# Pacific Horticultural and Agricultural Market Access Program (PHAMA)

Technical Report 17: Results of trials to confirm fruit fly non-host status for Polynesian Plum (Spondias dulcis) (FIJI12)

31 JULY 2012

Prepared for

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# BIOSECURITY AUTHORITY OF FIJI

**OFFICIAL REPORT**

Report on the Confirmatory Tests to Demonstrate the Efficacy of Commercial Force Hot Air as a Post-Harvest Quarantine Treatment for Eggs of *Bactrocera passiflorae*, Artificially Infested into Matured *Spondias dulcis* (wi).

# CONTENTS

**Aim**

To demonstrate that the commercial operation of the HTFA chambers, based on a shut-down of the heating cycle when the probed fruit (largest fruit by weight) located in the prescribed coldest location (the centre top of the bin or crate), reaches the kill value of at least 47.2$^{o}$C held for 20 minutes, will ensure complete kill of the most heat tolerant stage (early eggs) of the most tolerant species *Bactocera passiflorae* seeded in fruit of *Spondias dulcis* inside the chamber.

**Introduction**

The HTFA treatment of 47.2$^{o}$C held for 20 minutes is an approved quarantine treatment for fruit flies in Fiji. Fruit and vegetables considered to have potential for export are identified by exporters and growers and are tested by the Ministry of Primary Industries. *Spondias dulcis* or 'wi' as it is commonly known was identified as one such product by Nature's Way Co-operative Ltd. This test is being conducted to demonstrate that the HTFA treatment will be efficacious for a complete kill of the most heat tolerant stage (eggs younger than 10 hours old) of the most heat tolerant species, *B. passiflorae* (Frampton 1996).

The largest 'wi' fruit will be probed and placed in the coldest location of the chamber. Fruit seeded with the early eggs (also called infested fruit) will be placed around the probed fruit in the coldest spots. More than 10,000 eggs will be seeded into 100 fruit and it is mandatory that the exact final temperature is identified.

The 'wi' used for this test have been harvested from a number of trees near Agriculture offices, private homes and villages. The fruit are at the mature green stage, firm, undamaged and are of export quality. All fruit are free of pesticides.

This confirmatory test is being conducted using the same confirmatory test procedures that were conducted for eggplant, papaya, mangoes and breadfruit (Tora and Leweniqila 2001) and operating the commercial HTFA as stated in the Biosecurity HTFA Procedure Manual (2011). The HTFA facility is currently certified and approval was issued by NZMAF on 5$^{th}$ July, 2011 following an audit.

The project is funded by the Ministry of Primary Industries (MPI) and the Pacific Horticultural and Agricultural Market Access (PHAMA) Program respectively in the presence of the New Zealand Ministry of Agriculture officials.

**Materials**

*B. passiflorae* eggs younger than 10 hours old, 'wi' (*S. dulcis*) at mature-green stage, papaya domes, fine-tipped paint brush, plastic pipette, petri dishes, black filter paper, tissue paper, plastic beakers (500ml), counters, opaque containers, masking tape, permanent markers, scissors, styrofoam boxes, 70% alcohol, cotton wool, scalpel and blade, 4L containers and lids with central opening, sterilised sawdust, wire gauze, 100ml containers, sieve (2 mm), microscope, camera and data sheets.

**Methodology**

Origin of fruits

Fruits were harvested two days before the test was conducted. All fruits were sourced from trees that were free of insecticides, miticides, herbicides or fungicides and were all of export quality and at the mature stage.

At the Koronivia Research Station

From the bulk of the fruits harvested for testing, 120 of the largest fruit to be seeded were washed, weighed and numbered. 20 of these were used as control fruit. All fruits were securely sealed in styrofoam boxes and transported by road to the HTFA facility in Nadi.

Insects and Egg Collection (fruit fly culture laboratory at the Koronivia Research Station)

Eggs were extracted from the mass rearing laboratories at the Koronivia Research Station of the Ministry of Primary Industries. The fruit fly laboratory colonies were fed on sugar, water, enzymatic hydrolysed protein and Klebsiella pneumonia bacteria. The larvae were reared on a papaya based diet consisting of blended papaya, Torula Yeast and nipagin. Rejuvenation of colonies are carried out at least once a year and adults that were sexually mature (i.e. 3-4 weeks old) were used to produce eggs for the test. The laboratory colonies total adult population is capable of producing 50 000 eggs in a two hour period.

4 egging papaya domes were placed in two healthy *B. passiflorae* cages (two domes per cage), at 1am Wednesday 14th December, 2011. Three hours later, the egging domes were removed. Eggs were then washed from the dome using a spray of water from a hand operated sprayer; collected and percent egg hatch was prepared. Eggs were placed on moist black filter paper in petri dishes. The petri dishes were well sealed using masking tape, and tightly packed in opaque plastic containers in preparation for transport to the Commercial HTFA Unit at Nature's Way Co-operative Ltd.

The eggs were transported by plane to Nadi, accompanied by a fruit fly technician.

At the HTFA facility in Nadi

A second sample of eggs for percent egg hatch was prepared (refer to Appendix 2, Plate 2).

The 120 fruit that were pre-selected for seeding were wiped with 70% alcohol. Care was taken to ensure that the numbers were not wiped off. Using a scalpel and grafting knife, flaps/wedges were made at two lateral points on each side of the fruit. A fine-tipped paintbrush was used to gently place approximately 50 eggs under each flap/wedge and secured in place with masking tape.

The chamber used for the treatment has 4 temperature probes. Fruit centre temperature is measured throughout the treatment by probing the largest fruits which are placed in cold-spots and the base of the probes secured with masking tape to prevent unnecessary air and water contact with the probe. The 100 infested fruit were distributed around the probed fruits in single layers in each lug and heated air is forced over the fruit and through the open lattice bottoms. The HTFA treatment was operated following the "1996 Quarantine Procedures for the Commercial Heat Treatment Chamber, Nadi Airport" (Revised May 2011) approved by MAF.

Hydro-cooling

After treatment, fruits were hydro-cooled immediately by spraying the fruits with water. This continued until temperature reached ambient (below 30$^{o}$C).

Control fruits

The control fruits were infested with eggs at the same time as the treated fruits. They were held at 25$^{o}$C in a screened air-conditioned room during treatment.

After HTFA Treatment

Once fruit reaches ambient temperature after treatment, probes were removed and treated fruits off loaded from the chamber. The treated fruit were gently placed in styrofoam boxes and securely sealed with masking tape. These were transported back to the Koronivia fruit fly laboratory where each infested fruit were placed on sterilised sawdust in secure plastic containers, 1 fruit per container, for an incubation period of 14 to 17 days. At the conclusion of the incubation period, the fruit were dissected and the number of immature stages and adults were recorded.

**Results & Discussions**

Efficacy of treatment is measured by adult emergence from treated and control fruits.

A. Tables Showing Percent Egg Hatches

Table 1: Total Egg Production After 3 Hours

| Date | Cage No. | No. of Eggs Produced | Percent Egg Hatch | |
|---|---|---|---|---|
| | | | KRS | Nadi |
| 14/12/2011 | Bp 603 | 30,981 | 61.0% | 71.3% |
| 14/12/2011 | Bp 605 | 11,598 | 82.7% | 87.0% |

Time in to collect eggs from Cage Bp 603 & Bp 605: 1:00 am

Time Out to end extraction of eggs of Cages Bp 603 & Bp 605: 4:00am

Table 2: History of Egg Hatch Test

| Date | Cage no. | Percent egg hatch |
|---|---|---|
| 18/11/2011 | Bp 603 | 95% |
| 18/11/2011 | Bp 605 | 96% |

B. Table Showing Results of Fruit Fly Development in Treated Fruit

| Fruit No. | Fruit Wt. (g) | Bin No. | Maturity | No. of Pupae | Wt. 100 Pupae | Total Wt. | Adults | Comments |
|---|---|---|---|---|---|---|---|---|
| 1 | 191.86 | 25 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 2 | 141.41 | 25 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 3 | 163.03 | 25 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 4 | 143.33 | 25 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 5 | 150.49 | 25 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 6 | 142.27 | 17 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 7 | 127.11 | 17 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 8 | 145.47 | 17 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 9 | 157.64 | 17 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 10 | 144.88 | 17 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 11 | 130.03 | 17 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 12 | 163.98 | 19 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 13 | 137.42 | 19 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 14 | 137.61 | 19 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 15 | 146.23 | 25 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 16 | 160.28 | 17 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 17 | 143.58 | 20 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 18 | 137.03 | 20 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 19 | 128.63 | 19 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 20 | 133.40 | 25 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 21 | 134.36 | 17 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 22 | 136.78 | 19 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 23 | 134.94 | 19 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 24 | 135.66 | 19 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 25 | 147.63 | 25 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 26 | 122.02 | 17 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 27 | 157.98 | 17 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 28 | 133.59 | 19 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 29 | 130.51 | 25 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 30 | 137.93 | 25 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 31 | 127.22 | 17 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 32 | 139.79 | 17 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 33 | 123.08 | 24 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 34 | 137.54 | 17 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 35 | 136.33 | 25 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 36 | 124.04 | 24 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 37 | 123.83 | 24 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 38 | 135.43 | 17 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 39 | 131.29 | 24 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 40 | 122.93 | 23 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 41 | 119.73 | 24 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 42 | 123.55 | 20 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 43 | 148.50 | 23 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 44 | 135.45 | 23 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 45 | 172.97 | 23 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 46 | 135.92 | 24 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 47 | 131.02 | 25 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 48 | 136.56 | 20 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 49 | 151.07 | 24 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 50 | 118.04 | 23 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 51 | 130.39 | 24 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 52 | 159.34 | 23 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 53 | 169.54 | 23 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 54 | 149.26 | 23 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 55 | 139.26 | 20 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 56 | 124.81 | 20 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 57 | 126.58 | 24 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 58 | 138.13 | 20 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 59 | 147.65 | 20 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 60 | 145.34 | 25 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 61 | 168.13 | 20 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 62 | 132.62 | 24 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 63 | 132.52 | 24 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 64 | 134.70 | 20 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 65 | 155.18 | 19 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 66 | 164.23 | 23 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 67 | 133.76 | 25 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 68 | 137.45 | 20 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 69 | 134.64 | 24 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 70 | 136.36 | 20 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 71 | 128.37 | 20 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 72 | 169.05 | 23 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 73 | 141.82 | 24 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 74 | 115.72 | 23 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 75 | 126.39 | 23 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 76 | 127.46 | 23 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 77 | 121.24 | 17 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 78 | 131.63 | 25 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 79 | 146.90 | 17 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 80 | 135.82 | 19 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 81 | 143.88 | 19 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 82 | 129.55 | 19 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 83 | 131.48 | 24 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 84 | 128.17 | 24 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 85 | 150.55 | 19 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 86 | 148.97 | 23 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 87 | 149.03 | 23 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 88 | 169.57 | 24 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 89 | 130.89 | 24 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 90 | 130.89 | 17 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 91 | 129.40 | 20 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 92 | 119.55 | 19 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 93 | 118.16 | 19 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 94 | 153.67 | 23 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 95 | 127.93 | 25 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 96 | 130.92 | 19 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 97 | 120.57 | 20 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 98 | 124.48 | 20 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 99 | 114.14 | 17 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |
| 100 | 139.32 | 23 | Mature green | Nil | Nil | Nil | Nil | Dead unhatched eggs |

It was observed that during dissection, all eggs were unhatched and dead. During the sieving process, there were no pupae collected. The fruit were also seen to have deteriorated, with signs of mould around the incisions made during the seeding process.

C. Table Showing Results of Fruit Fly Development in Control Fruit

| Fruit No. | Fruit Wt. (g) | Maturity | No. of Pupae | Pupal Wt. (g) | Adults | | | Comments |
|---|---|---|---|---|---|---|---|---|
| | | | | | ♂ | ♀ | Total | |
| 1 | 151.62 | Mature green | 34 | <10 | 3 | 2 | 5 | 29 unemerged |
| 2 | 147.69 | Mature green | 7 | <10 | 0 | 1 | 1 | 6 unemerged |
| 3 | 128.16 | Mature green | 16 | <10 | 3 | 2 | 5 | 11 unemerged |
| 4 | 122.13 | Mature green | 4 | <10 | 0 | 0 | Nil | 4 unemerged |
| 5 | 122.96 | Mature green | Nil | <10 | 0 | 0 | Nil | 24 larvae |
| 6 | 139.84 | Mature green | Nil | <10 | 0 | 0 | Nil | 7 dead larvae |
| 7 | 127.17 | Mature green | 31 | <10 | 2 | 0 | 2 | 29 unemerged |
| 8 | 131.62 | Mature green | 45 | <10 | 2 | 5 | 7 | 38 unemerged |
| 9 | 138.33 | Mature green | 5 | <10 | 0 | 0 | Nil | No emergence |
| 10 | 121.98 | Mature green | 35 | <10 | 2 | 1 | 3 | 32 unemerged |
| 11 | 148.22 | Mature green | 18 | <10 | 3 | 2 | 5 | 13 unemerged |
| 12 | 121.60 | Mature green | 24 | <10 | 1 | 1 | 2 | 22 unemerged |
| 13 | 120.41 | Mature green | 18 | <10 | 0 | 0 | Nil | 13 larvae |
| 14 | 120.32 | Mature green | 15 | <10 | 1 | 0 | 1 | 14 unemerged |
| 15 | 143.53 | Mature green | 3 | <10 | 0 | 0 | Nil | 4 dead larvae |
| 16 | 138.39 | Mature green | 26 | <10 | 3 | 4 | 7 | 1 larva |
| 17 | 128.96 | Mature green | 25 | <10 | 0 | 0 | Nil | No emergence |
| 18 | 124.72 | Mature green | 8 | <10 | 0 | 0 | Nil | No emergence |
| 19 | 127.76 | Mature green | 26 | <10 | 1 | 0 | 1 | 25 unemerged |
| 20 | 135.38 | Mature green | 18 | <10 | 1 | 0 | 1 | 17 unemerged |

At the end of the incubation period, it was noted that though there were a few pupae, majority of the larvae were still in their 3$^{rd}$ instar. These were left for another 3 to 5 days to pupate before they were rechecked.

In addition to the 20 control "wi" fruits, 2 ripe papaya fruit weighing 940g were used. From this, 33 pupae were collected of which 9 adults emerged.

**The treatment print-out (Appendix 1) confirmed that all probes had reached 47.2$^{o}$C.**

**Conclusion**

Based on the results above, heating the fruit core to 47.2$^{o}$C and holding it at that temperature for 20 minutes is an effective quarantine treatment in killing the early eggs (eggs younger than 10 hours old) of *Bactrocera passiflorae* infested in wi.

**Recommendation**

It is therefore recommended that this treatment be approved as an acceptable quarantine treatment for *Spondias dulcis* from Fiji.

**References**

MAF Regulatory Authority Standard 155.02.03: Specification for the Determination of Fruit Fly Disinfestations Treatment Efficacy (Lo – 155.02.02 is for non host testing).

Frampton C.M and Evans A. 1996. Report on statistical analysis of heat tolerance studies of *Bactrocera passiflorae* and *B. xanthodes*. A report prepared for the Ministry of Agriculture, Fisheries & Forests, Fiji.

1996 Quarantine Procedures for the Commercial Heat Treatment Chamber, Nadi Airport. Revised 2011.

Tora Vueti E and Leweniqila L, 2001. Status of Fruit Flies in Fiji. Secretariat of the Pacific Community, Fiji.

**Appendix 2 (**Photos of the Confirmatory test process)

Plate 1: Harvesting 'wi' at the Lakena Agriculture Office.

Plate 2: 11,598 *B.passiflorae* eggs collected in 3 hours from cage no. Bp 605

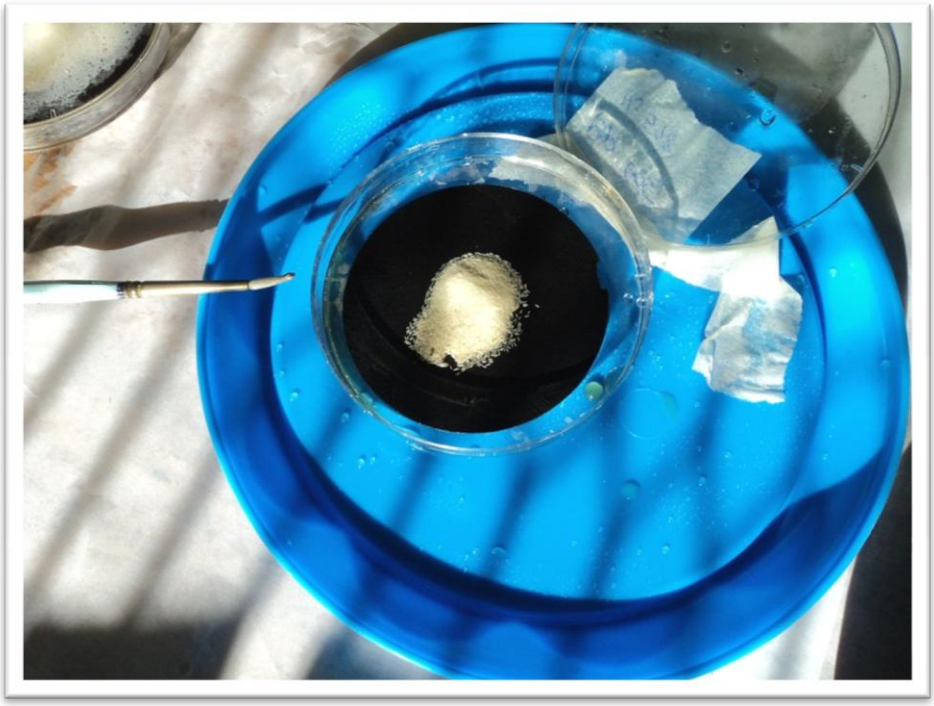

Plate 3: Preparation of second sample of eggs for percent egg hatch determination, Nadi.

Plate 4: Cutting a wedge in which fruit fly eggs will be seeded.

Plate 5: Unloading bins after treatment.


Plate 6: Placing treated fruit into styrofoam box.

Plate 7: Treated fruit placed over sterilized sawdust for incubation

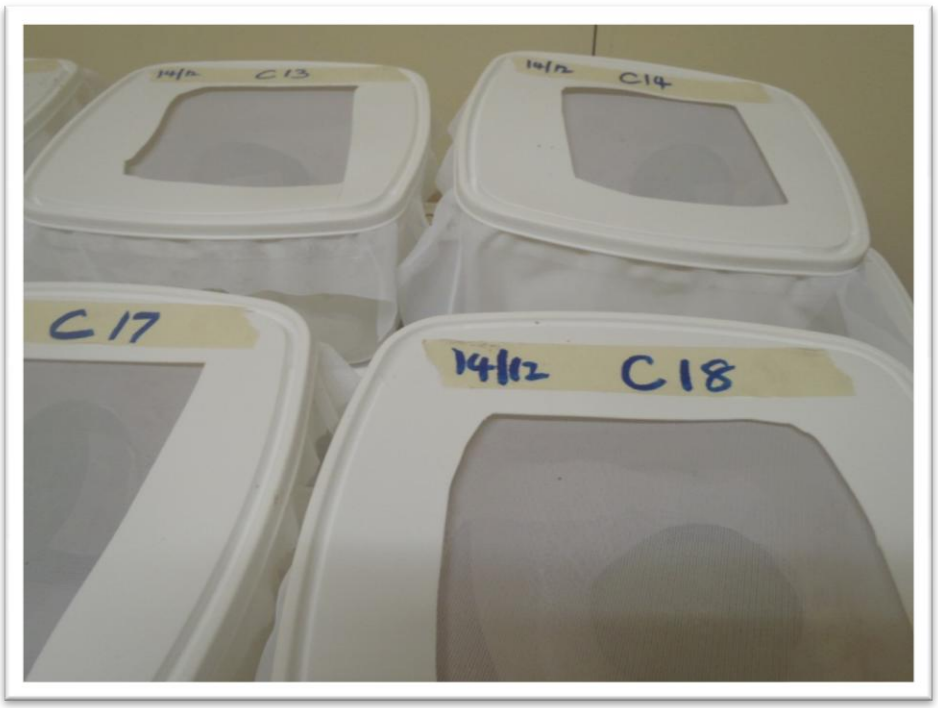


Plate 8: Treated fruit tightly sealed in labelled containers.

Plate 9: Fruit held in laboratory for incubation at 25°C.


Plate 10: Treated fruit after 14 days.

Plate 11: Unhatched eggs in treated fruit after 14 days.



Plate 12: Examining unhatched eggs under microscope.

Plate 13: Dead unhatched eggs in treated fruit confirmed under microscope.

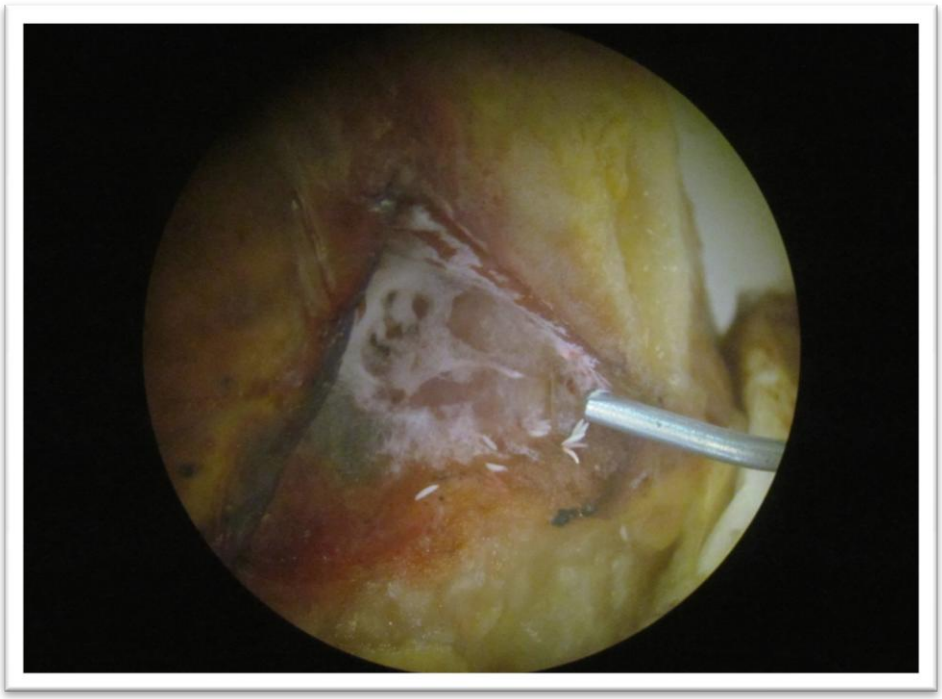


Plate 14: Dissecting treated fruit.

Plate 15: Sieving sawdust for pupae collection.


Plate 16: Rechecking control fruit.

Plate 17: Third instar larva on control fruit.

## Limitations

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