worth 2,027.63 million bahts in 2015 and 2016, respectively. Thus, it is certain mangoes are demanded in international markets. However, fruit flies play an important role in the quality of mango production as well as the exportation which many countries required the mango to be free from those flies. Montree and Ocha (1998) reported that a value of more than 1,000 million bahts per year of agricultural products was damaged by fruit flies.

Fruit fly is in the Diptera order and Tephritidae family. It consists of two wings and halteres, which are minute drum-stick shape balance organs developed from the second pair of wing (hindwings) of an insect. Fruit fly wings are transparent in which veins are highly visible. It has bright yellow chest. There are many important species of fruit flies in Thailand.



Six species of fruit flies were reported by San (1986), while Montree (1993) and Montree and Ocha (1998) reported more than 10 species.

*Bactrocera dorsalis* (Hendel) is a quarantine pest of many countries as the eggs were laid in the fruits and the larvae feed inside the fruits that make them very difficult to detect and control. Fruit fly has a very wide host range that attack economic crops especially fruit crops with the mean of damage that make it is difficult to control. There are a lot of countries list fruit flies as their quarantine pests. As a result, it constrains the exportation of agricultural products, especially fresh fruits including fruits of vegetables that are the host of these flies. Some countries required phytosanitary measures in importing fresh fruits from Thailand to treat against these fruit flies. The post-harvest treatments that are effective against fruit flies such as irradiation, fumigation, vapor heat treatment and hot water immersion treatment. Thailand is successfully treated against fruit fly in mango, mangosteen to Japan and Korea with vapor heat treatment and using irradiation for mangosteen, rambutan, longan and lychee to the USA. However the investment and operating cost for vapor heat treatment and irradiation is very expensive. Hot water immersion treatment is another option that is easy to operate, lower investment and operational cost.

Hot water immersion treatment to control fruit flies in mangoes is an effective and widely used in many countries, especially in Latin America to control Mexican Fruit Fly, *Anastrepha ludens* (Loew). It has been accepted as quarantine treatment by the Animal and Plant Health Inspection Service (APHIS), United State Department of Agriculture (USDA) since 1987. Hot water treatment for Mexican Fruit Fly is conducted at water temperature of 46.1-46.5°C for 65-110 min depending on weight and varieties of mangoes (Sharp et al., 1998). With hot water immersion, the heat transfer occurs from the water to the skin of fruit and from the skin through the flesh then to the center of the fruit. The heat transfer from the water to skin is faster than the skin to center transfer. The rate of heating from the skin to the center of the fruit with hot water immersion is substantially faster than VHT (Vapor heat treatment) and FHAT (Forced hot-air heating) of the same temperature (Couey, 1989; Stewart et al., 1990; Jordan, 1993). Hot water immersion has a number of advantages which include: relative ease of use by the industries, short treatment time, reliable and accurate monitoring of fruit and water temperatures (Sharp, 1994). Another important advantage of hot water immersion technology from an economic point of view is that the cost of atypical commercial system is approximately 10% that of a commercial VHT system (Jordan, 1993)

This research aims to determine optimum temperature and duration of hot water immersion treatment for complete controlling fruit flies larvae in mango for exporting purposes; to develop method of hot water immersion treatment to meet plant quarantine treatment standard. The efficacy of hot water immersion treatment test was on oriental fruit fly, *B. dorsalis* in mango variety Nam Dorkmai.

## Material and Method

### Material

1. Oriental fruit fly, *B. dorsalis* adult stage, egg stage and 1st instar larvae
2. Insect cage, insect container and plastic feeding box
3. Filter paper, parafilm, brush, cotton ball, tweezers, tissue paper
4. Cork borer
5. Mango of variety Nam Dorkmai
6. Memmert hot water boiler model WNB 22 (Interworld Highway, LLC dba

Tequipment 205 Westwood Avenue. Long Branch, NJ 07740)

7. Stainless steel hot water boiler, 2.53 meters long, 1.35 meters wide and 0.6 meters high, has a heating system heater and continuous temperature, with a microprocessor control unit. The temperature is measured with a PT 100 (Class A) probe.

8. 2 decimal weight scales
9. Temperature probes
10. Penetrometer
11. Digital refractometer
12. Data Logger

### Method

**1. Test Mango Fruits:** Mango of variety Nam Dorkmai was used in this experiment.

#### 1.1 Fruit characteristics

The horticultural characteristic of Nam Dorkmai mango is described below;

1.1.1 Fruit shape: oblong elliptic, basal base of fruit is prominence than slightly taper to apex

1.1.2 Size: medium (Length 16.0 cm, Major dia. 7.2 cm and Minor dia. 6.9 cm)

1.1.3 Weight: 300-500 g

1.1.4 Shoulders: prominent, dorsal shoulder was sloping and ventral shoulder was curving

1.1.5 Sinus: very shallow or absent

1.1.6 Beak: acute and small

1.1.7 Apex: acute

1.1.8 Skin:

- Color<sup>a/</sup>; coloration of nature green stage is Green group 139 C., coloration of full ripeness is Yellow-Orange group 22C

- Surface; glands numerous, film of fine powder covering the fruit skin

- Thickness; thin about 1.1 mm

1.1.9 Pulp:

- Color; Yellow-Orange

- Texture; soft and fine, thickening pulp with firm

- Flavor; luscious like jasmine's odour
- Taste; very sweet, Brix value about 19-20°Brix
- Juice; abundant or much
- Fiber; scanty
- General quality; excellent

#### 1.1.10 Stone:

- Shape; same as in the fruit
- Body; flat and thin
- Fiber; abundant

<sup>a/</sup> The pulp color is checked by comparing with R.H.S Color Chart of the Royal Horticultural Society, London.

#### 1.2 Fruit sizes

The test fruit of mango was determined fruit size. The size of Nam Dorkmai mango was categorized into 5 grades based on their weight as following;

Size	Weight (g)
1. Extra-large (XL)	> 420
2. Large (L)	360 - 420
3. Medium (M)	300 – 359
4. Small (S)	250 - 299
5. Super small (SS)	< 250

Nam Dorkmai mangos used in the experiment come from GMP farms from several locations such as Chachoensao, Chonburi, Prachuap-Khiri-Khan, Chiang-Mai and Chiang-Rai province. 85% ripeness mango fruits were used in disinfestation tests. They were cleaned, inspected for no symptoms or damage from fruit fly then sizing and grading. The fruits were kept in the fruit fly free room. Upon arrival at the laboratory, mangoes were graded depending upon the specific requirement of the experiment and selected again for their uniformity in ripeness before the experiment.

The fruits that used in the experiment were medium and large size.

## 2. Test Insect

Oriental fruit fly, *B. dorsalis* used in this experiment was collected from infested mangoes, *Mangifera indica* L., in Amphoe Pak Chong, Nakhon Ratchasima province and Amphoe U Thong, Suphanburi province. Infested fruits were held until larvae emerged and pupated. Pupae were placed in rearing cages and young individual fly was identified under a stereomicroscope before initiating mass rearing. The population of the oriental fruit fly was propagated using mass rearing technique. Then, the colony was collected and maintained for the experiment at laboratory of Pest Management Group, Plant Protection Research and Development Office, Department of Agriculture, Bangkok.

## 2.1 Fruit fly mass rearing technique

**Rearing Room:** The colonies of oriental fruit flies was held in temperature and humidity controlled room at  $26.10 \pm 1.27^\circ\text{C}$  and  $69.07 \pm 3.25$  % relative humidity (RH) and photoperiod of 12:12 (L:D) h. The photoperiod occurred from 6:00 a.m. to 6:00 p.m. The lighting system of rearing room was provided by 32 watts fluorescent lamps affixed to ceiling above the cages. The photo-phase occurred from 6:00 to 18:00 and a light-dark cycle of L:D 12:12. In addition, 1 lamp of 15 watts is attached to allow dim light to mimic the natural condition of sun during dawn and dusk to help stimulate mating. The photo-phase occurred from 5:30-6:00 and 18:00-18:30.

**Adult:** Approximately 20,000 adult *B. dorsalis* flies were housed in each of the 16-mesh wire screen cages (65.5x69x77 cm). They fed on artificial diet consisting of 10 parts sugar, 1 part enzymatic protein hydrolysate (Amber series 100) and 1 part yeast extract by weight. Diet was placed in a shallow plastic dish on the cage floor. Water was supplied by placing a plastic bottle (16 cm in diameter by 7.5 cm in height) with perforated lid on filter paper on the screened top of the cage. The filter paper was moistened by absorbing water through three holes (1 mm) punched on the bottle lid. Adult flies were replaced every 6 weeks. After that the remaining flies were destroyed and the cage was cleaned and prepared for new emerging flies. About 5 cages with a population of approximately 20,000 flies per cage and 10 cages with a population of approximately 2,000 flies per cage (35x50x35 cm) were continuously maintained throughout the study.

**Egg collecting:** A perforated polyethylene container (17 cm in height, tapering downward from 7 to 5.5 cm diameter) was used as an egg receptacle inside each adult cage. Eggs were deposited through 0.4 mm hole punched through the side of the container. To provide and ovipositional stimulus and to prevent eggs from desiccating, inside of egg receptacle was moistened with a solution of orange juice diluted with water. Eggs were collected once a week beginning 15 days after adult emergence and placed on the larval diet the same day. Egg collecting was done by placing egg receptacles inside the cage. The eggs inside containers were washed under running water into a fine mesh cloth from which they were transferred into a beaker. Eggs were kept under water until they were seeded on the larval diet. Periodic checks on hatching rate were made by placing samples with a small camel hair brush on moist blotting paper held in petri dishes and recording the number of hatched eggs.

**Larval diet:** Larval diet based on corn flour was used for rearing the oriental fruit fly, *B. dorsalis* (Watanabe et al. 1973). The formulation now used at the laboratory in preparing the media is as follow:

corn flour	50	g
sugar	5	g
Brewer's yeast	5	g



Butyl p-hydroxybenzoate	0.15	g
toilet paper	3	g
HCl (conc.)	0.2	ml
distilled water	85	ml

Approximately 900 g of diet was placed in each plastic tray (23x32x5 cm). The eggs were seeded on the top of 2 tissue paper strips (5.5x11.0 cm) place across the surface of the larval diet. Each tray received about 0.4 ml of egg. Eggs were transferred on the larval diet by using a fine brush. The diet tray was covered by the inverted tray to maintain the humidity, and was held for pupation.

**Pupal Handling:** Mature larvae began leaving the diet at 6 day after egg transfer, and at this time the cover was removed and trays were placed in pupae-collecting boxes (43x74x23 cm) containing moist sawdust (to encourage pupation). Pupal stage was 8-10 days, and 2 days before expected emergence, the pupae were separated from the sawdust by sieving through a 20-mesh screen sieve, 20,000 pupae placed in plastic tray (23x32x5 cm) and transferred to new cage for adult emergence

**Quality control:** Oriental fruit fly, *B. dorsalis* laboratory colonization must have routine quality control check in every generation. Therefore, every generation of *B. dorsalis* have the records of hatching rate, emerging rate, pupae weight and sex ratio to monitor the quality of the flies.

2.2 Preparation of infested mango with Oriental fruit fly, *B. dorsalis* for the experiment.

Nam Dorkmai mangoes infested with oriental fruit fly were obtained by two methods.

**2.2.1 Artificial infestation method:** It was carried out by directly infesting certain stage of developmental stage of fruit fly into the fruit flesh. Prior to artificial infestation, fruits were prepared by using the following techniques. A small piece of mango pulp was cut off from the mango by using 1 cm diameter cork borer and 1 cm depth into the fruit flesh (Figure 1). A thin layer of mango flesh was cut off from the plug so that there was some space for the eggs and larva to be housed. The plug was inserted back into the hole after egg or larva was transferred into the fruits. Then masking tape was put on the plug to prevent the larva leaving the fruit.

**2.2.2 Forced infestation method:** Forced infestation tested fruits were prepared by allowing gravid female to lay eggs directly (Figure 2) into full ripe mango fruits. The infested cage is a 16 mesh wire screening cage (35x50x35 cm). Approximately 2,000 adult flies with male and female ratio of 1:1 were held in the cage. Forced infestation method was used to prepare infested fruits for only large-scale confirmation test. Infestation was carried out by using the following techniques (Unahawutti at el., 1991). Each mango fruit was wrapped up with clear plastic bag. The bag is tightly fastened to fruit surface with masking tape. Five punctures were made on one side with insect pin through the flesh to

facilitate oviposition. The female flies were forced to oviposit into fruit pulp only through the holes. Ten test fruits were placed inside the cage and the holes was exposed to the flies. The oviposition was done 40 hours before treatment and the exposure time was 25 minutes. Upon completion of exposure time, the fruits were taken out from fruit flies cage. Infested fruits were held.

### **2.3 Test insect preparation**

There were two stages of fruit fly that were used in the experiment, egg stage and first instar larva.

**2.3.1 Egg preparation:** Eggs of the oriental fruit fly, *B. dorsalis* used in disinfestation test were 24 hour old. The infestation was done one day before the experiment. Eggs were collected from existing population by using previously mentioned techniques. Adult flies were allowed to lay eggs for 30 min. Eggs were transferred to 200 ml beaker containing distilled water. Unfertilized eggs which were floating on the water were discarded. Newly laid eggs were transferred and counted on moist black blotting paper held in petri dishes. Eggs were counted under magnifier and then transferred to mango pulp by using a fine camel's hairbrush then close the opening with the same piece of fruit pulp and seal with masking tape. Each test fruit was infested with 100 eggs. Subsequently, infested fruits were kept in temperature controlled room at 25-27°C until used. All infested fruits were left for 1 hour before subjecting to treatment.

**2.3.2 First instar larva preparation:** The 1st instar was obtained by collecting eggs 40 hour prior. Eggs were transferred to wet muslin cloth placed inside plastic container (12x18x4.5 cm) with small amount of water inside and kept it in insect rearing room at 25-27°C until egg hatched. After hatching, some 1st instar larvae were burrow through the muslin cloth into water and some still remains on the cloth. Then the cloth was dipped in distilled water to collect more larvae. 100 larvae were counted under magnifier and kept in petri dish containing water. They were poured into fine mesh cloth and transferred in to each test fruit by using camel's hairbrush. All infested fruits were left for 3 hour before subjecting to treatment to allow larvae to burrow inside the fruit.

### **3. Treatment facility**

3.1 Water bath for small scale was conducted by using Memmert® water bath, model: WNB 22. Water bath is electrically heated and electronically controlled. Heating positioned is on three sides around the tank to ensure a natural water circulation of the water inside, thus securing an optimal uniform temperature distribution. The temperature of the water is continuously controlled by a microprocessor-controller. The temperature is measured by using a PT 100 temperature sensor (4-wire circuit). The components of temperature control are controlled by integrated malfunction-recognition (Figure 3).

3.2 Water bath for large scale was conducted by using stainless steel tank (2.53x1.35 x0.6 m) capable of holding 12 baskets, capacity of 20 kilogram per basket. The temperature of

the water is continuously controlled by a microprocessor-controller. The temperature is measured using a PT 100 (Class A). Water circulation system using water pump size 40 l /min for uniform temperature distribution. (Figure 4)

### **3.3 Calibration of resistance thermometers**

All the sensors were calibrated periodically with a certified precision quality thermometer at temperature 47°C. The test was conducted by dipping all sensors and standard thermometer into temperature controlled water bath. The test was conducted at 47°C of water bath.

Water temperature was allowed to stabilize for 0:20 minutes before data recording began. The actual temperature of water was confirmed with the standard thermometer. Temperature readings of all sensors were recorded every 0:05 minutes. Calibration was conducted regularly.

### **3.4 Monitoring of fruit temperature**

Fruit temperatures were determined by using platinum resistance thermometer PT 100, protective tube length 70 mm and protective tube diameter 3 mm. The sensors were connected to 6 points-multifunction hybrid recorder (Shinko, model: HR-700).

### **3.5 Determination of mortality and holding conditions after treatment and observation**

After the treatment, the fruit temperature was cooled down by force air cooling for 30 min. All the fruits were individually placed in organandy bag. Then kept them separately in plastic container (33x41x12 cm) and covered with fine mesh clothes to prevent reinfestation. They were held in temperature controlled room at 25-27°C until observation. To facilitate larval survival and pupation, test fruits which were obtained by forced infestation method were longitudinally cut 1 or 2 days after treatment. Treatment mortality was determined by dissecting the fruits within 4-5 days after treatments and counting the number of dead and alive larvae. The numbers of live larvae in each fruit were recorded. Corrected mortality was calculated by using Abbott's formula (Abbott, 1925). The data was analyzed for Probit 9 (Polo-Plus, POLO for Windows, LeOra Software, 1007 B St., Petaluma, CA 94952, USA).

## **5. Preliminary disinfestation test to determine the treatment temperature and exposure period of hot water immersion treatment to disinfest oriental fruit fly, *B. dorsalis* in mangoes**

Lapasathukul et al., (2002) studied heat tolerance of immature stages of 4 fruit fly species in Thailand, *B. carambolae*, *B. dorsalis*, *B. papayae* and *B. pyrifoliae* by immersion eggs, 1st, 2nd and 3rd instar larvae in hot water at 45°C at various holding time. It was found that 1st instar larvae of each fruit fly species were more heat tolerance than eggs, 2nd and 3rd instar larvae. Kaneyuki et al. (2014) found that early- and mid-aged first instars being more tolerant than late-aged at 45°C. Furthermore, Kaneyuki et al. (2016) report that at 45°C

found that heat tolerance decreased with increasing age of 1st and 2nd instar *B. dorsalis*, but increased with increasing age of 3rd instars, with 1st instars being the most tolerant .

Based on this result, the efficacies of hot water immersion treatment for this experiment were tested against 1st instar larvae and egg stage which are the most resistance developmental stage.

Artificial infestation method for the tested fruits was prepared. The stages of fruit fly to be tested were egg and first instar. The fruit used were medium and large size (310-420 g). The treatments in the Memmert® water bath, model: WNB 22 were 43°C, 44°C, 45°C, 46°C, 46°C+5 min, 46°C+10 min, 46°C+15 min and 46°C+20 min. The tested fruits were then dipped in the water bath one treatment at a time. 5 replications were performed. Each replication comprised of 3 treated fruits of egg infestation, 3 treated fruits of first instar larva infestation. There were total 270 treated fruits and 30 fruits (15 fruits of egg infestation and 15 fruits of first instar larva infestation) as control (non-treated).

#### **6. Large scale disinfestation test for oriental fruit fly in Nam Dork Mai mangoes**

Large scale disinfestation test was carried out to assess the effectiveness of potential quarantine schedule which the based on the result in the preliminary test.

The treatment to be tested was 46°C for 10 min in commercial water bath. Artificial and forced infestation test fruits were prepared as described in 2.2.1, 2.2.2 and 2.3.2. This experiment aimed to disinfest first instar larva of oriental fruit fly in mangoes not less than 30,000 larva. Three replications were performed in this experiment. Each replication comprised of one hundred artificial infested fruits, fifty forced infested fruits and 144 filler fruits were used in the experiment. The total hot water dipped fruits were 294. All the fruits were divided into 12 plastic baskets. One basket comprised of 8 artificial infested fruits, 4 forced infested fruits and 12 filler fruits. The rest of 4 artificially infested fruits and 2 forced infested fruits were randomly place in the baskets. And there were 20 artificial infested fruits and 10 forced infested fruits to be used as control (non-treated). Total number of the treated fruit was 450 fruits or approximately 55,850 of treated 1st instar larva and 90 control (non-treated) or approximately 11,170 of control 1st instar

#### **7. Fruit injury test**

This study is to determine the symptoms of thermal injury in mango. The tests were carried out by using medium to large size mango ( 310-420 g).

7.1 Small scale test: The mangoes were place in plastic basket (24x30x17 cm) then dipped in water bath, Mamert ®WNB 22 at the temperature 48.5°C. Ten treated fruits and ten control (non-treated) were used in each treatment. The treatments were done when the innermost temperatures reach 48.5°C, 48.5°C+10 min and 48.5°C +20 min. Then the treated fruits were air cool for 30 min.

#### **7.2 Simulation test**

This study is to determine the quality of treated fruits that simulated in the exportation by air and by sea and compare the various quality characteristic with the non-treated fruits. Twenty fruits of each were used in the study. The fruits were treated at the innermost temperature of 46°C for 10 min. Air cooling for 30 min after the treatment then put in foam net and placing the box (32x45x13 cm) with 4 holes of 2.5 cm in diameter on both sides which covered with fine mesh. The fruits were kept in temperature controlled room at 10°C for 7 and 14 days.

#### 1. Percentage of weight loss

Comparison between the mango fruits that were hot water immersion treatment immersed of 10 fruits and the mango fruits that did not hot water immersion treatment (control) of 10 fruits. Each fruit was weighed at 0, 7 and 14 day after storage. The weights were recorded. Percentages weight loss were calculated using the following equation:

Percentage weight loss =  $\frac{[(\text{pretreatment fruit weight} - \text{post storage fruit weight})/\text{pretreatment fruit weight}] \times 100$

The percentages of weight loss of the fruits were averaged and analyze statistical compare the mean by T-test

#### 2. Brix value

Comparison between the mango fruits that were hot water immersion treatment immersed of 9 fruits and the mango fruits that did not hot water immersion treatment (control) of 9 fruits. Method 3 fruits/time (use the same set of mango as the firmness), with the method of squeezing juice from mango meat for analysis of total soluble solid (TSS) in units of degrees of brix. Measurement of brix value from mango meat using a digital refractometer (DBX-30, Atago Co., Ltd., Tokyo, Japan). Brix value of each method was measured into 3 replicates at 0, 7 and 14 day after storage. Brix value of the fruits were averaged and analyze statistical compare the mean by T-test

#### 3. Firmness

Comparison between the mango fruits that were hot water immersion treatment immersed of 9 fruits and the mango fruits that did not hot water immersion treatment (control) of 9 fruits. Each fruit was divided into 2 sides. Each side was subdivided into 3 parts: top, middle, and bottom. Firmness of each piece was measured into 3 replicates using penetrometer at 0, 7 and 14 day after storage. The firmnesses of the fruits were averaged and analyze statistical compare the mean by T-test

### **Time and place**

The study began in October 2013, and ended in September 2018

Experiments were performed at:

- Laboratory Pest Management Group, Plant Protection Research and Development Office, Department of Agricultural



- Laboratory Crop Processing Research and Development Group, Postharvest and Processing Research and Development Division, Department of Agricultural
- The packing vegetables and fruits of V.S. Freshco Company Limited

## Result and Discussion

### Preliminary disinfestation test

The mortality of eggs and 1st instar larvae of the oriental fruit fly, *B. dorsalis* were presented in Table 1. There was 100 percent mortality of eggs stage at the innermost temperature 46°C and at 46°C with various holding times, 46°C+5 min, 46°C+10 min, 46°C+15 min and 46°C+20 min. At the temperature 43°C, 44°C and 45°C the mortality was 61.94, 95.59 and 99.35 percent respectively. For 1st instar larvae stage was 100 percent mortality of eggs stage at the temperature 46°C maintained at this temperature for 5 min (46°C+5 min.) to 46°C maintained at this temperature for 20 min (46°C+20 min.). At the temperature 43°C, 44°C, 45°C and 46°C the mortality was 78.29, 85.89, 98.16 and 99.93 percent respectively.

### Large scale confirmatory test

The result showed that to obtain probit 9 security (required to kill 99.99683%), eggs stage need temperature 44.46°C and 57.43 minutes while 1st instar larvae stage need temperature 45.07°C and 60.62 minutes. Lapasathukul et al., (2002) reported that estimated lethal dipping time for egg and 1st instar larvae stage of *B. dorsalis* in hot water at 45°C was 17.73 and 29.58 min. respectively. The result show that 1st instar larvae stage of *B. dorsalis* was most tolerance same this experiment. According to the result, 1st instar larvae stage of *B. dorsalis* was the most tolerance. The estimated lethal immersion temperature and immersion time is 45.07°C and 60.62 minutes for kill 1st instar larvae stage of *B. dorsalis* at 99.99683%. However, temperature at the innermost of mango at 46°C+10 min or immersion temperature at 46°C for 70 min and cooling in air blowers for 30 minute was chosen for further investigation. The results are in a well-agreement with a study performed by Thanaaet al. (2011) suggest that treatment of hot water at 46°C for 90 min can disinfest fruit flies without any effect on the quality of mango fruits.

Table 2 showed time for innermost temperature of mango fruit under full loading conditions to 46°C and to be kept at 46°C for 10 min. The number of fruits and treated 1st instar larva used in this test were given in Table 3.

Figure 5 showed the innermost temperature of mango fruit during immersion in hot water at 43°C, 44°C, 45°C, 46°C, 46°C+5 min, 46°C+10 min, 46°C+15 min and 46°C+20 min, and cooling in air blowers for 30 minute when reading of temperature of 3 sensor fruits receiving required temperature and exposure periods.

### Fruit injury test

#### Small scale test:

The results on the quality of mangoes that were treated at 48.5°C for 0, 10 and 20 min showed that there were no different from the control in weight loss (Table 4) and Brix value (Table 5). There was no symptom of spongy tissue in the treated fruits which conformed to the study by Unahawutti et al. (1991) that modified vapor heat treatment mango at 48.5°C for 30 min did not show symptom of spongy tissue in Nam Dorkmai, Rad and Pimsaen Dang mango. Disease symptom (black spot) was found in the mangoes that were kept for 14 days. There were small black spots in the treated mangoes at 48.5°C for 10 and 20 min (Figure 6).

#### **Simulation test**

The quality of treated mangoes that were kept at temperature controlled room at 10.05±1.35°C and 74.32±5.18 % RH for 7 and 14 days showed that there were no statistic difference from the control mangoes in term of weight loss (Table 6), Brix value (Table 7) and flesh firmness (Table 8)

#### **Discussion**

The study of hot water immersion treatment of Nam Dorkmai mango infested with Oriental fruit fly, *B. dorsalis* for Export. It was found that hot water immersion treatment at fruit innermost temperature of 46.0°C and maintained at this temperature for 10 min is effective against the 1st instar larva which is most heat tolerance stage of *B. dorsalis*. It does not affect the quality of Nam Dorkmai mango fruits.

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**Table 1** Mortality (%)<sup>1/</sup> of egg and 1st instar larvae of the oriental fruit flies, *Bactrocera dorsalis* (Hendel) after treatment in hot water immersion at difference temperature and immersion periods.

Stage	Treatment <sup>2/</sup>	Time (min.) <sup>3/</sup>	Number treated	Number dead	Corrected mortality <sup>4/</sup> (%)
Egg	Control	0	1,500	118	0
	43°C	50	1,500	974	61.94
	44°C	55	1,500	1,439	95.59
	45°C	57	1,500	1,491	99.35
	46°C	60	1,500	1,500	100
	46°C+5 min	65	1,500	1,500	100
	46°C+10 min	70	1,500	1,500	100
	46°C+15 min	75	1,500	1,500	100
	46°C+20 min	80	1,500	1,500	100
1 <sup>st</sup> instar larva	Control	0	1,500	35	0
	43°C	50	1,500	1,182	78.29
	44°C	55	1,500	1,286	85.89
	45°C	57	1,500	1,473	98.16
	46°C	60	1,500	1,499	99.93
	46°C+5 min	65	1,500	1,500	100
	46°C+10 min	70	1,500	1,500	100
	46°C+15 min	75	1,500	1,500	100
	46°C+20 min	80	1,500	1,500	100

<sup>1/</sup> Combined data of 5 replicates.

<sup>2/</sup> Inoculation egg stage 100 eggs per fruit and 1<sup>st</sup> instar larva stage 100 individuals per fruit.

<sup>3/</sup> Timing for immersion hot water was initiation when the temperature of the water in water bath stabilized until fruits were considered receiving required temperature.

<sup>4/</sup> Mortality was corrected by using Abbott's formula

**Table 2** Time for innermost of mangoes “Nam Dorkmai” to attain 46°C+10 min.

Rep	Sensor fruit weight (g)	Loading	Time <sup>1/</sup> (h)
1	370	Full (108 kg)	1:10
	378		
	378		
2	372	Full (110 kg)	1:11
	376		
	378		
3	374	Full (113 kg)	1:13
	376		
	378		

<sup>1/</sup> Time for fruit innermost of 3 sensor fruits to attain target temperature and time

**Table 3** Survival of 1st instar larvae of the oriental fruit flies, *Bactrocera dorsalis* (Hendel) in mangoes treated with hot water immersion at the innermost temperature of 46°C for 10 min.

Rep.	Infestation method	No. test fruit		No. alive individual in control	Estimated treated population <sup>a/</sup>	No. survivors <sup>b/</sup>
		Control	Treatment			
1	1st instar larvae infestation	20	100	1,960	9,800	0
	forced infestation	10	50	1,763	8,815	0
	total	30	150		18,615	0
2	1st instar larvae infestation	20	100	1,940	9,700	0
	forced infestation	10	50	1,699	8,495	0
	total	30	150		18,195	0
3	1st instar larvae infestation	20	100	1,954	9,770	0
	forced infestation	10	50	1,854	9,270	0
	total	30	150		19,040	0
Total		90	450	11,170	55,850	0

<sup>a/</sup> The estimated treated populations were calculated from number of larvae in untreated fruits.

<sup>b/</sup> Survival of larvae was determined 4 days after treatment.

**Table 4** Weight loss (%) of mangoes “Nam Dorkmai” after hot water immersion at 48.5 °C holding times at 0, 10 and 20 min. and 7 and 14 days storage at 26.10±1.35°C, 75±5% RH

Day storage	Treatment	Weight loss (%) <sup>1/</sup>		
		0 min.	10 min.	20 min.
7	48.5°C	7.07	6.19	6.18
	control	5.25		
	t-test 48.5°C vs. Control	ns	ns	ns
14	48.5°C	12.23	12.37	12.23
	control	11.78		
	t-test 48.5°C vs. Control	ns	ns	ns

<sup>1/</sup>Value are mean of 10 fruits (treatment), and 10 fruits (Control), ns=non-significant  
\*=significant at 5% level

**Table 5** Total soluble solid (°Brix) of mangoes “Nam Dorkmai” after hot water immersion at 48.5°C holding times at 0, 10 and 20 min. and 7 and 14 days storage at 26.10±1.35°C, 75±5 % RH

Day storage	Treatment	Total soluble solid (°Brix) <sup>1/</sup>		
		0 min.	10 min.	20 min.
7	48.5°C	16.73	16.83	16.87
	control	16.97		
	t-test 48.5°C vs. Control	ns	ns	ns
14	48.5°C	17.67	17.53	17.60
	control	17.29		
	t-test 48.5°C vs. Control	ns	ns	ns

<sup>1/</sup>Value are mean of 10 fruits (treatment), and 10 fruits (Control), ns=non-significant  
\*=significant at 5% level

**Table 6** Weight loss (%) of mangoes “Nam Dorkmai” after hot water immersion at 46°C holding times at 10 min. and 7 and 14 days storage at 10.05+1.35°C, 74.32+5.18 % RH

Day storage	Treatment	Weight loss (%) <sup>1/</sup>		
		Rep. 1	Rep. 2	Rep. 3
7	46°C+10 min	5.10	6.96	5.97
	control	5.57		
	t-test 46°C+10 min vs. control	ns	ns	ns
14	46°C+10 min	13.32	13.53	13.14
	control	12.72		
	t-test 46°C+10 min vs. control	ns	ns	ns

1/ Value are mean of 10 fruits (treatment), and 10 fruits (Control), ns=non-significant  
\*=significant at 5% level

**Table 7** Total soluble solid (°Brix) of mangoes “Nam Dorkmai” after hot water immersion at 46°C holding times at 10 min. and 7 and 14 days storage at 10.05+1.35°C, 74.32+5.18 % RH

Day storage	Treatment	Total soluble solid (°Brix) <sup>1/</sup>		
		Rep. 1	Rep. 2	Rep. 3
7	46°C+10 min	16.75	16.81	16.97
	control	16.42		
	t-test 46°C+10 min vs. control	ns	ns	ns
14	46°C+10 min	17.85	17.94	18.12
	control	17.79		
	t-test 46°C+10 min vs. control	ns	ns	ns

1/ Value are mean of 10 fruits (treatment), and 10 fruits (Control), ns=non-significant  
\*=significant at 5% level



**Table 8** Firmness level (N) of mangoes “Nam Dorkmai” after hot water immersion at 46°C holding times at 10 min. and 7 and 14 days storage at 10.05±1.35°C, 74.32±5.18 % RH

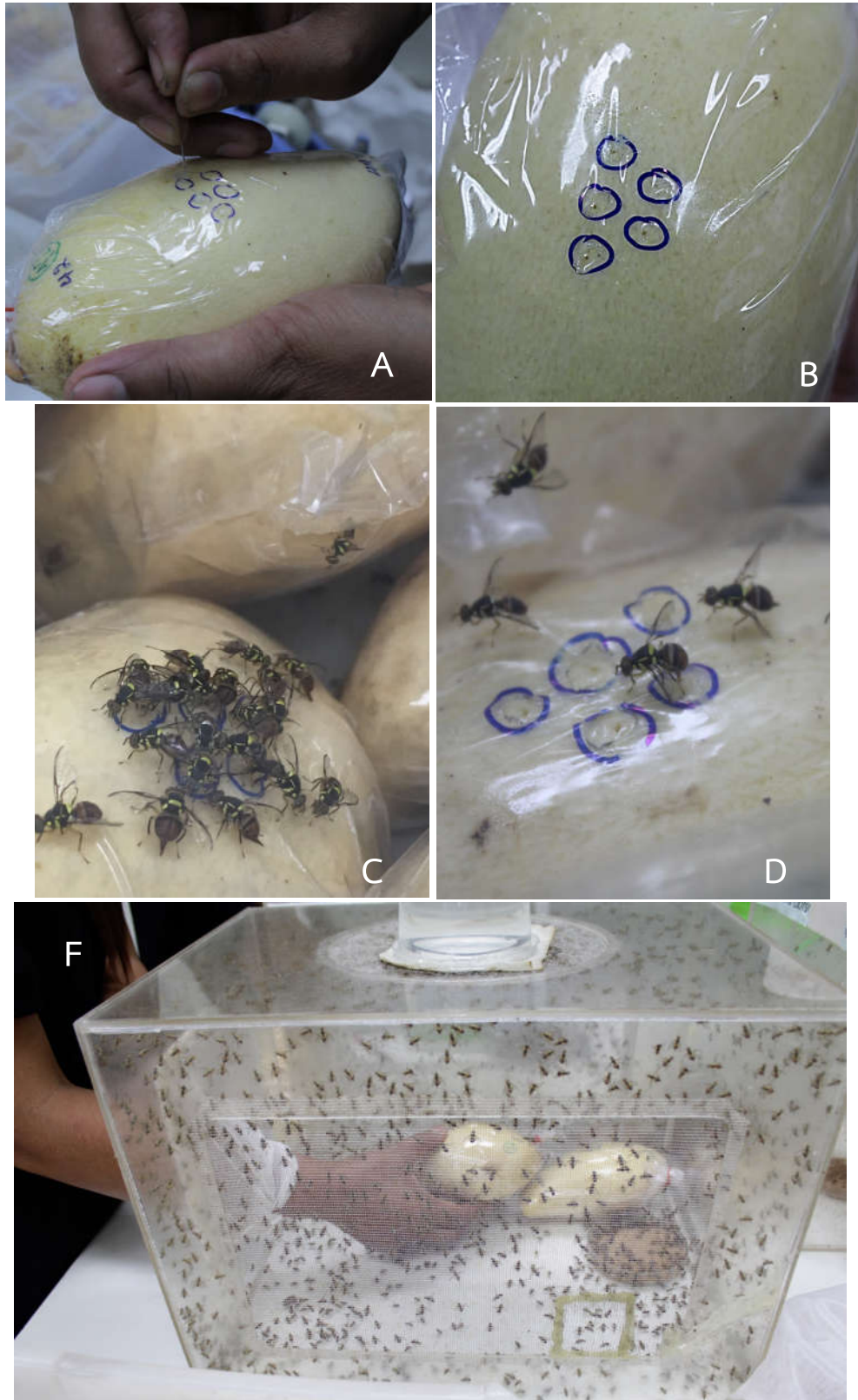
Day storage	Treatment	Firmness level (N) <sup>1/</sup>		
		Rep. 1	Rep. 2	Rep. 3
7	46°C+10 min	16.44	16.47	16.11
	control	17.58		
	t-test 46°C+10 min vs. control	ns	ns	ns
14	46°C+10 min	10.25	10.17	10.56
	control	11.94		
	t-test 46°C+10 min vs. control	ns	ns	ns

1/ Value are mean of 10 fruits (treatment), and 10 fruits (Control), ns=non-significant

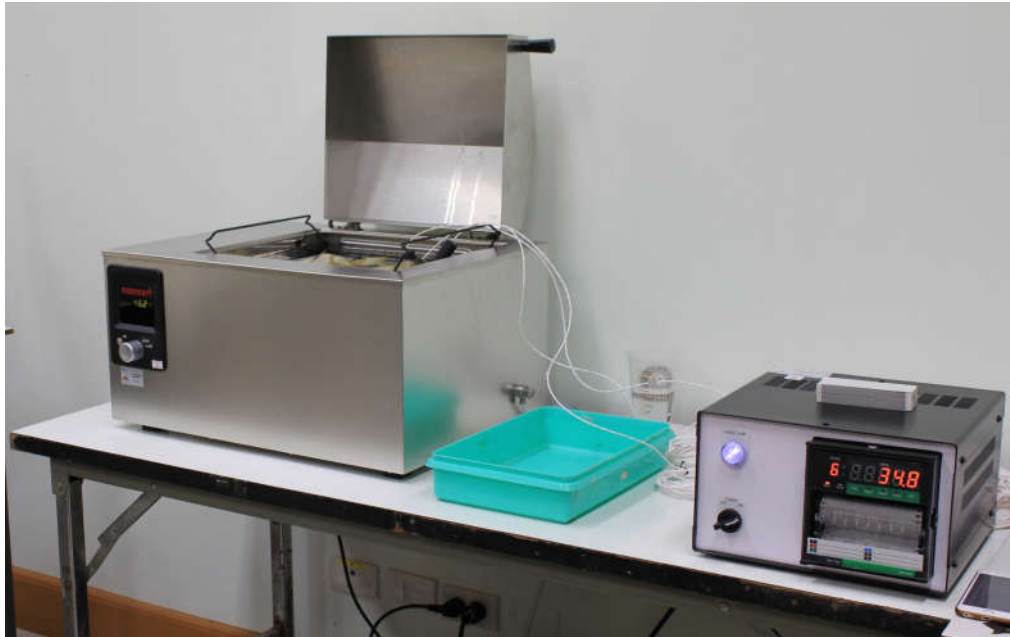
\*=significant at 5% level



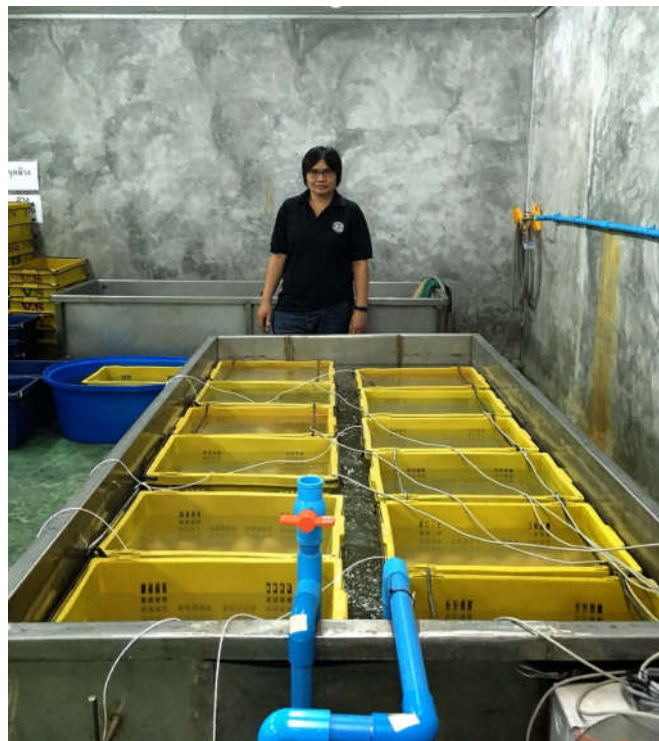
**Figure 1** Preparation of infested mangoes; artificial infestation method; (A-B) Holes were made by 1 cm diameter cork borer at 1 cm (C) Transfer the eggs on the hole (D) Close with masking tape



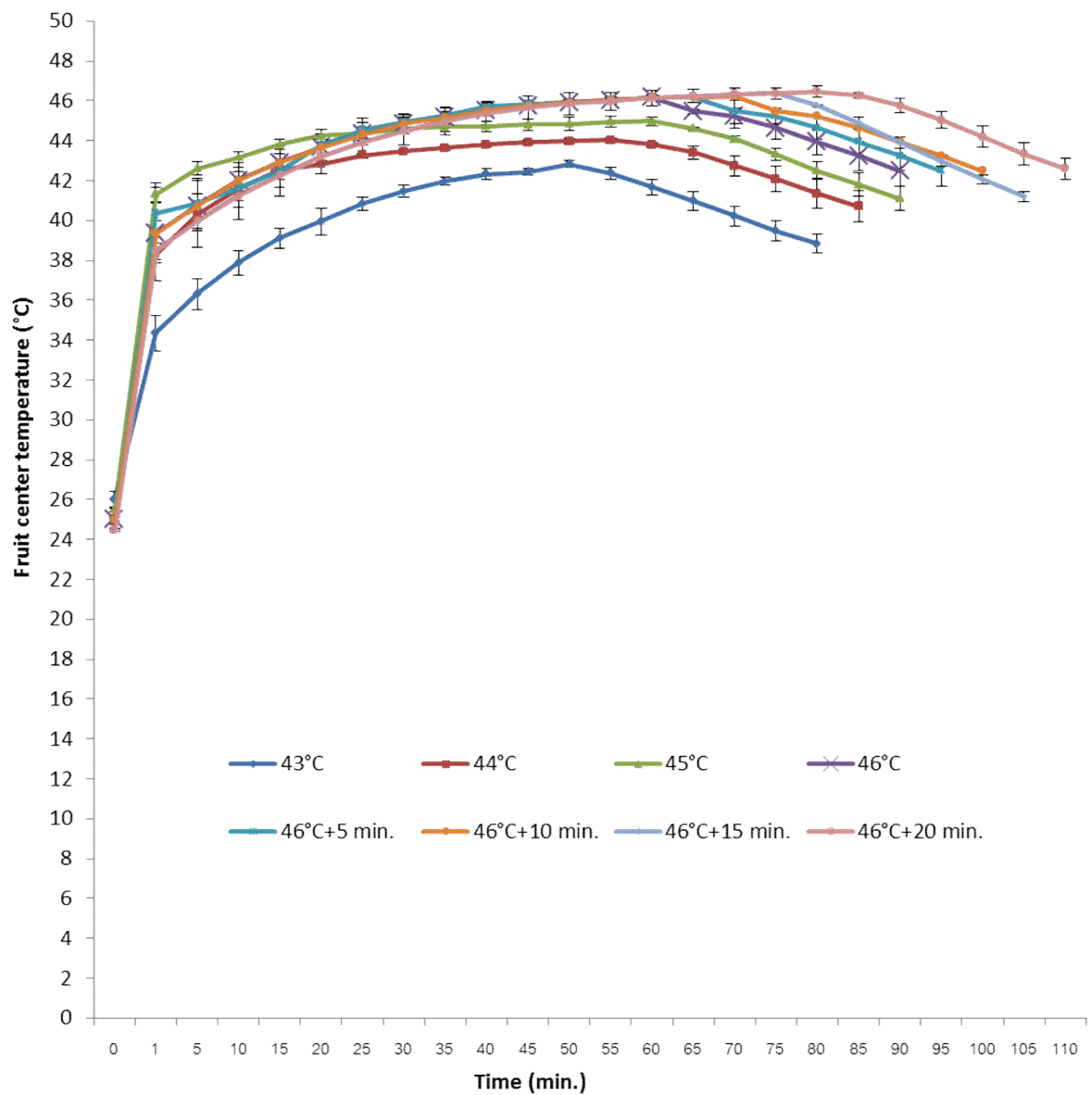
**Figure 2** Preparation of infested mangoes; forced infestation method; (A-B) Each mango fruit was wrapped up with clear plastic bag and made five punctures by insect pin (C-D) Female laying eggs (F) After 25 min., take the mango fruits out of the insect age



**Figure 3** Water bath for small scale was conducted by using Memmert® water bath, model: WNB 22.

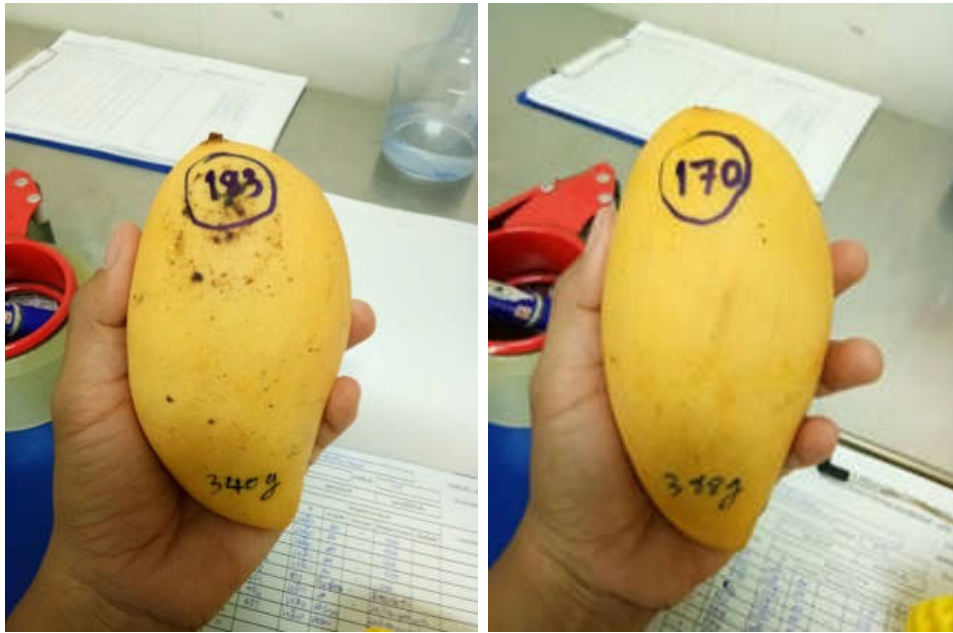


**Figure 4** Water bath for large scale was conducted by using stainless steel tank (2.53 m long by 1.35 m wide by 0.6 m deep) capable of holding 12 baskets.



**Figure 5** Innermost temperature of mango fruit during immersion in hot water at 43°C, 44°C, 45°C, 46°C, 46°C+5 min, 46°C+10 min, 46°C+15 min and 46°C+20 min, and cooling in air blowers for 30 minute.





**Figure 6** Disease symptom; Black spot on mangoes “Nam Dorkmai” after hot water immersion at 48.5°C holding times at 10 and 20 min. and 14 days storage at 26.10±1.35 °C, 75±5 % RH