Final Report

Alternative disinfestation for market access for crops affected by tomato potato psyllid

Project leader:

Dr. Sonya Broughton

Delivery partner:

Department of Primary Industries and Regional Development

Project code:

VG17015

Project:

Alternative disinfestation for market access for crops affected by tomato potato psyllid (VG17015)

Disclaimer:

Funding statement:

This project has been funded by Hort Innovation, using the vegetable research and development levy and contributions from the Australian Government. Hort Innovation is the grower-owned, not-for-profit research and development corporation for Australian horticulture.

Publishing details:

ISBN 978-0-7341-4850-6

Published and distributed by: Hort Innovation

Level 7
141 Walker Street
North Sydney NSW 2060

Telephone: (02) 8295 2300

www.horticulture.com.au

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Public summary

Tomato potato psyllid (TPP), *Bactericera cockerelli* (Sulc.) is a serious pest of solanaceous crops and some Convolvulaceae crops and is associated with extensive economic losses. TPP is a vector of a bacterial plant pathogen, *Candidatus liberibacter solanacearum* (CLso), which causes psyllid yellows disease in tomato, potato, capsicum, eggplant and tamarillos, and zebra chip disease in potato. It is native to western United States and Mexico and was first found in New Zealand in 2006 and on the Australian territory of Norfolk Island in 2015. The first detection of TPP on the Australian mainland occurred in the Perth metropolitan region of Western Australia (WA) in February 2017. To date, TPP is limited to WA and zebra chip disease has not been found in WA.

Another destructive pest species that also shares some plant hosts species with TPP is the Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Wiedemann). Limited to WA where it is endemic, Medfly is associated with significant economic losses indirectly affecting market access of horticulture commodities. Industry stakeholders in the eastern states and territories are concerned about the impact of both pests if these were to spread to their jurisdictions. The current strategy is to confine both pests to their current distribution, prevent their spread, surveillance, and minimise their impact on domestic and international trade. Presently, methyl bromide (MB) fumigation is used as an endpoint postharvest treatment option against TPP and Medfly in most produce. So, developing additional postharvest disinfestation treatments against both pests in their common host crops would support trade from WA.

Three alternative treatments were investigated: low dose methyl bromide (LDMB), ethyl formate (EF) and ionising radiation. Several treatments using LDMB, EF and Vapormate® were identified that were effective in killing more than 30,000 insects with no survivors that satisfied the quarantine standard of 99.99% control. Probit 9 level of control was achieved with four LDMB doses against Medfly eggs in red capsicum, 24 mg/L at 21°C for 5.5 h, 24 mg/L at 13°C for 7.5 h, 32 mg/L at 21°C for 5.5 h and 32 mg/L at 13°C for 6 h and with two new 2 h-doses of 16 and 24 mg/L at 21°C against TPP eggs. For TPP nymphs and adults, a probit 8.719 level of control was achieved with the two new 2h-doses against. EF dose of 40 mg/L + 15% CO_2 effectively killed TPP eggs in laboratory scale trials. A probit 8.719 level of control was achieved with Vapormate® dose of 54 mg/L at 10°C for 2 h. X-ray irradiation dose ≥350 Gy resulted in mortality of TPP eggs and nymphs and rendered unviable eggs from females irradiated at ≥400 Gy. The data supporting the treatments contained in this report are the main outcomes of this project. This information is sufficient to gain and maintain access to interstate markets and sufficient basis for negotiating international access and trade.

Keywords

Tomato potato psyllid, Mediterranean fruit fly, disinfestation treatments, market access, low dose methyl bromide, ethyl formate, phytosanitary irradiation.

Introduction

Tomato potato psyllid (TPP), *Bactericera cockerelli,* (Sulc.) is an exotic pest that was reported in the Australian mainland in February 2017. So far, it is limited to Western Australia (WA). The incursion of TPP has serious ramifications for several horticulture industries, particularly in the restricted movement of plant material from WA. Some of the economically important crops affected are capsicum, chilli, eggplant, and tomato. These crops are also host to Mediterranean fruit fly (Medfly), *Ceratitis capitata,* (Wiedemann) which is an endemic pest species to WA. There are various recognised phytosanitary measures in place for host produce and plant products, and often, to gain market access into the eastern states or internationally, end-point treatments are required to export produce.

Currently, methyl bromide (MB) fumigation is a standard postharvest disinfestation treatment against both pests and is recognised as an approved phytosanitary treatment for all life-stages of TPP by Australia and its trading partners like Japan. MB fumigation can disinfest internal and external pests. Although MB has been phased out globally for most of its use, it is controlled under the Montreal Protocol by many countries. In Australia, it is exempt for quarantine preshipment, feedstock application and certain critical use exemptions. However, it is not widely used in Australia for capsicum and tomato as it is phytotoxic at higher doses. An alternative would be using lower doses with longer exposure times that can reduce environmental impacts, the associated costs of using MB, fruit injury, and chemical residue. Reducing MB doses can also reduce total treatment time by halving venting time for fumigation chambers. Use of low dose methyl bromide (LDMB) was successfully demonstrated for Medfly in tomato with lower doses of 24 mg/L at 13°C for 7.5 h or at 21°C for 5.5 h and 32 mg/L at 13°C for 6 h or 21°C for 5.5 h being effective against eggs and larvae (VT12000). The efficacy of LDMB has also been shown to be effective against Queensland fruit fly in summer fruit (SF12016) and citrus (CT14008). However, the LDMB dose rates against TPP are unknown.

The TPP incursion in WA prompted a large biosecurity emergency response in 2017 - 2018 which was managed by the Department of Primary Industries and Regional Development. The transition to management (T2M) phase of the response had demonstrated the potential of ethyl formate (EF) as an alternative disinfestation treatment. Generally Recognised as Safe (GRAS), EF is a fumigant that is primarily effective on external pests but is not known to control internally feeding insects such as fruit fly. The work on EF during T2M, found it to be effective against nymphs and adults of TPP. However, the EF doses suitable for those life-stages did not affect eggs. Hence, it is necessary to develop EF treatments in combination with other gases such as carbon dioxide that are known to enhance EF toxicity.

Another alternative treatment is the use of ionising radiation. Irradiation may either completely kill or prevent an insect from further development however, effective dose rates against TPP need to be identified. The benefits of using irradiation are general worldwide quarantine acceptance, approval of generic doses and no or minimal damage to produce. For fruit flies, a generic dose of 150 Gy is accepted. Since TPP falls in the higher generic dose of 400 Gy, specific data to reduce it needs to be provided.

The overall objectives of the project are to ensure continuity of supply of Australian vegetables to consumers and to ensure containment of TPP to its current distribution range. Specifically, the project aims to carry out research into alternative disinfestation treatments to support trade of fresh produce from WA. The project will conduct necessary trials to satisfy interstate quarantine requirements and demonstrate that fumigation (LDMB and EF) and irradiation treatments can be used to disinfest both, Medfly and TPP in capsicum, chilli, eggplant, and tomato. Proof of disinfestation must demonstrate control of at least 30,000 insects with no survivors at specified treatment level. This involves series of trials that would be carried out, first, to investigate insect development through life history studies, followed by most tolerant life-stage (MTS) trials using various doses and finally by large-scale trials. Life history studies are done to determine the days required to obtain a desired life-stage, and numbers of fruits required to achieve the desired insect numbers. MTS trials include all life-stages tested against a range of dose and time to identify the stage that is hardest to control. Effective dose rates are then identified on the MTS after which large-scale trials are done to provide confidence by satisfying quarantine requirements at probit 8.7 or higher with no survivors from at least 30,000 test insects. This work is complemented by fruit injury and chemical analysis tests to ensure residues are within the maximum residue limits for that produce.

The treatments developed in WA will benefit other states by limiting the spread of TPP to their jurisdictions and may directly benefit other states if TPP were detected in their jurisdiction. The outcome of the project will be the approval of the treatment schedules developed from the data packages generated in this project by interstate and overseas quarantine. Fumigation and irradiation schedules will be shared with the disinfestation teams in other jurisdictions.

Methodology

2.1 Test fruit

Organic truss tomato (*Solanum lycopersicum*) and purple broad eggplant (*S. melongena*) were obtained from Mitri Hydroponics and Perth Organics, WA, respectively, whereas red cayenne chilli (*Capsicum frutescens*) and red capsicum (*C. annuum*) were sourced from local markets.

2.2 Test insects

For Mediterranean fruit fly (Medfly, MFF), *Ceratitis capitata*, eggs and three larval life-stages were tested whereas for tomato potato psyllid (TPP), *Bactericera cockerelli*, eggs, five nymphal life-stages and adults were tested. The MFF used in this project is maintained at DPIRD's fruit fly insectary where a million flies are artificially reared from eggs each week. Adult flies reared in cages are allowed to lay eggs through terylene mesh fabric on either side of the walls. Eggs drop into troughs filled with clean water. A collection of 24 h-old eggs is used to inoculate an artificial paper rearing medium in a separate rearing room. Mature pupae are collected two weeks later into sand. Pupae are sieved and used to prepare new cages where they hatch into adults. Adequate food and water are provided to flies. Quality control is carried to ensure egg hatch rate, pupal emergence, egg volumes collected every week. Adult flies used for trials are 2–3-week-old, fertile and produce eggs that have high viability, generally over 85% hatch rate. The genetic vigour and fitness of the collection is maintained by replenishing the stock by collecting and rearing wild flies every 12 to 18 months (Appendix 1).

The TPP used in this project is also maintained at DPIRD's insectary in growth rooms where adult psyllids in terylene mesh fabric cages are allowed to feed on tomato plants and lay eggs. Cages are replenished with fresh tomato plants every week. Nursery stock of tomato plants is constantly maintained in glasshouses and plants are deflowered to promote vegetative growth (Appendix 2).

2.3 Experimental test methods

All tests were replicated three or more times.

2.4 Fruit infestation

For MFF, both, natural and artificial methods were used to infest fruit (Appendix 3). Capsicum, chilli and truss tomato were naturally infested while eggplant was artificially infested. For natural infestation, fruit was pin-pricked and placed in monolayer on metal trays that were introduced into cages containing about 50,000 gravid females for 2 to 3 hours. For artificial infestation, fruit was cored 2-3 cm deep and about 10 g paper media inoculated with 500 µL packed volume of eggs was placed inside. The cored skin was lightly placed on top and multipurpose gap filler was used to seal the wound. Infested fruit was used for insect development studies, MTS and large-scale trials.

For TPP, tomato leaves set up in cup cage assemblies were used (Appendix 3). Tomato leaflet with terminal leaves and at least two pairs of primary leaves were excised from healthy plants, placed in vials containing water-soaked dental wicks fastened with a stretched layer of Parafilm®. Vials were held in place by adhesive and the cup was covered with either an inverted cup or a lid with mesh on a 5 sq. cm window for air exchange. A collection of 10 to 12-day-old fertile adults was aspirated and introduced into cups containing tomato leaves and allowed to lay eggs for 24 to 72 hours. Either adults, eggs, or early nymphal stages after aspirating adults were used in trials. For trials with late nymphs, individual nymphs were manually transferred onto leaf surface.

2.5 Insect development studies

Life history studies were done to track MFF development in infested fruit held at 26°C and RH 50-60%. Fruit was dissected and the numbers of eggs and larvae, live and dead, were recorded daily until pupation. Number of pupae retrieved from each fruit was also recorded. These data provided number of days required to obtain the desired life-stage and number of fruits required for large-scale trials.

For insect development studies on TPP, cup cage assemblies as described above were held at 24°C ±1°C, RH 50-60%, L:D 16:8. Leaves were examined under the microscope and the number of eggs and nymphs were recorded daily.

2.6 Ethyl formate (EF) fumigation

EF (97.5% purity laboratory reagent, Sigma-Aldrich) trials were done in glass desiccators and all trials were done at 10°C (Appendix 4). This temperature was chosen as it is generally used for storage of vegetables used in this project and falls in the recommended range (capsicum and chilli is 7-13°C, eggplant is 10-14°C and tomato is 7-15°C depending on its ripeness). Glass desiccators (6.9 - 7 L) containing magnetic stirrer bar at the bottom, a circular wire gauge for holding samples, a disposable plastic straw for injecting EF and a lid having a self-sealing septum were used for fumigation. Prior to fumigation, volume of air displaced was calculated and was removed with a gas tight syringe. Modified screw thread tubes with cone and y-arm were adapted to fit into the desiccator lids for gas sample collection.

Trials in desiccators were conducted to identify most-tolerant TPP life-stage, effective EF doses and effectiveness of those doses on MFF. For TPP, at first, preliminary and confirmatory trials were done using 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5% EF for 1 h and 2 h to test tolerance of eggs, small and large nymphs, and adults, and identify the MTS. The MTS was then exposed to 0.1, 0.5, 1, 1.25, 1.5, 2 and 2.5% EF for 1 h, 2 h, 3 h and 4 h to identify an effective dose rate that was sufficient in achieving complete mortality. The MTS was subsequently exposed to a range of lower EF doses (0.6, 0.8, 1, 1.15, 1.2, 1.25% at 1 h and 2 h) in combination with 15% CO_2 (dry ice). A suitable dose (1.25% + 15% CO_2 for 2 h) was identified that resulted in complete mortality of MTS and had no fruit injury. This dose was used in large-scale desiccator trials. It was also tested on all life-stages of MFF in tomato.

Vapormate® (BOC Gases Ltd), a mixture of 16.7% EF and 83.3% CO_2 (Gas code 279) supplied in FE size cylinder was used against the MTS of TPP in 68 cu. m ISO refrigerated container trials (Appendix 5). Two doses were tested, 1.32% (42 mg/L) and 1.7% (54 mg/L) for 2 h at 10°C. The fans at the back of the container were kept on during fumigation to ensure gas distribution. The container was serviced prior to trials to check for air leakage and temperature sensors were calibrated. Vaporiser unit was setup as per manufacturer's recommendation and a serviced set of scales were used. A total of nine gas lines were setup. Three gas lines were along the front, middle and rear position at three different heights in air. The remaining six gas lines were inside bulk fruit boxes at three varying heights in pallets containing TPP samples interspersed with bulk fruit. Portable Grant Squirrel meter/logger with 16 channels (Model 2020, Cambridge, UK) were used to record temperatures in large-scale container trials and temperature was recorded every minute. There were 15 temperature probes. Three were along the front, middle and rear position at three different heights in air. Six each were in fruit and free air space inside the bulk fruit cartons containing TPP samples at three different heights across two pallets. Concentrations of EF and CO_2 were measured on portable multi-gas detector devices Microtector II G460 and G999 after the first 30 min and then every 15 minutes. Container was vented for a minimum of half an hour before retrieving samples.

2.7 Low dose methyl bromide (LDMB) fumigation

All LDMB trials were conducted at commercial fumigation facilities, Tritan Fumigation in Canning Vale. Three temperature-controlled fumigation chambers of 100 cu. m were used (Appendix 6). LDMB was used in MTS, confirmatory and large-scale trials. Two dose rates, 24 mg/L at 21°C for 2h and 32 mg/L at 21°C for 2 h were used for MTS trials on MFF in capsicum, chilli and eggplant. Once the MTS was identified, confirmatory trials were then carried out on all three fruits using the MTS and four doses identified to be effective on tomatoes previously (VT12000). These were, 24 mg/L at 21°C for 5.5 h, 24 mg/L at 13°C for 7.5 h, 32 mg/L at 21°C for 5.5h and 32 mg/L at 13°C for 6 h. All four doses were then tested against the MTS in red capsicum in large-scale trials. These four doses and two additional new doses, 16 and 24 mg/L at 21°C for 2 h were also tested in confirmatory trials against all TPP life-stages. The two new doses were then used in large-scale trials against all TPP life-stages.

For MTS and large-scale trials, each fumigation chamber was used as a replicate. For confirmatory trials however, due to limited availability of rooms, a chamber consisted of three spatial replicates. In MTS and confirmatory trials, MFF infested fruit was held in gridded tote boxes that were setup in an alternating direction on top of each other in a stack. Portable Grant Squirrel meter/logger with 16 channels (Model 2020, Cambridge, UK) were used to record temperatures throughout the trials. In all trials, there were three air temperatures probes (accuracy ±0.01°C) affixed at three varying heights and four probes that were inserted through the flesh to measure fruit pulp temperature. There were four additional air probes in the bulk fruit cartons containing TPP samples in large-scale trials. Both air and fruit temperatures were recorded every ten minutes in confirmatory trials on both pests and large-scale trials on MFF, and every minute in large-scale trials on TPP. Gas was introduced when desired fruit temperature was achieved. SquirrelView® software was used to view and download calibration and trial temperature data. Calibration data was used to correct trial temperature data. There were three gas lines: back, middle and in the front of the room. Gas readings were recorded and averaged every 30 minutes on Fumiscope to assess the need for any top-ups.

2.8 Phytosanitary irradiation (PI) trials

PI trials were done using Radsource RS2400Q X-ray irradiator used in the Sterile Insect Technique (SIT) facility located at DPIRD (Appendix 7). It is routinely used to deliver SIT Medfly pupae for use in eradication program by the Primary

Industries and Regions South Australia. The RS2400Q is a high capacity, single chamber design with an integrated multi-canister carousel technology and has a dose uniformity >98%. It requires 4000W power, has a dose rate of 12 Gy/min (0.46 g/mL) and a dose uniformity ratio of 1.3 to 1.5. It holds six 830 mL canisters having 10.2 cm diameter and about 12 Gy/min dose rate. All PI trials were done on TPP only. Doses of 50, 100, 150, 200, 250, 300, 350 Gy were tested against TPP eggs and nymphs whereas, doses of 400, 500 and 600 Gy were used against adults. For testing adults, 2-week-old fertile males and females were aspirated from a healthy cage into cup-cages for irradiation. Irradiated adults were then placed at 10°C to slow down so that females could be identified under a stereomicroscope and transferred into a cup-cage assembly, one female per cup, and allowed to oviposit on tomato leaf for 48 h. Females were then retrieved and transferred into new cup-cage assemblies and their survival was monitored daily. The eggs that were laid in the 48 h period were monitored daily for egg hatch and development. For each dose, a minimum of 1400 eggs, 900 small and large nymphs each and 30 females across six replicates were tested.

2.9 Most tolerant life-stage (MTS) and confirmatory trials

Trials done on MFF

WA-grown organic capsicum, chilli, and eggplant was sourced from the local markets for infesting with MFF. For MTS trials, to confirm the presence of the desired MFF life-stage and numbers of individuals, dissections were done prior to LDMB fumigation. Both control and fumigated fruit was held at 26°C for two to three weeks after which sand was sieved to recover and record number of pupae. All three larval stages and eggs were treated in MTS trials. A minimum of 1200 individual life-stages were tested across three replicates. For confirmatory trials, the MTS of eggs (minimum age of 48 h) was used. Three spatial replicates in a fumigation chamber were done with each replicate consisting of 100 fruits, so a total of 300 fruits were infested. Three replicates of control fruits were setup. A minimum of 17,000 viable eggs were treated and 3,000 pupae retrieved from controls. Total number of pupae from control and treated fruits were recorded upon sieving. Five fruit dissections were done prior to fumigation to confirm egg-lay and record the average number of eggs. Another five fruit dissections were done on day 5 to record the number of hatched eggs, live and dead 1st instars. These data were used to estimate the number of total viable eggs treated.

Trials done on TPP

Tomato leaf cup cage assemblies with the desired TPP life-stage were used in MTS and confirmatory fumigation trials. In EF trials, a minimum of 150 nymphs and 300 eggs and adults were tested. In LDMB confirmatory trials, a minimum of 4,900 eggs, 5,000 nymphs and 1,700 adults were tested. Three spatial replicates in a fumigation chamber were done with each replicate consisting of three or four cup cage assemblies. Three replicates of 10 cup cages for control were setup. Nymphs and adults were gently prodded with a fine paint brush and if any movement was recorded, they were considered alive whereas lack of movement was considered dead. Live and dead nymphs were recorded in treatment and controls. Nymphs and adults were assessed 24 h after treatments. Eggs were assessed 10 days post treatment to ensure enough time was provided for eggs to hatch. An egg was considered dead if no nymph hatched from it and it remained intact at the end of 10 days.

2.10 Large-scale trials

Trials done on MFF

WA-grown organic red capsicum and green capsicum as bulk fruit was sourced from the local markets. Treatment fruit was naturally infested, and extra fruit was prepared for dissections to provide data on viable eggs, post-fumigation photos and residue analysis. Treatment fruit was marked prior to infesting for easy identification at time of trials. It was spread across each bulk fruit carton on every layer on the pallet during LDMB fumigation. Control fruit was setup in gridded tote boxes that were covered with a mesh lid and placed on sand boxes at 26°C (Appendix 6). Treatment fruit was also setup for pupae collection the same way as controls after fumigation. All fruit was slit open on day five to allow for the juice to escape and avoid larvae from drowning. Sand was sieved several times over four weeks. Pupae were collected and stored in the freezer for counting at a later stage. Fruit used for temperature probes was equilibrated at 26°C prior to trials to correspond to treatment fruit temperature. Largescale trials were done on MFF eggs in red capsicum for all four doses described in section 2.7.

Trials done on TPP

Largescale trials using two new LDMB doses (section 2.7) were done on eggs, nymphs, and adults (Appendix 6). For eggs and nymphs, in each replicate, there were a total of 70 cups each and for adults there were 100 cups interspersed amongst bulk fruit across pallets. Green capsicum sourced from the local markets was used as bulk fruit. Mortality assessment was done as described previously.

Largescale trials using EF (with dry ice) and TPP eggs were done in desiccators using the dose rate described in section 2.6. Three replicated trials each consisting of 30 cups were done. Vapormate® on TPP eggs were done in 68 cu.m refrigerated shipping container with either kiwi or apple as bulk fruit. In each replicate there were a total of 26 cups with TPP samples that were placed inside bulk fruit cartons across pallets.

Loading factors

Loading factor for LDMB and Vapormate® were calculated based on room size, fruit weight and carton sizes. For LDMB, the bulk fruit boxes used were standard ventilated cartons for fresh produce of 210 mm (h) x 285 mm (w) x 430 mm (l) *i.e* 25.7355 L. A pallet comprised of 40 cartons, eight cartons per layer and five layers high. Each box contained 12 kg of green capsicum. There were six pallets used. A total of 240 cartons with a total of 2,880 kg fruit was used per room. The total volume used for 240 cartons was 240 x 25.7355 / 1000 = 6.18 cu.m. The room size was 100 cu.m. The loading factor in weight was 2,880 kg / 100 cu.m = 28.8 kg/cu.m, whereas, in volume expressed as percentage was (6.18 cu.m / 100 cu.m) x 100 = 6.18%.

For Vapormate® trials using apple as bulk fruit, the standard ventilated box dimensions were 175 mm (h) x 380mm (w) x 580 mm (l) *i.e* 38.57 L. A pallet comprised of 48 cartons, four cartons per layer and 12 layers high. Each box contained 12.7 kg of fruit. There were four pallets used. A total of 192 cartons with a total of 2,438 kg fruit was used per replicate. The total volume used for 192 cartons was 192 x 38.57 / 1000 = 7.41 cu.m. The room size was 68 cu.m. The loading factor in weight was 2,438 kg / 68 cu.m = 35.85 kg/cu.m, whereas, in volume expressed as percentage was (7.41 cu.m / 68 cu.m) x 100 = 10.5%.

In Vapormate® trials where kiwi fruit was used, the standard ventilated box dimensions were 190 mm (h) x 300mm (w) x 400 mm (l) *i.e* 22.80 L. A pallet comprised of 70 cartons, 10 cartons per layer and seven layers high. Each box contained 10 kg of fruit. There were four pallets used. A total of 280 cartons with a total of 2,800 kg fruit was used per replicate. The total volume used for 280 cartons was 280 x 22.80 / 1000 = 6.38 cu.m. The room size was 68 cu.m. The loading factor in weight was 2,800 kg / 68 cu.m = 41.18 kg/cu.m, whereas, in volume expressed as percentage was (6.38 cu.m / 68 cu.m) x 100 = 9.38%.

For both fumigation trials, fruit boxes were arranged to have air space in the centre, to mimic export packing, held firmly with corner boards and strapped together when required to keep in place.

2.11 Quality assessment

Fruit injury tests and visual observations were made on days 1, 7 and 14 post-fumigation on fruit stored at 10°C. Fruits were checked visually for any damage or injury such as soft or sunken spots, discoloration, blemishes, and fungal growth. Photographs of fruit were taken on each of these time points. Capsicum, chilli, eggplant, and truss tomato treated with 1.25% EF and 15% CO_2 were assessed for hardness and weight. To see if addition of CO_2 yielded differences in physicochemical parameter of truss tomato, in addition to weight and hardness, pH, °BRIX and fresh tomato colour index (TCI_f) were compared between control fruit and fruit treated with EF with and without CO_2 (Appendix 8). For TCI_f, colour co-ordinates (*l*, *a*, *b*) ($TCI_f = 2000 \times a^*/(l^* \times (a^{*2} + b^{*2})^{1/2})$) (Gómez, 1997) were recorded. Irradiated truss tomato was assessed for hardness, weight, °BRIX and TCI_f.

2.12 Chemical analysis

For methyl bromide residue analysis, both red and green capsicum were exposed to fumigation by placing these in the front, middle and back of the room in three replicates corresponding to the three fumigation rooms during large-scale trials. All fumigated fruit was aerated for a day before freezing after which it was transported in ice-filled Styrofoam boxes to the Scientific Services Division of ChemCentre, a national accredited laboratory for residue analysis. For each dose, fruit type and location in the room, fruit was bulked, and analysis was done for the presence of bromomethane in 38 fumigated and untreated control samples using GC-MS.

2.13 Data analyses

The corrected mortality with 95% confidence for MFF was calculated using methods of Couey and Chew (1986) and Food Organisation of the United Nations (2016). Corrected mortality was based on the estimated number of insect life-stages treated and the number of surviving pupae. For TPP, percentages of mortality were corrected according to the Abbott's formula (Abbott, 1952) using natural mortality in untreated controls as the correcting factor. Data for mortality due to EF treatment was analysed by probit regression (Finney, 1971). Probit link function between numbers responded and logarithm of the dose was used as the complementary log-log and logit-link function did not significantly reduce residual deviance. Quality assessment and irradiation data for various treatments were analysed with Student's or Welch's t-tests or one-way analysis of variance (1-ANOVA) at least significant difference level (95%) where appropriate. When ANOVAs were significant, means were separated using the Tukey's HSD test ($p<0.05$) to identify differences. Where appropriate, data were squared root transformed ($\surd x+0.5$) to minimise variation before subjected to statistical analyses. Data were reversed transformed for presentation. The time-mortality data for irradiation were analysed for survival analysis using Kaplan-Meier estimators and the differences between curves were compared by the log-rank test with Bonferroni correction. Hazard ratios were calculated using Cox's proportional hazards regression model where HR=1 means equal or no effect of treatment versus control. Statistical analyses were performed in GenStat 20th edition (VSN International 2022) and MedCalc Software (Version 19.4).

Results and Discussion

3.1 Insect development studies

For MFF, data indicated that in all fruits, eggs began to hatch on day 3 (Figure 1). In capsicum, presence of each instar stage found was as follows: 1st instars day 3-6; 2nd instars day 4-7, 3rd instars day 6-8. More than 50% of each life-stage was as follows: eggs day 1-2; 1st instar day 3-4; 2nd instar 5-6; 3rd instar day 7-8. Non-feeding 3rd instars were observed on day 8. Average number of pupae emerged from capsicum were 47 per fruit (*n*=32). In chilli, presence of each instar stage found was as follows: 1st instars day 3-5; 2nd instars day 4-9, 3rd instars day 6-9. More than 50% of each life-stage was as follows: eggs day 1-2; 1st instar day 3-4; 2nd instar 5-6; 3rd instar day 7-9. Non-feeding 3rd instars were observed on day 9. Average number of pupae emerged from capsicum were 21 per fruit (*n*=68). In eggplant, presence of each instar stage found was as follows: 1st instars day 3-5; 2nd instars day 4-6, 3rd instars day 5-6. More than 50% of each life-stage was as follows: eggs day 1-2; 1st instar day 3; 2nd instar 5; 3rd instar day 6. Large numbers of non-feeding 3rd instars observed on day 7 making it difficult to count and hence not shown in the figure below. Average number of pupae emerged from eggplant were 185 per fruit (*n*=40). In tomato, presence of each instar stage found was as follows: 1st instars day 3-5; 2nd instars day 4-6, 3rd instars day 5-6. More than 50% of each life-stage was as follows: eggs day 1-2; 1st instar day 3; 2nd instar 4-5; 3rd instar day 6. Large numbers of non-feeding 3rd instars observed on day 7 making it difficult to count and hence not shown in the figure below. Average number of pupae emerged from tomato were 84 per fruit (*n*=89).

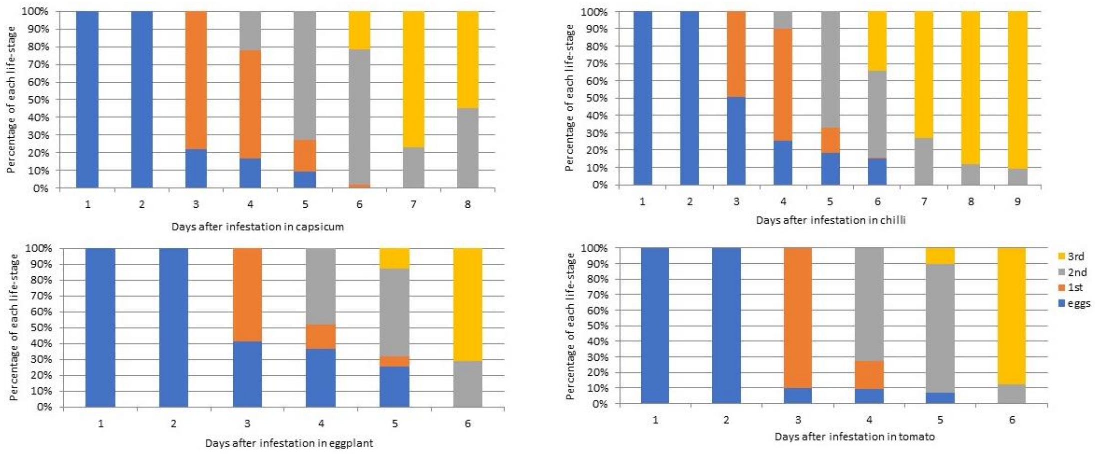

Figure 1. Life history of Mediterranean fruit fly in capsicum, chilli, eggplant and tomato.

Data for TPP life-stage development indicated that eggs began to hatch on day 5 (Figure 2). Average hatch rate was 85% (SEM ± 0.1, *n=10*). Presence of each instar stage found was as follows: 1st instars day 5-8; 2nd instars day 6-11; 3rd instars day 8-11. More than 50% of each life-stage was as follows: eggs day 1-4; 1st instar day 5-6; 2nd instar 7-8; 3rd instar day 11. Psyllid sugars were conspicuous from day 8 as 3rd instars developed. Presence of 4th instar on day 11 was seen however, its development could not be studied beyond that point due to fungal growth on accumulated psyllid sugars.

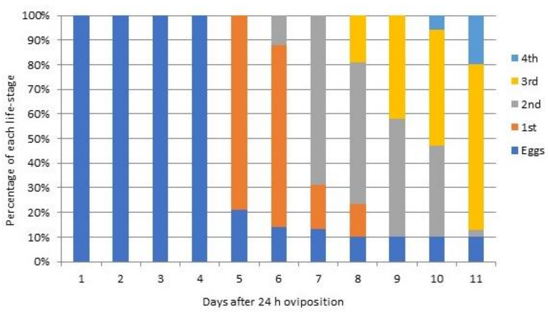

Figure 2. TPP development on tomato.

3.2 Preference of TPP for oviposition on fruit calyx and leaf

TPP is known to be associated with above ground plant parts including leaf, stem and calyx on fruit thereby making transmission *via* fruit likely during interstate movement. However, it is not clear whether eggs and nymphs on persistent calyx of fruit are viable. Choice and no-choice tests, indicated that fruit and calyx were poor substrates for oviposition, did not support egg-hatch or nymphal development as compared to foliage.

In no-choice tests, TPP laid eggs on calyx and in fewer instances on flesh of the fruit closer to the calyx with practically none laid on tomato flesh. There were greater numbers of eggs laid on leaf than fruit (Table 1). Between the calyx of the two fruits, more were laid on capsicum. By day 5, eggs began to hatch on all substrates. However, by day 8 hatched nymphs on calyx were dead while those on leaf continued to develop.

Table 1. Comparison of the number of TPP eggs laid on either tomato leaf or tomato and capsicum fruit (including calyx) in no-choice tests and mean egg hatch.

Substrate	Total no. of eggs laid	Mean no. of eggs laid (±SEM)	Mean percent egg hatch (±SEM)
Capsicum	213	42.6 ± 2.87	30.36 ± 3.53
Tomato	106	21.20 ± 3.68	14.21 ± 4.98
Leaf	487	97.40 ± 2.00	80.82 ± 2.00

In choice tests, when provided with a choice between leaf and fruit, TPP preferred to lay eggs on leaf (Table 2). None of the psyllids that hatched on fruit were alive beyond days 8 and 10 on capsicum and tomato, respectively.

Table 2. Mean number of TPP eggs laid on tomato and capsicum fruit (including calyx) or tomato leaf in choice tests and mean egg hatch.

Substrate	Total number of eggs laid on fruit	Total number of eggs laid on leaf	Mean no. of eggs laid on fruit (±SEM)	Mean no. of eggs laid on leaf (±SEM)	Mean egg hatch on fruit (%)	Mean egg hatch on leaf (%)
Leaf + capsicum	95	330	19.00 ± 2.61	66.00 ± 4.93	16.31 ± 1.56	78.90 ± 1.29
Leaf + tomato	109	334	21.80 ± 1.85	66.80 ± 4.19	9.14 ± 2.68	80.55 ±0.76

3.2 Ethyl formate fumigation

TPP: Trials using a total of 12,874 TPP life-stages and various EF doses were done to identify a dose range and exposure time that is effective in causing TPP mortality (Table 3). A total of 4200 adults, 2100 small and large nymphs each and 4,474 eggs were tested across three replicates at EF doses ranging between 0 and 16 mg/L. Complete mortality of both adults and nymphs was achieved with EF ≥ 10 mg/L (0.3%) at both 1 h and 2 h exposure times. Results indicated eggs were the most tolerant life-stage and hence higher doses were tested.

Table 3. Estimated response of TPP life-stages exposed to EF for 1-2 hours at 10°C in laboratory trials.

			Model parameters						Model fit			Lethal dose mg/L (LL and UL at 95% CI)					
TPP	Time	n	Intercept	±SE	Slope	±SE	R^2		X^2	df	p	LD50	LL	UL	LD99	LL	UL
Adult	1h	2100	-8.37	0.78	12.07	1.04	97.86		9.09	4	0.06	4.90	2.70	7.10	12.75	10.55	14.95
	2h	2100	-7.85	0.70	11.53	0.96	98.23		7.07	4	0.13	4.05	2.05	6.05	10.10	8.10	12.10
Small nymphs	1h	1050	-6.66	0.56	10.88	0.88	98.94		3.69	4	0.45	4.07	0.57	7.57	10.70	7.21	14.21
	2h	1050	-6.59	0.56	10.93	0.89	99.14		2.80	4	0.59	3.98	0.78	7.18	10.47	7.27	13.67
Large nymphs	1h	1050	-6.74	0.57	11.01	0.89	98.79		4.68	4	0.32	4.99	0.57	7.57	10.47	6.97	13.97
	2h	1050	-6.65	0.57	11.13	0.92	99.16		2.95	4	0.57	4.75	0.68	7.28	10.23	6.93	13.53
Eggs	1h	2244	-6.85	1.12	4.14	1.03	98.46		2.74	4	0.60	10.50	5.50	15.50	41.25	36.25	46.25
	2h	2230	-2.86	0.38	1.12	0.40	99.34		2.85	4	0.91	8.88	4.38	13.38	39.15	36.45	43.65

Over 10,000 eggs were treated with EF doses ranging from 0 to 80 mg/L at various exposure times (Table 4). Complete mortality was achieved with EF ≥ 40 mg/L (1.25%) in 2-4 h and with ≥ 64 mg/L (2%) in 1 h.

Table 4. Estimated response of TPP eggs exposed to EF for 1-4 hours at 10°C in laboratory trials.

			Model parameters					Model fit			Lethal dose mg/L (LL and UL at 95% CI)					
TPP	Time	n	Intercept	±SE	Slope	±SE	R^2	X^2	df	p	LD50	LL	UL	LD99	LL	UL
Eggs	1h	2639	-7.61	0.73	4.83	0.45	96.46	2.49	5	0.08	10.99	9.49	12.49	41.89	40.29	43.49
	2h	2593	-8.24	0.77	6.09	0.51	97.27	3.14	5	0.12	9.85	8.05	11.65	39.85	37.95	41.75
	3h	2611	-6.00	0.60	4.80	0.40	97.51	1.36	5	0.23	7.00	5.00	9.00	36.50	34.30	38.70
	4h	2600	-5.10	0.52	4.29	0.35	96.98	1.72	5	0.25	6.50	4.60	8.40	32.70	32.70	36.30

Subsequently, to check if the addition of CO_2 to EF also yielded equal or greater egg mortality, TPP eggs were exposed to an EF dose range of 0 to 40 mg/L for 1-2 hours and 10-15% CO_2 (Table 5). Both exposure times were found to be effective in preventing egg hatch. However, to ensure the effectiveness of treatment on mixed age of eggs, 1.25% EF + 15% CO_2 for 2 h was found to be suitable and was advanced for laboratory based large-scale trials on mixed stages of eggs. Treated eggs were found to dry out and shrivel losing their form as compared to control eggs (Figure 3).

Table 5. Estimated response of TPP eggs exposed to EF and CO_2 for 1-2 hours 10°C in laboratory trials.

			Model parameters					Model fit			Lethal dose mg/L (LL and UL at 95% CI)					
TPP	Time	n	Intercept	±SE	Slope	±SE	R^2	X^2	df	p	LD50	LL	UL	LD99	LL	UL
Eggs	1h	2276	-5.15	3.35	6.25	2.38	98.52	1.20	5	0.88	9.61	7.81	11.41	43.34	41.04	45.64
	2h	2305	-5.41	4.72	6.94	3.38	96.58	1.77	4	0.78	6.50	6.50	10.50	41.05	37.75	44.35

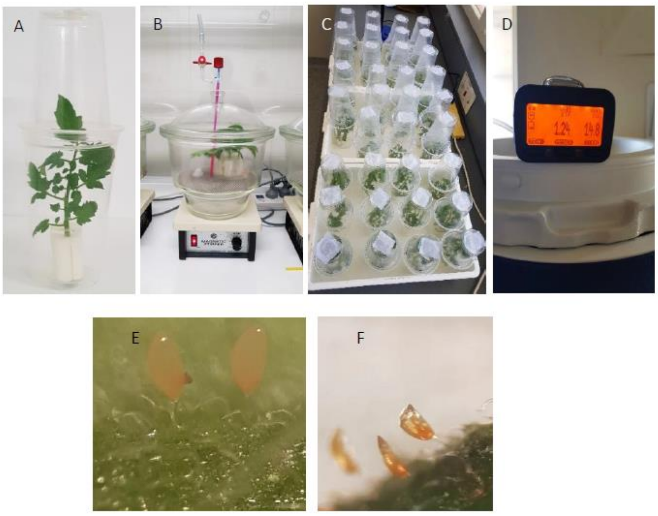

Figure 3. Ethyl formate treatment of TPP. 1A. Cup cage assembly consisting of tomato leaf with TPP eggs. 1B. EF+CO_2 in desiccators. 1C. Cup cage assemblies held in controlled environment before and after fumigation. 1D. Concentration of EF and CO_2 measured on a portable multi-gas detector device Microtector II G460. 1E and 1F. Control and fumigated eggs, respectively.

MFF: In MFF life-stages treated with and without CO_2, it was found that addition of CO_2 to EF increased MFF mortality than with EF-only (Table 6). Eggs were the most tolerant followed by 3rd instars, 1st instars and finally 2nd instars. With EF-only, the resulting mortality for MFF eggs, 3rd, 1st and 2nd was 8%, 21%, 25% and 31%, respectively, whereas, with EF and CO_2 it was 10%, 28%, 36% and 41%, respectively. This suggested that the EF + CO_2 treatment that was effective in causing 100% TPP egg mortality can cause some MFF mortality but cannot provide quarantine level of mortality.

Table 6. Mortality of MFF life-stages in tomato after 1.25% EF ± 15% CO_2 exposure for 2 h at 10°C.

EF dose rate	Average number of pupae (survivors) post treatment for:				Average percent mortality post treatment for:				95% CI (Range)			
	Eggs	1st instar	2nd instar	3rd instar	Eggs	1st instar	2nd instar	3rd instar	Eggs	1st instar	2nd instar	3rd instar
Control	330	345	343	333	-				-			
1.25%EF -CO_2	305	259	238	264	7.67	25.09	30.61	20.74	6.81-8.53	23.90-26.29	28.92-32.30	20.22-21.26
1.25%EF +CO_2	297	222	203	238	10.19	35.81	40.91	28.46	9.53-10.86	33.86-37.76	40.40-41.42	26.76-30.15

3.3 Low dose methyl bromide fumigation

MFF: Results of most-tolerant life-stage trials on MFF in capsicum, chilli and eggplant showed mortality of all life-stages (Table 7). Lowest mortality was found in fruit infested with eggs indicating eggs were the most tolerant followed by 3rd, 2nd and finally 1st instars for all three fruits. Confirmatory trials were done on MFF eggs in the three fruits using the four doses 24 mg/L at 21°C for 5.5 h, 24 mg/L at 13°C for 7.5 h, 32 mg/L at 21°C for 5.5 h and 32 mg/L at 13°C for 6 h. Results indicated that all doses were successful in achieving 100% egg mortality with no pupariation (Table 8). These doses were advanced for largescale trials on MFF eggs in red capsicum.

Table 7. Mortality of MFF life-stages in capsicum, chilli and eggplant after low dose methyl bromide fumigation.

			Number of pupae				Corrected percent mortality			
Dose	**Exposure**	**Ct product**	**Eggs**	**1st instar**	**2nd instar**	**3rd instar**	**Eggs**	**1st instar**	**2nd instar**	**3rd instar**
					Capsicum					
Control for 24 mg/L	*Not applicable*		4782	4390	3223	3158	*Not applicable*			
24 mg/L	2h	48	3389	2622	1698	2045	29.13	40.27	47.32	35.24
Control for 32 mg/L	*Not applicable*		4807	3842	3235	3235	*Not applicable*			
32 mg/L	2h	64	3122	1940	1389	1855	35.05	49.51	57.06	42.66
					Chilli					
Control for 24 mg/L	*Not applicable*		1220	1257	1524	1749	*Not applicable*			
24 mg/L	2h	48	816	560	516	968	33.11	55.45	66.14	44.65
Control for 32 mg/L	*Not applicable*		1400	516	1752	1835	*Not applicable*			
32 mg/L	2h	64	737	968	413	791	47.36	67.43	76.43	56.89
					Eggplant					
Control for 24 mg/L	*Not applicable*		2890	3788	3378	3343	*Not applicable*			
24 mg/L	2h	48	1916	1680	1448	1900	33.70	55.65	57.13	43.16
Control for 32 mg/L	*Not applicable*		3064	3788	3390	3482	*Not applicable*			
32 mg/L	2h	64	1737	1348	1138	1683	39.90	64.41	66.31	49.66

Table 8. Mortality of MFF eggs in capsicum, chilli and eggplant after low dose methyl bromide fumigation across three replicates.

°C air¨	°C fruit¨	Ct product	Estimated corrected number of viable eggs in 300 treatment fruits*§	Estimated corrected number of pupae in 300 control fruits*	Pupae in treated fruit	Observed mortality (%)	Eggs control at upper 95% CL	Pupae control at upper 95% CL
Capsicum								
24 mg/L at 21°C for 5.5h								
21.83	21.5	132	95,398	26,553	0	100	99.99683	99.9
24 mg/L at 13°C for 7.5h								
13.41	13.41	180	95,093	26,825	0	100	99.99683	99.9
32 mg/L at 21°C for 5.5h								
21.98	21.48	176	87,395	24,444	0	100	99.99	99.9
32 mg/L at 13°C for 6h								
12.04	13.09	192	83,754	23,311	0	100	99.99	99.9
Chilli								
24 mg/L at 21°C for 5.5h								
21.83	21.5	132	17,433	3,349	0	100	99.9	99.9
24 mg/L at 13°C for 7.5h								
13.41	13.41	180	19,264	3,546	0	100	99.9	99.9
32 mg/L at 21°C for 5.5h								
21.98	21.48	176	18,415	3,470	0	100	99.9	99.9
32 mg/L at 13°C for 6h								
12.04	13.09	192	18,666	3,472	0	100	99.9	99.9
Eggplant								
24 mg/L at 21°C for 5.5h								
21.83	21.5	132	95,921	18,543	0	100	99.99683	99.9
24 mg/L at 13°C for 7.5h								
13.41	13.41	180	92,135	17,639	0	100	99.99	99.9
32 mg/L at 21°C for 5.5h								
21.98	21.48	176	94,880	18,338	0	100	99.9968	99.9
32 mg/L at 13°C for 6h								
12.04	13.09	192	105,913	19,101	0	100	99.99683	99.9

¨ Air and fruit temperatures are common for all three fruits as trials were conducted in one chamber with three spatial replicates.
* Corrected as per Couey and Chew (1986), De Lima et al. (2017) and FAO (2016 a,b).
§ Based on fruit dissection data prior to fumigation (*n* = 5).

TPP: The four doses listed above along with two new shorter doses, 16 and 24 mg/L at 21°C for 2 h, were tested on all TPP life-stages in confirmatory trials. There were no survivors recorded from all the treatments with eggs, nymphs and adults. Data in Table 9 is across three replicates and the treated life-stages were corrected for control mortality which ranged from 9 to 10% for eggs, 13% for nymphs and 3 to 10% for adults. Number of treated insects were corrected as per methods of Couey and Chew (1986), De Lima et al. (2017) and FAO (2016 a,b). The two new shorter doses were advanced to largescale trials on all TPP life-stages.

Table 9. Mortality of TPP life-stages after low dose methyl bromide fumigation across three replicates.

TPP lifestage	C°air	C°fruit Capsicum	C°fruit Chilli	C°air	C°fruit Eggplant	Corrected number of insects treated*	Survivors	Observed mortality (%)	Control at upper 95% CL
	Air temperature for trials with capsicum and chilli together			Air temperature for trials with eggplant only					
24 mg/L at 21°C for 5.5h (Ct product = 132)									
Eggs	21.80	21.50	21.50	21.74	21.40	4,791	0	100	99.9
Nymphs						5,399	0	100	99.9
Adults						1,673	0	100	99.9
24 mg/L at 13°C for 7.5h (Ct product = 180)									
Eggs	13.30	13.41	13.41	13.02	13.25	4,841	0	100	99.9
Nymphs						5,387	0	100	99.9
Adults						1,900	0	100	99.9
32 mg/L at 21°C for 5.5h (Ct product = 176)									
Eggs	22.00	21.48	21.11	21.60	21.64	4,959	0	100	99.9
Nymphs						5,307	0	100	99.9
Adults						1,765	0	100	99.9
32 mg/L at 13°C for 6h (Ct product = 176)									
Eggs	12.00	13.09	12.98	13.27	13.18	5,526	0	100	99.9
Nymphs						5,355	0	100	99.9
Adults						1,983	0	100	99.9
16 mg/L at 21°C for 2h (Ct product = 32)									
Eggs	22.00	20.88	**			7,623	0	100	99.9
Nymphs						7,417	0	100	99.9
Adults						2,998	0	100	99.86501
24 mg/L at 21°C for 2h (Ct product = 48)									
Eggs	22.00	20.74	**			7,769	0	100	99.9
Nymphs						8,391	0	100	99.9
Adults						3,090	0	100	99.9

* Corrected as per Couey and Chew (1986), De Lima et al. (2017) and FAO (2016 a,b).

** Temperature data not applicable as trials were done only with capsicum.

3.4 Low dose methyl bromide largescale fumigation

MFF: Results indicated that across three replicates, for each treatment, over 279,00 estimated viable eggs were treated with no survivors (Table 10) thereby satisfying quarantine of 99.99683% with over 78,000 pupae retrieved from respective controls satisfying quarantine of 99.99% at 95% confidence level.

Table 10. LDMB largescale fumigation of MFF eggs in capsicum using four doses across three replicated trials.

°C air	°C fruit	Estimated corrected number of viable eggs in 600 treatment fruits*§	Estimated corrected number of pupae in 600 control fruits*	Pupae in treated fruit	Observed mortality (%)	Eggs control at upper 95% CL	Pupae control at upper 95% CL
24 mg/L at 21°C for 5.5h							
22.48	20.92	279,845	78,951	0	100	99.99683	99.99
24 mg/L at 13°C for 7.5h							
8.72	12.91	281,814	79,507	0	100	99.99683	99.99
32 mg/L at 21°C for 5.5h							
22.60	20.79	282,256	79,632	0	100	99.99683	99.99
32 mg/L at 13°C for 6h							
9.16	13.27	280,358	79,096	0	100	99.99683	99.99

* Corrected as per Couey and Chew (1986), De Lima et al. (2017) and FAO (2016 a,b).

§ Based on fruit dissection data prior to fumigation (*n* = 10).

TPP: Both the shorter 2h doses resulted in no survivors from over 30,000 individuals providing 99.99% control at 95% confidence level (Table 11). Across the two doses, control mortality was < 11% for eggs and nymphs, and ≤1% for adults.

Table 11. LDMB largescale fumigation of TPP eggs, nymphs, and adults across three replicated trials.

TPP life-stage	°C air	°C air in fruit box	°C fruit	Corrected total insects treated*	Survivors	Observed mortality (%)	Eggs control at upper 95% CL
16 mg/L at 21°C for 2h							
Eggs	15.53	19.13	20.99	120,913	0	100	99.99683
Nymphs				32,768	0	100	99.99
Adults				31,500	0	100	99.99
24 mg/L at 21°C for 2h							
Eggs	16.44	16.34	21.06	130,751	0	100	99.99683
Nymphs				31,424	0	100	99.99
Adults				31,517	0	100	99.99

* Corrected as per Couey and Chew (1986), De Lima et al. (2017) and FAO (2016 a,b).

3.5 Ethyl formate and Vapormate® largescale fumigation

In largescale desiccator trials done on TPP eggs that used EF and dry ice, complete prevention of egg hatch was achieved using 40 mg/L EF + 15% CO_2 for 2 h at 10°C with no survivors found in 44,370 number of treated viable eggs equating to a mortality of 99.99% at the 95% level of confidence level. Control mortality averaged at 11% across three replicates. These results formed the basis for Vapormate® trials.

Two doses were tested for Vapormate®, 42 mg/L and 54 mg/L for 2 h at 10°C (Table 12). The dose of 42 mg/L resulted in a high level of control on TPP eggs with 99.97% mortality however, it did not provide quarantine security. A higher dose was applied to account for sorption or leakage. The dose of 54 mg/L resulted in no survivors from over 30,000 eggs tested satisfying the quarantine standard of 99.99% at probit 8.719. In both the largescale fumigation trials, the number of treated insects were corrected as per methods of Couey and Chew (1986), De Lima et al. (2017) and FAO (2016 a,b).

Table 12. Largescale fumigation using 42 mg/L and 54 mg/L Vapormate® for 2h at 10°C against TPP eggs across three replicated trials.

Dose	°C air	°C fruit	EF	CO_2	Total number of eggs	Hatched eggs	Egg mortality at upper 95% CL
Control	*Not applicable*				39,435	34,659	12.11
42 mg/L	10.08	9.76	42	10.24	45,616*	14	99.97
54 mg/L	10.62	10.48	54	13.43	42,190*	0	99.99

* Corrected as per Couey and Chew (1986), De Lima et al. (2017) and FAO (2016 a,b).

3.6 Irradiation

Eggs and nymphs were irradiated at 0 to 350 Gy and assessed for mortality and deformities. Eggs irradiated above 150 Gy did not hatch whereas at 50, 100 and 150 Gy eggs hatched and developed until 3rd to 4th instar nymphs (Table 13). Small nymphs irradiated above 100 Gy did not survive whereas at 50 and 100 Gy developed into 4th to 5th instars and died 5 days post-irradiation. Small nymphs irradiated at 200, 250, 300, 350 Gy did not develop into large nymphs and died (Table 13). Large nymphs irradiated 50, 100, 150, 200, 250 Gy developed into adults, but none developed into adults at 300 or 350 Gy (Table 13). There were deformities seen in adults that developed from small and large nymphs (Table 14 and Appendix 9); either the venation on wings was not fully formed or wings were deformed. Abdomen of dead nymphs appeared dark brown. Appendages of both small and large dead nymphs appeared brownish or burnt, while body appeared pale green or discoloured and dehydrated. Based on these preliminary results it can be speculated that a dose ≥350 Gy would be required to achieve mortality of eggs and nymphs.

Fecundity (number of eggs laid) of irradiated and untreated females, hatch rate and mortality were recorded. Data indicated that eggs laid by females irradiated at 500 and 600 Gy were significantly lower than controls (Table 15). Moreover, none of the eggs laid by females irradiated at 400, 500 and 600 Gy hatched into nymphs. Survival curves for each dose and controls were significantly different with hazard ratios indicating that exposure to irradiation increased the risk of mortality (Table 15 and Figure 4).

Table 13. Percent mortalities of irradiated TPP eggs and nymphs.

Dose (Gy)	Number of treated individuals (*N*)	Abbott's corrected mortality*§ (%)	[Mean ± SE]	95% CI (Range)	
		Eggs ($F_{7,40}$ = 2.25, $p<0.0001$)			
0	1514	11.50 a	1.02		
50	1520	43.86 b	1.48	43.68	44.04
100	1499	90.82 c	1.20	90.67	90.97
150	1506	93.73 c	1.44	93.55	93.91
200	1518	100 d	0	100	100
250	1496	100 d	0	100	100
300	1505	100 d	0	100	100
350	1504	100 d	0	100	100
		Small nymphs ($F_{7,40}$ = 2.25, $p<0.01$)			
0	1015	27.29 a	3.44		
50	987	37.94 b	4.36	37.27	38.61
100	1005	52.44 b	7.22	51.35	53.53
150	1012	100 c	0	100	100
200	1004	100 c	0	100	100
250	992	100 c	0	100	100
300	1007	100 c	0	100	100
350	1003	100 c	0	100	100
		Large nymphs ($F_{7,40}$ = 2.25, $p<0.01$)			
0	1015	29.02 a	3.76		
50	987	32.13 b	3.83	31.54	32.72
100	1005	51.88 c	6.88	50.82	52.94
150	1012	69.54 cd	1.91	69.25	69.83
200	1004	84.70 de	1.65	84.45	84.95
250	992	98.47 e	1.00	98.35	98.59
300	1007	100 e	0	100	100
350	1003	100 e	0	100	100

*Abbott's corrected mortality was applied to account for natural mortality (eggs that failed to hatch) in controls.
§Means with different letters are significantly different (1-way ANOVA, Tukey's HSD *p*<0.05).

Table 14. Percent deformity in irradiated TPP nymphs.

Dose (Gy)	Number of individuals alive after irradiation (*N*)	Deformity (%)§	[Mean ± SE]	95% CI (Range)	
		Small nymphs ($F_{2,9}$ = 28.41, $p<0.01$**)			
0	738	3.39 a	2.86	3.18	3.59
50	458	21.40 bd	3.27	21.10	21.70
100	351	27.35 cd	3.41	26.99	27.71
		Large nymphs ($F_{5,30}$ = 19.62, $p<0.01$**)			
0	719	3.06 a	1.28	2.83	3.29
50	488	11.68 a	0.72	11.52	11.84
100	346	24.57 b	1.96	24.06	25.07
150	219	43.84 c	1.88	43.23	44.45
200	110	79.09 d	1.06	78.61	79.57
250	11	90.91 e	1.00	89.81	92.01

§One-way ANOVA, **significant statistical difference with *p*<0.01. Means with the same letter are not significantly different from each other (Tukey's HSD, *p*<0.05).

Table 15. Fecundity, hatch rate and Kaplan Meier survival analysis of irradiated adults.

Dose	Fecundity* ($F_{3,116}$=22.58, p<0.0001)	LL (95%CI)	UL (95%CI)	Egg hatch rate(%)	Mean survival	LL (95%CI)	UL (95%CI)	X^2	df	Significance	Hazard ratio**	LL (95%CI)	UL (95%CI)
Control	34.37 a	32.39	36.34	88	15.97	15.82	15.99	318.34	3	p<0.001	*Not applicable*		
400 Gy	31.97 a	26.37	37.57	0	13.75	13.44	14.06				16.78	13.53	20.80
500 Gy	21.83 b	16.61	27.06	0	12.16	11.84	12.49				28.33	22.86	35.12
600 Gy	10.67 c	6.53	14.81	0	12.23	11.90	12.55				27.89	22.50	34.57

* One-way ANOVA. Means with the same letter are not significantly different from each other (Tukey's HSD, p<0.05).

** Hazard ratio is in comparison to unirradiated controls.

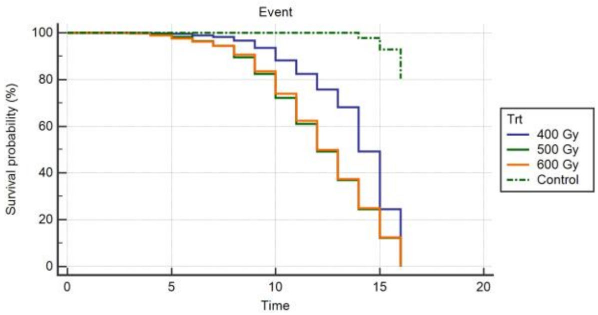

Figure 4. Survival curves of irradiated and control female TPP using Kaplan-Meier estimators' log-rank test.

3.7 Quality assessments

EF and Vapormate® treated fruit

Capsicum, chilli, eggplant and truss tomato treated with 1.25% EF + 15% CO_2 (dry ice) and Vapormate® held at 10°C were checked visually for any damage or injury such as soft or sunken spots, discoloration, blemishes and fungal growth post-treatment. Visual observations indicated that the treatment did not cause physical damage or injury to the flesh of the fruit on days 1, 7 and 14 days at 10°C (Appendix 10 and Appendix 11). No blemishes, discoloration, soft or sunken spots or rots were seen on the flesh for most fruits. On day 14, some wrinkling on capsicum and eggplant was seen on treated fruit but it was also seen on untreated control fruit. Similarly, there were a few fungal spots on treated and untreated tomato which may be due to longer-term storage. The persistent calyx on treated eggplant and truss tomato was found to suffer browning and was dry as compared to control fruits. There was occasional browning on eggplant skin closer to the calyx by day 14. Based on these observations, treated tomato may be stored between seven to 10 days and eggplant and capsicum up to day 14 without compromising fruit quality. The fruit hardness of treated fruit did not significantly differ (p>0.05) from controls for any fruit until (data not shown).

For all four fruits treated with 1.25% EF + 15% CO_2 (dry ice), the differences in mean weight or hardness of treated and untreated control on days 1, 7 and 14 post-treatment were not statistically significant (Appendix 12). Additionally, for truss tomato, there were no significant differences (p>0.05) in diameter, pH and °BRIX of treated and untreated on days 1, 7 and 14 post-treatment. The TCI_f for treated truss tomato was slightly greater than that of control; however, it was not significantly different to control suggesting that tomato flesh colour quality was unaffected (Appendix 12).

LDMB treated fruit

Visual observations for fruit injury, blemishes, discoloration, soft spots etc. and measurements for hardness of fumigated fruits held at 10°C were recorded on days 1, 7 and 14. For all fruits, except eggplant, there were no statistically significant differences (p>0.05) in hardness between treated and untreated control on days 1, 7 and 14 post-treatment (Appendix 13). For eggplant, there were significant differences in hardness on days 7 and 14 for all four treatments. The external appearance of fumigated fruit varied depending on the dose, fruit, and the number of days it was stored at 10°C. Of all three fruits tested, chilli stored well until day 14 post-fumigation with all four doses. Capsicum stored well until day 14 after fumigation at all doses except 24 mg/L at 13°C for 7.5 h and 32 mg/L at 13°C for 6 h where some fungal growth initiated by day 14. Eggplant fumigated with all four doses stored well until day 7 but by day 14 showed fungal growth and skin browning (Appendix 14).

For the two new doses, in addition to capsicum, chilli and eggplant, truss tomato was also tested. It was found that for all fruits there was no visual damage (Appendix 14), and the fruit hardness of treated fruit did not significantly differ (p>0.05) from controls for any fruit until day 14 except for eggplant (data not shown). Discoloration on the flesh of treated eggplant was seen in addition to fungal growth on calyx but that was also present on control fruit. There was more damage on eggplant stored until day 14 that were treated at 24 mg/L than at 16 mg/L at 21°C for 2 h. Overall, eggplant fumigated with all six doses stored well only until day 7 but by day 14 showed fungal growth and skin browning. Storing eggplant beyond day 7 influenced fruit hardness and compromised its visual appearance for all six treatments and the fruit is not edible due to fungal growth.

Irradiated fruit

Seven doses, 50, 100, 150, 200, 250, 300 and 350 Gy were assessed for effects of irradiation on truss tomato quality. At 7 days after irradiation, visual observations were made in addition to recording hardness, weight, pH, °BRIX and colour co-ordinates (*l*, *a*, *b*) to deduce fresh tomato colour index (TCI_f) between treated and control fruits). Results indicated that there were no significant differences (p>0.05) in hardness, pH, °BRIX and TCI_f of treated fruit in comparison to control fruit. On the other hand, fruit weight of control was higher and significantly different than 300 and 350 Gy (Appendix 15). Visual observations indicated that the treatment did not cause any physical damage or injury and there were no differences observed in comparison to control fruit after 10 days of storage at 10°C (Appendix 16).

3.7 Chemical analysis

For each dose, fruit type and location in the room, fruit was bulked, and analysis was done for the presence of bromomethane in 38 fumigated and untreated control samples using GC-MS. The maximum residue limit permissions as recognized by FSANZ (Standard 1.4.2) is 50 mg/kg for capsicum. The residue analysis data (detection limit of 0.1 mg/kg) indicated that the residue levels of <0.5 mg/kg were below the permitted limits in untreated controls and red and green capsicums sampled from any of three locations within the fumigation chamber at doses of 24 mg/L at 13°C for 7.5 h, 24 mg/L at 21°C for 5.5 h, 32 mg/L at 21°C for 5.5 h, 32 mg/L at 13°C for 6 h, 16 mg/L at 21°C and 24 mg/L at 21°C for 2 h (ChemCentre Report 21S2411). Thus, none of the treatments exceeded the permitted maximum residue limit.

Outputs

The project was successful in developing several treatments at a commercial scale that could be used as alternative disinfestation options in crops affected by TPP and Medfly. Based on the information gathered in this project, data package demonstrating the effectiveness of LDMB disinfestation treatment Medfly and TPP in capsicum will be developed for submission to SDQMA. Effectiveness of ethyl formate and Vapormate® as alternative treatments could also be presented for registration to APVMA and FSANZ. During the project, research results were communicated to industry (Vegetables WA), funding bodies, SDQMA and growers through articles, seminars and meetings are listed below.

- Project reference group meetings held on 11th December 2020, 28th May 2021, 07th December 2021 and 17th July 2022.
- Insect research comes full circle. WA Grower Magazine, Vegetables WA, Spring 2019, Vol. 54 (3):46-47.
- Tomato Potato Psyllid, Fall Armyworm and Exotic Leafminers Webinar Series. Vegetable Industry Webinar Series. https://www.youtube.com/playlist?list=PL4cJZzAUfvKAtqZd-vEbGlIuA8pB1caqj
- Western Australian Research on TPP develops treatments for market access of affected crops. WA Grower Magazine, Vegetables WA, Spring 2020, Vol. 55 (3): 20-21.
- Alternative TPP and Medfly disinfestation treatments for WA produce. WA Grower Magazine, Vegetables WA, Spring 2022, Vol. 57 (3): 41-43.

Outcomes

The project resulted in key outcomes on practical, feasible and non-damaging end-point disinfestation treatment for both Medfly and TPP using low dose methyl bromide, ethyl formate and irradiation. Major findings of the research are as follows.

- Low dose methyl bromide treatments were identified that can be effectively used against Medfly and TPP in their common solanaceous hosts, capsicum, chilli, eggplant, and tomato. Medfly eggs were found to be the most tolerant life-stage.

- Four low dose methyl bromide treatments can be applied effectively against Medfly; these were 24 mg/L at 21°C for 5.5 h, 24 mg/L at 13°C for 7.5 h, 32 mg/L at 21°C for 5.5 h and 32 mg/L at 13°C for 6 h.
- These doses can also be applied effectively against TPP eggs, nymphs, and adults.
- Two shorter doses of 16 mg/L and 24 mg/L at 21°C for 2h were found to be effective against all the life-stages of TPP and are lower than the currently recognised lowest dose of methyl bromide recommended in the ICA-04 for TPP.
- The application of two short and four long exposure doses did not compromise fruit quality or its external appearance. Longer-term storage of fumigated fruit at 10°C to mimic shelf or in-transit conditions can be achieved for chilli, capsicum and tomato at all doses for up to 14 days. Eggplant is sensitive to fumigation injury and can be stored well until day 7.
- The methyl bromide residues from fumigated and untreated fruit are not different and within the recognised MRLs (FSANZ, 2022).
- Ethyl formate with and without carbon dioxide can effectively control TPP life-stages but is not as effective on Medfly which is an internal pest. The egg stage of TPP is the most tolerant life-stage. In the presence of carbon dioxide, EF yielded better control of TPP eggs with doses above 40 mg/L+ 15% CO_2 for 2 h at 10°C.
- Two doses of Vapormate®, 42 mg/L and 54 mg/L for 2 h at 10°C. The dose of 42 mg/L resulted in a high level of control of TPP eggs however, it did not provide quarantine security. This dose can be used an end-point treatment with other control measures such as high pressure washing or systems approach. The dose of 54 mg/L satisfied quarantine standard and can be applied for effective disinfestation of TPP.
- X-ray irradiation of doses greater than 350 Gy can be applied to achieve mortality of TPP eggs and nymphs. Doses ≥400 Gy reduce the lifespan of adults and eggs from irradiated females are not viable.
- Fruit quality due to irradiation is not affected as there is no difference in tomato fruit quality parameters such as hardness, Brix°, color or pH of irradiated and untreated fruit.

Monitoring and evaluation

Milestone outputs supplied through the Monitoring and Evaluation Plan of the project were as below.

- M102 (31-05-2020). This report provided information on: TPP and Medfly development in capsicum, chilli, eggplant and tomato, and comparative response of TPP against ethyl formate by testing the most tolerant lifestage.
- M103 (30-11-2020). This report identified TPP eggs to be the most tolerant lifestage and identified a suitable dose of ethyl formate and carbon dioxide. It also reviewed doses for fruit injury on capsicum, chilli, eggplant, and tomato.
- M104 (31-05-2021). Largescale semi-commercial ethyl formate trials on capsicum and tomato and irradiation tests were initiated and completed.
- M105 (31-11-2021). Comparative response of TPP and Medfly against low dose methyl bromide by testing mpost tolerant lifestage initiated at commercial fumigation service.
- M106 (30-05-2022). Largescale low dose methyl bromide trials were completed for TPP and Medfly, residue data was obtained for commercial treatments and effective irradiation doses against TPP were identified. There is opportunity for future research to test successful doses that work against Medfly also work against endemic fruit fly species in horticulture produce such as grape, citrus and berries.

Recommendations

- Low dose methyl bromide fumigation is an effective alternative treatment to disinfest Medfly and TPP without causing phytotoxicity and could be used as a treatment option for both pests in their common hosts. The two new 2 h shorter doses of 16 and 24 g. per cu. m at 21°C provide quarantine security, are lower than the currently recognised doses on ICA-04 for TPP and could be used in future. These doses would reduce environmental impacts, the associated costs of using MB, fruit injury, and chemical residue. The low dose methyl bromide treatments that are effective against Medfly may also be effective against endemic species such as *Bactrocera aquilonis* and *B. jarivisi* but this requires further research.

- Ethyl formate (Vapormate®) is environmentally friendly, not phytotoxic, requires shorter treatment times to disinfest TPP and has a potential for market access of capsicum, chilli, eggplant and tomato.
- Since both treatments satisfied quarantine standards, WA could request consideration from other states for this treatment to be considered effective and developed into an Interstate Certification Arrangement or Approved Quarantine measure.
- Irradiation can offer an alternative treatment that is chemical-free, environmentally friendly, not phytotoxic and requires shorter treatment time. Doses ≥400 Gy are potentially detrimental to eggs and nymphs and yield unviable eggs from irradiated females. Further research on reciprocal crossing, ovarian dissections along with largescale trials to satisfy quarantine requirements are required.
- Registration of alternative treatment with regulatory bodies such as APVMA and FSANZ may be explored to facilitate market access of host produce affected by Medfly and TPP.

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Intellectual property

No project IP or commercialisation to report.

Acknowledgements

The project was co-funded by Hort Innovation and the Department of Primary Industries through the Vegetables industries R&D levy. The project was supported by Mr. Rohan Prince, Director of Horticulture & Irrigated Agriculture (DPIRD) and Dr. Sonya Broughton, Chief Plant Biosecurity Officer (DPIRD). The project work was supervised by Dr. Francis De Lima, Director, AgHort Solutions Pty Ltd. Trials were done by Dr. Vineeta Bilgi, Mrs. Emma Mansfield, Mr. Juan Sorrequieta and Mr. Christopher Fell. Irradiation trials were assisted by Mr. Ian Lacey, Jeremey Lindsey and Ms.

Rose Fogliani. Commercial trials with methyl bromide were completed at Tritan Fumigation kindly assisted by Mr. Yohan Vales de Menezes. Casual staff support was provided by Ms. Olivia Shaw-Szymanski, Ms. Ida Mosberg, Mr. Joonathan Adamoniene, Mr. Alex Van Hooran, Mr. Lachlan Halliday and Mr. Thompson Cross. Industry support was provided by Mr. John Shannon and Mr. Callum Fletcher (AusVeg), and Mr. Manus Stockdale, VegetablesWA.

Appendices

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Appendix 3. Natural and artificial infestation of fruit for Medfly life-stages and TPP cup cage assembly
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Appendix 15. Physicochemical parameters of untreated control and irradiated truss tomato.
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Appendix 1. Rearing of Mediterranean fruit fly colony

1. Origin:

The Medfly (Mediterranean fruit fly) colony at the Department of Primary Industries and Regional Development, South Perth, was first established in April 1983 from infested citrus collected from Carnarvon. The genetic fitness is maintained by replenishing the existing colony with wild flies collected every 12 to 18 months. Collection has been from locations in south-west Western Australia including Belmont, Bindoon, Bridgetown, Canning Vale, Cannington, Carmel, Chittering, Cloverdale, Collie, Donnybrook, Dwellingup, Harvey, Kalamunda, Katanning, Maddington, Mandurah, Manjimup, Nannup, Perth Hills, Roleystone, Serpentine, and Stoneville. In 2019 and 2020 the colony was renewed with wild flies from collections in Perth and Manjimup. The fruits from which wild flies have been obtained include apples, guavas, mandarins, nectarines, oranges, pears, plums, and peaches. The identity of the colony has been validated from 1991 to 2006 through supply of colony flies to international laboratories (International Atomic Energy Authority, Sibersdorf, Austria (IAEA), Italy (University of Pavia) and the USA (USDA laboratories in Hawaii, Florida, and Washington DC), New Zealand Plant Research Institute as well as nationally by State and Commonwealth Institutions in Australia.

2. Rearing methods:

Colony is maintained by artificially breeding new stock from eggs each week. Adult females oviposit through the mesh cloth on the sides of the cages and eggs are collected in water troughs. A 24 h egg collection is introduced to an artificial rearing medium and rearing trays are held in separate larval rearing room at 21°C and RH 65%. Mature pupae are collected in sand 13-16 days after oviposition. The pupae are sieved out of the sand and new fly cages are prepared in adult rearing room at 26°C and RH 50%. Adults emerge in 3 to 5 days. Emerging adults feed on yeast, sugar and water that is provided regularly. After a cage has remained in the adult colony room for 4 weeks it is cleaned out, sterilised and renewed with fresh pupae. When more adult flies or eggs are required for experimental work, additional cages and or larvae rearing trays are prepared to give insects of known age.

(i) **Adults**: Approximately 6.5 L of pupae are introduced into the adult rearing cages. Cage dimensions are 200 cm (length) x 150 cm (height) x 40 cm (depth). The adult flies that emerge are fed on a diet of yeast hydrolysate, crystalline sugar and water all placed in separate containers. The quantities consumed by an adult colony over a 4-week period are approximately, 840 g yeast hydrolysate, 2500 g crystalline sugar and 12 litres water. It is expected that a large proportion of the water is lost in evaporation.

Number of adult flies emerging per cage: 250,000 - 300,000 viable adult flies emerge/cage.

Sex ratio: 50:50

Number of cages in routine colony: 4 cages are constantly maintained. Every week the cage that is approximately 4 weeks old is renewed with a fresh cage. When more eggs or adults are required for experimental purposes the colony size is increased to 6 -8 cages.

Rearing room conditions: Constant temperature 26 ± 1.0°C; 60 - 65% RH and a darkness:light cycle of 15-16:8-9 h is constantly maintained in the adult breeding room.

Method of egg collection: The eggs are deposited through the sides of the cages by the adult females into sterilised water about 10 days after their emergence from the puparium and mating of the adult flies.

Quantity of eggs collected: Approximately 20-60 mL /day/cage.

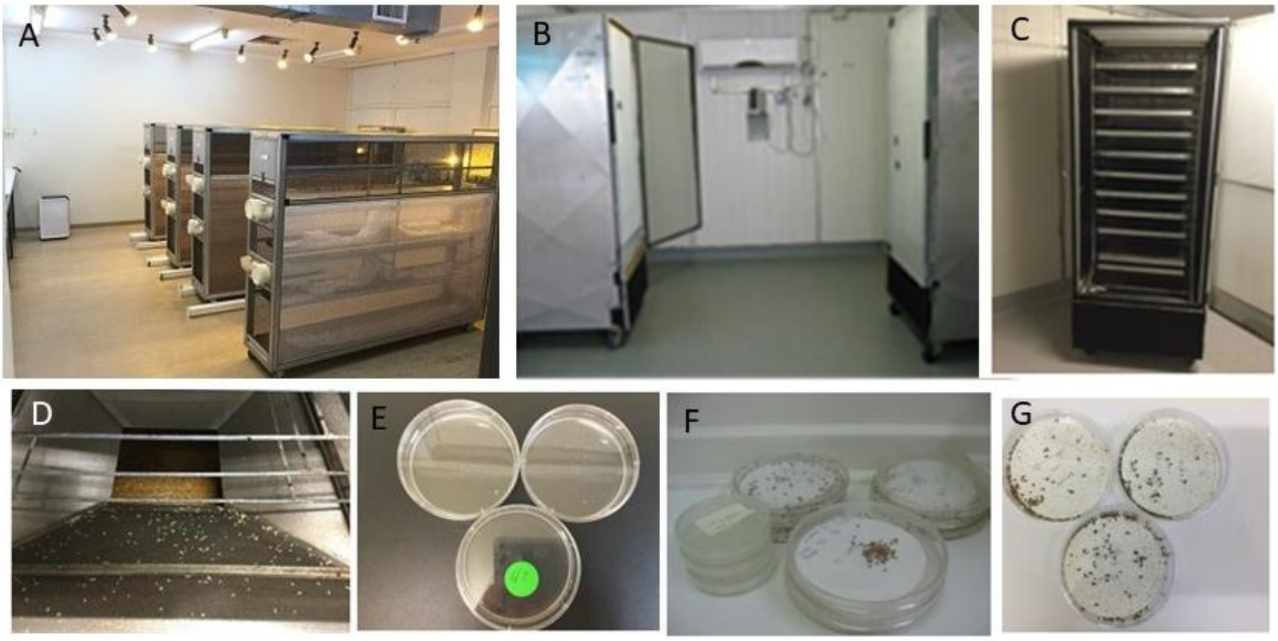
(ii) **Larvae:** The larval rearing is done on an artificial medium based on sterilised untreated paper pulp. Special larval rearing cages are prepared, and larval rearing is done in a separate room from the adult colony. The following ingredients are mixed in the specified proportions into a homogenous mix, which is then spread on 11 stainless steel trays for egg deposition.

Medium:

3.5 kg Paper pulp
6.0 kg Crystalline cane sugar
3.0 kg Yeast hydrolysate
50 g Methyl propylhydroxybenzoate (Nipagin)
50 g Propylhydroxybenzoate (Nipasol)
50 g Sodium benzoate
20 ml Hydrochloric acid
21 litres Water. Final pH = 5.4

Quantity of medium: Approximately 32 kg of medium is made in each mix and about 2.8 to 2.9 kg per tray (tray size: 53x53x3 cm) is used. Eleven trays are prepared each week.
Quantity of eggs inoculated: 5 mL/tray x 8 trays/cage (number of eggs inoculated: approximately 150,000 eggs/tray x 11 trays/cage).
Rearing Room conditions: 26°C ±1.0°C; 60 - 65% RH
Period of developmental stages:
Eggs: 2 days
1st - 3rd instar larvae: 6 - 8 days.
Pupae: 10 - 11 days.
Adults: 4 - 6 weeks.
Rate of egg hatch: approx. 60 - 90%
Rate of larval pupation: 80 - 90%
Collection of pupae in larval rearing cage: The 3rd instar larvae jump out of the trays into the bottom of the cage and pupate in sterilised sand in trays (53x53x3 cm).

(iii) **Pupae:**
Maturation of pupae: The trays containing larvae in sand (53x53x3 cm) are transferred to another cage for 5-6 days until most of the larvae have pupated. This pupal maturation cage is in the same controlled environment room as the larvae. Thereafter they are transferred to the adult colony cages for emergence as described above.
Number of pupae reared: Approximately 200,000 pupae/tray x 4 trays x cage = approx. 800,000 pupae (10 litres).
Rearing temperature/humidity: 26°C ±1.0°C; 60 - 65% RH.
Rate of emergence: Approximately 90%.



Medfly adult rearing cages (A), larva rearing cabinets (B and C), pupae collection tray (D), quality control for egg hatch (E) and adult emergence from pupae (F and G).

Appendix 2. TPP colony

Tomato plant nursery maintained in greenhouse (A), water-powered fertiliser injector system (B), irrigation station controller (C), drip irrigation (D), TPP rearing cages inside growth room, TPP eggs on tomato leaf (F), late nymph (G), young adults (H and I), and adults (J).

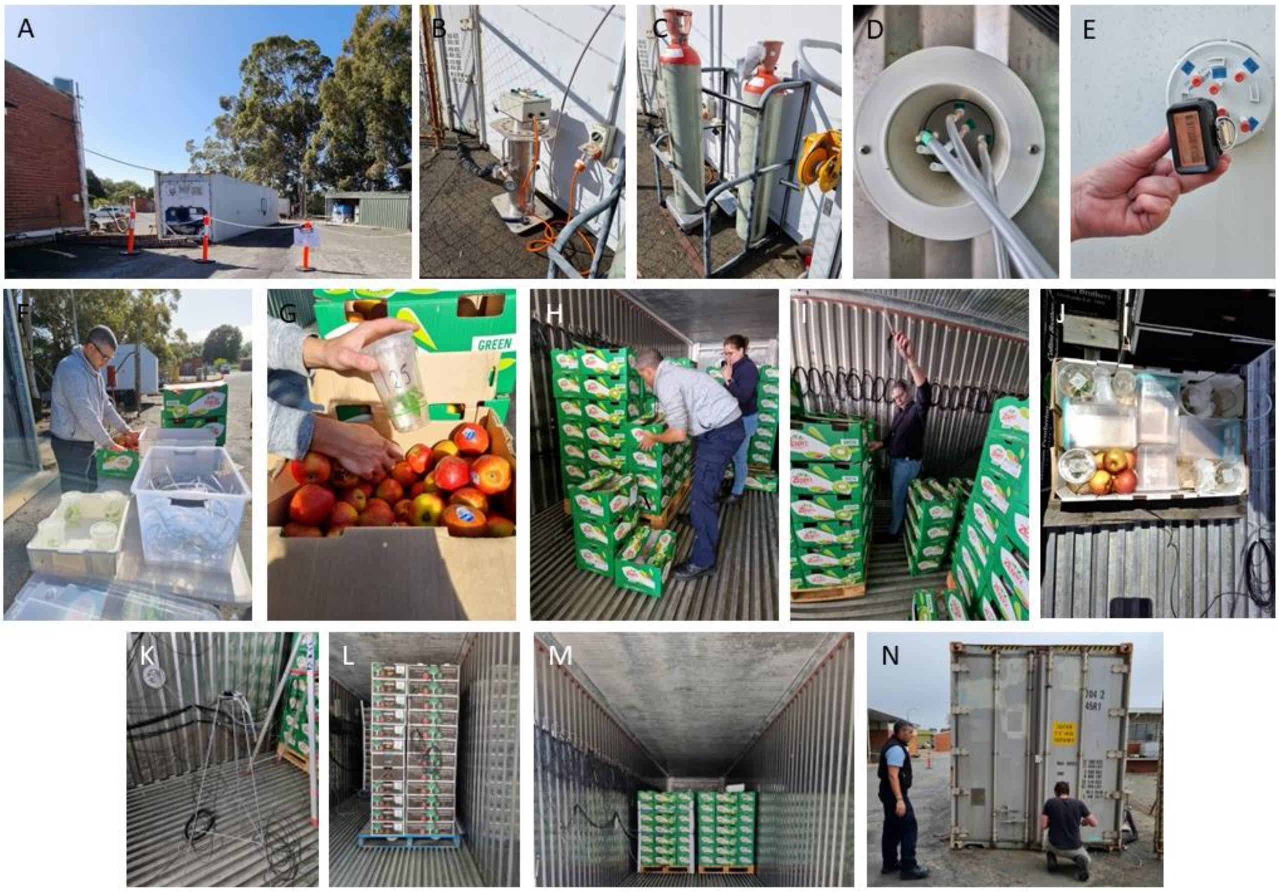
Appendix 3. Natural and artificial infestation of fruit for Medfly life-stages and TPP cup cage assembly.

Natural infestation of capsicum (A) and chilli (B) in adult Medfly cages, paper media prepared for artificial infestation of Medfly in eggplant (C and D), capsicum fruit setup after infestation (E), nonfeeding 3rd instars collected in sand (F and G), TPP cup cage assembly (H), tomato leaflets setup in vials (I), and cup cage assemblies prepared for trials.

Appendix 4. EF fumigation

EF fumigation trials in desiccators (A), tomato leaves with TPP life-stages (B), fumigation on fruit (C), and portable gas detector Microtector II G-460 for EF and CO_2 (D).

Appendix 5. Vapormate® fumigation



Vapormate® trials conducted in a 40-foot refrigerated shipping container at DPIRD (A), vaporiser (B), gas cylinders on weighing scale and in stock (C), gas lines from the container (D) attached to tubes used for gas sampling using the Microtector II G-460 and G-600 (E), TPP cup cage assemblies being prepared (F) to be loaded inside bulk fruit (G), bulk fruit with samples being loaded on pallets (H), temperature probes being prepared (I) to record fruit and air temperature (J and K), pallet ready for fumigation (L and M), and container being sealed (N).

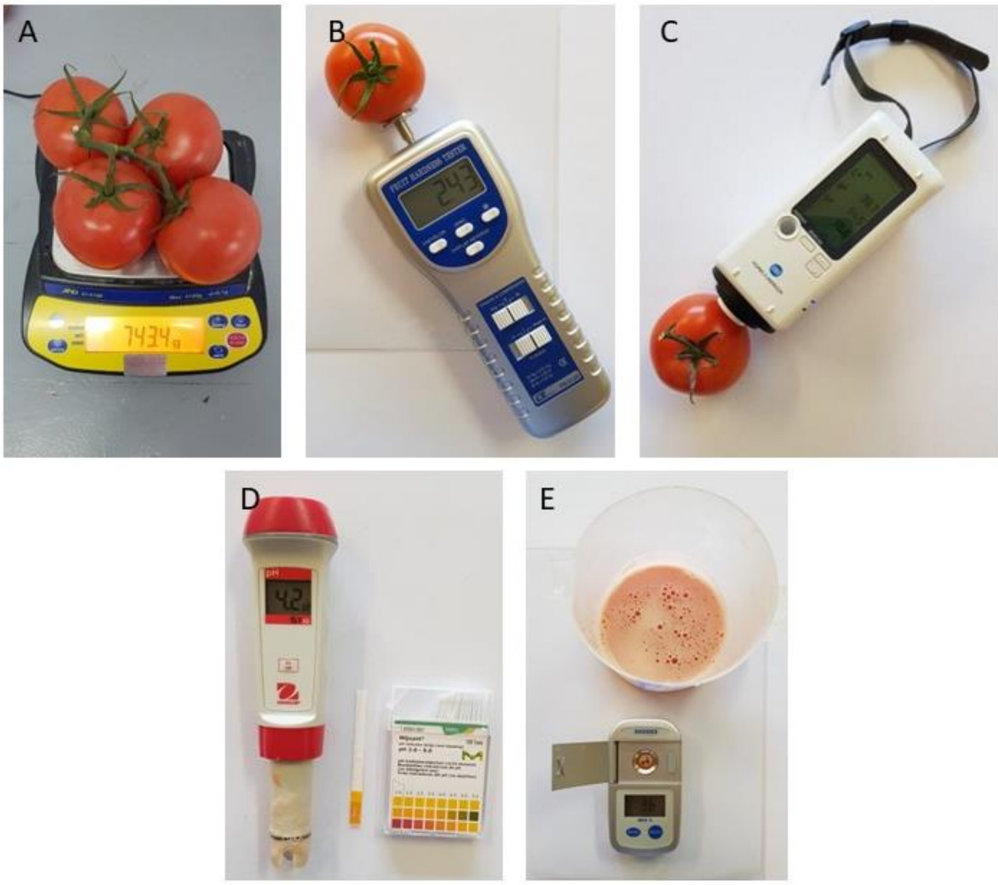
Appendix 6. Low dose methyl bromide fumigation

Temperature probes prepared for fumigation trials (A), probes inserted into fruit (B, C, D), temperature data being recorded during trials (E), trials in progress inside fumigation rooms (F), vapouriser and gas line setup (G), gas lines for each room (H), Fumiscope (I), ventilation (J) and temperature (K) control systems, natural infestation of capsicum (L), setup of capsicum infested with Medfly eggs in controlled environment (M) and TPP cup cage assemblies prepared for fumigation trials.

Appendix 7. Phytosanitary irradiation

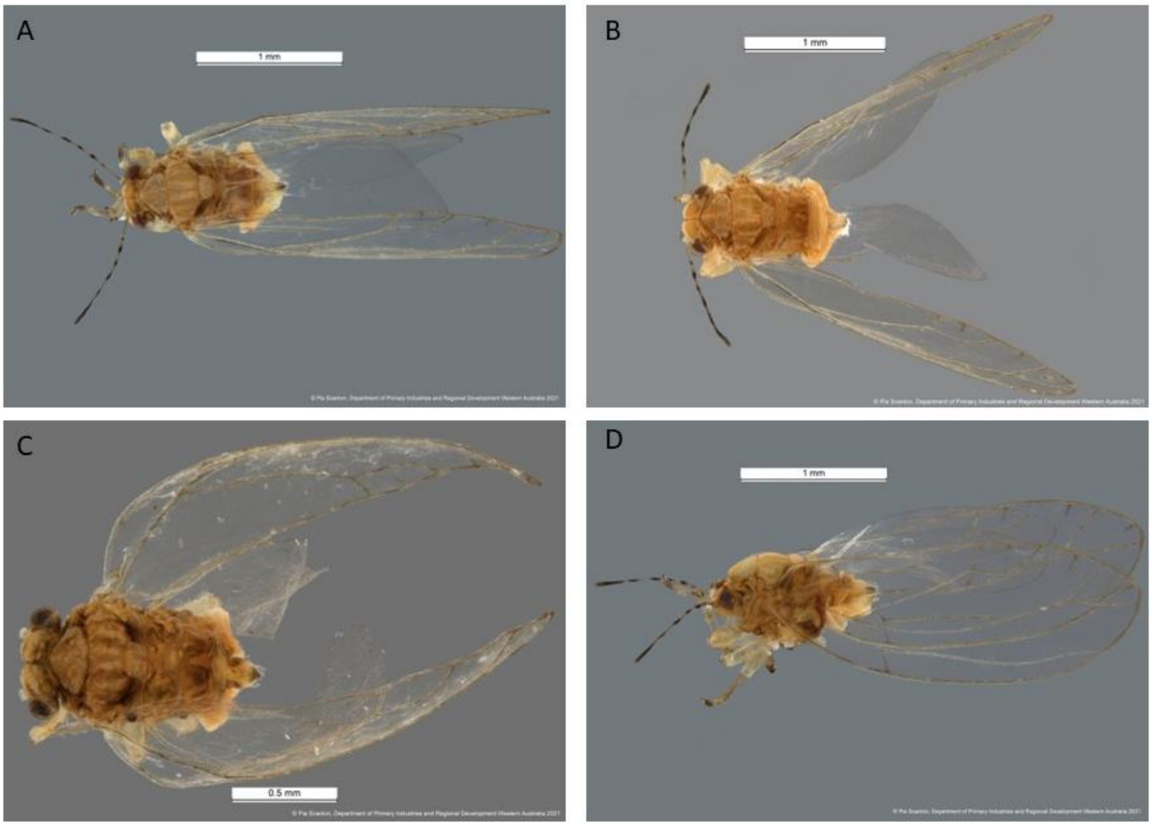
Radsource RS2400Q X-ray irradiator (A) loaded with TPP samples (B), staff preparing TPP samples (C) and operating the irradiator (D).

Appendix 8. Fruit quality tests



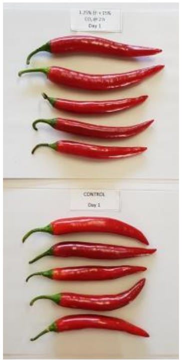
Data being recorded for fruit weight (A), hardness (B), colour parameters (C), pH (D) and Brix°.

Appendix 9. TPP deformities post-irradiation



Dorsal (A, B, and C) and lateral (D) views of deformed TPP adults that developed from irradiated small or large nymphs.

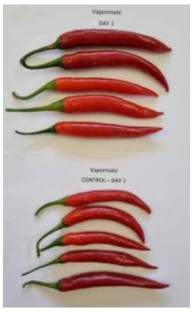
Appendix 10. External appearance of fruit fumigated (top) with 1.25% ethyl formate and 15% carbon dioxide at 10°C for 2h and untreated fruit (bottom) on days 1, 7 and 14.

	Days after fumigation		
Fruit	Day 1	Day 7	Day 14
Capsicum			
Chilli			
Eggplant			
Truss tomato			

Appendix 11A. External appearance of fruit fumigated (top) with 42 mg/L Vapormate® at 10°C for 2h and untreated fruit (bottom) on days 1, 7 and 14.

	Days after fumigation		
Fruit	Day 1	Day 7	Day 14
Capsicum			
Chilli			
Eggplant			
Truss tomato			

Appendix 11B. External appearance of fruit fumigated (top) with 54 mg/L Vapormate® at 10°C for 2h and untreated fruit (bottom) on days 1, 7 and 14.

	Days after fumigation		
Fruit	Day 1	Day 7	Day 14
Capsicum			
Chilli			
Eggplant			
Truss tomato			

Appendix 12. Physicochemical parameters of untreated control and fruit treated with 1.25% EF + 15% CO_2 for 2h.

Variable	Group	Mean (M)	Standard deviation (±SD)	*t**	*df*	*p*	95% CI (Range) Lower	Upper
Capsicum								
Hardness (kg/cm^2)								
Day 1	Treated	0.95	0.20	-0.94	7.99	0.37	0.77	1.13
	Control	1.07	0.20				0.90	1.25
Day 7	Treated	0.91	0.09	-2.04	6.05	0.09	0.93	0.99
	Control	1.09	0.17				0.94	1.24
Day 14	Treated	0.87	0.06	-1.88	7.70	0.10	0.81	0.92
	Control	0.93	0.05				0.88	0.97
Weight (gm)								
Day 1	Treated	294.14	12.47	-0.02	6.63	0.98	283.21	305.07
	Control	295.44	9.76				286.89	304.00
Day 7	Treated	294.8	8.96	-0.34	4.52	0.75	286.95	302.65
	Control	296.2	2.28				294.20	298.20
Day 14	Treated	295.6	10.06	-0.43	4.42	0.69	286.78	304.42
	Control	297.6	2.30				295.58	299.62
Chilli								
Hardness (kg/cm^2)								
Day 1	Treated	0.23	0.03	-1.61	5.27	0.17	0.21	0.25
	Control	0.28	0.06				0.22	0.34
Day 7	Treated	0.23	0.02	-1.62	5.27	0.17	0.22	0.34
	Control	0.28	0.06				0.21	0.25
Day 14	Treated	0.22	0.03	-0.38	7.64	0.71	0.20	0.25
	Control	0.23	0.04				0.20	0.26
Weight (gm)								
Day 1	Treated	19.94	2.66	0.22	7.99	0.83	17.60	22.27
	Control	19.58	2.58				17.32	21.84
Day 7	Treated	19.26	0.84	-0.95	5.14	0.39	18.52	20.00
	Control	20.26	2.20				18.34	22.19
Day 14	Treated	19.18	0.46	-1.21	5.07	0.28	18.78	19.58
	Control	19.90	1.24				18.81	20.99
Eggplant								
Hardness (kg/cm^2)								
Day 1	Treated	3.03	0.64	-0.23	7.55	0.82	2.47	3.59
	Control	3.11	0.50				2.67	3.55
Day 7	Treated	3.03	0.64	0.03	4.62	0.97	2.47	3.59
	Control	3.02	0.18				2.86	3.17
Day 14	Treated	3.34	0.42	1.44	4.81	0.21	2.97	3.71
	Control	3.06	0.13				2.94	3.18
Weight (gm)								
Day 1	Treated	431.46	25.37	-0.21	7.83	0.84	409.22	453.69
	Control	434.6	21.91				514.39	453.81
Day 7	Treated	429.2	16.71	0.38	6.11	0.72	414.55	443.85
	Control	423.2	31.24				395.81	450.58
Day 14	Treated	400.60	11.91	-0.31	8.00	0.76	390.16	411.04
	Control	402.94	12.07				392.36	413.52
Tomato								
Hardness (kg/cm^2)								
Day 1	Treated	1.94	0.03	0.50	7.88	0.63	1.92	1.97
	Control	1.93	0.03				1.90	1.96
Day 7	Treated	1.83	0.11	-1.42	4.44	0.22	1.73	1.93
	Control	1.91	0.03				1.88	1.93
Day 14	Treated	1.81	0.05	-1.61	7.38	0.15	1.77	1.86
	Control	1.87	0.06				1.81	1.93
Weight (gm)								
Day 1	Treated	190.10	4.88	-0.21	7.82	0.84	185.82	194.38
	Control	190.70	4.20				187.02	194.38
Day 7	Treated	180.17	5.77	-0.16	6.33	0.88	175.12	185.23
	Control	180.65	3.27				177.78	183.52
Day 14	Treated	168.40	2.52	-0.64	6.90	0.54	166.19	170.60
	Control	169.27	1.65				167.82	170.72
Diameter (mm)								
Day 1	Treated	67.75	1.5	-0.73	7.84	0.48	66.43	69.06

	Control	68.5	1.7				66.98	70.02
Day 7	Treated	67	0	.	.	.	67.00	67.00
	Control	67	0				67.00	67.00
Day 14	Treated	66.25	1.5	-1.58	8.00	0.15	64.93	67.56
	Control	67.75	1.5				66.43	69.07
pH								
Day 1	Treated	4.35	0.06	0.73	7.84	0.48	4.30	4.4
	Control	4.32	0.05				4.28	4.37
Day 7	Treated	4.37	0.05	-1.12	4.00	0.33	4.33	4.42
	Control	4.40	0				.	.
Day 14	Treated	4.35	0.05	0	8.00	1.15	4.30	4.40
	Control	4.35	0.05				4.30	4.40
°BRIX								
Day 1	Treated	3.88	0.10	-0.34	5.08	0.75	3.79	3.97
	Control	3.90	0.04				3.87	3.93
Day 7	Treated	3.87	0.05	-0.53	7.35	0.61	3.82	3.92
	Control	3.89	0.04				3.85	3.93
Day 14	Treated	4.14	0.08	-0.25	7.14	0.80	4.10	4.21
	Control	4.16	0.12				4.05	4.26
TCI_f								
Day 1	Treated	34.88	0.61	2.24	4.27	0.06	34.34	35.41
	Control	31.17	3.30				28.29	34.06
Day 7	Treated	35.75	4.23	0.52	7.56	0.61	32.04	39.46
	Control	34.49	3.30				31.59	37.39
Day 14	Treated	37.75	1.80	2.53	6.63	0.07	36.17	39.33
	Control	33.84	2.95				31.26	36.42

§ Student's or Welch's t-tests.

Appendix 13. Hardness of untreated control and fruit exposed to LDMB doses.

Variable	Group	Mean (M)	Standard deviation (SD)	$t^{§}$	*df*	*p*	95% CI (Range) Lower and Upper	
24 mg/L at 21°C for 5.5h								
Capsicum								
Day 1	Treated	1.57	0.09	-0.01	5	0.99	1.50	1.65
	Control	1.58	0.27				1.34	1.81
Day 7	Treated	1.58	0.17	0.03	7	0.98	1.43	1.73
	Control	1.58	0.26				1.35	1.81
Day 14	Treated	1.65	0.13	0.07	8	0.94	1.54	1.76
	Control	1.64	0.13				1.52	1.76
Chilli								
Day 1	Treated	0.82	0.6	-0.71	8	0.49	0.77	0.86
	Control	0.84	0.06				0.86	0.89
Day 7	Treated	0.83	0.04	0	8	1	0.79	0.86
	Control	0.83	0.04				0.79	0.86
Day 14	Treated	0.58	0.06	-0.44	8	0.67	0.53	0.62
	Control	0.59	0.07				0.5	0.66
Eggplant								
Day 1	Treated	2.82	0.88	-0.64	6	0.54	2.05	3.59
	Control	3.11	0.50				2.67	3.55
Day 7	Treated	2.08	0.28	-6.27	7	0.0004*	1.83	2.33
	Control	3.02	0.18				2.86	3.18
Day 14	Treated	1.75	0.18	-8.77	8	2.2e-05*	1.59	1.91
	Control	2.90	0.23				2.70	3.10
24 mg/L at 13°C for 7.5h								
Capsicum								
Day 1	Treated	1.60	0.19	-0.02	8	0.99	1.42	1.76
	Control	1.60	0.19				1.43	1.77
Day 7	Treated	1.68	0.14	-0.11	8	0.92	1.55	1.81
	Control	1.69	0.15				1.56	1.82
Day 14	Treated	1.51	0.11	0	5	1	1.41	1.61
	Control	1.51	0.04				1.47	1.55
Chilli								
Day 1	Treated	0.79	0.09	-0.08	8	0.94	0.71	0.87
	Control	0.80	0.07				0.74	0.86
Day 7	Treated	0.83	0.05	-0.98	7	0.36	0.78	0.87
	Control	0.86	0.06				0.82	0.88
Day 14	Treated	0.53	0.06	-0.54	8	0.60	0.48	0.50
	Control	0.56	0.06				0.59	0.61
Eggplant								
Day 1	Treated	2.81	0.71	-1.03	7	0.33	2.18	3.43
	Control	3.20	0.45				2.08	3.60
Day 7	Treated	2.70	0.16	-2.96	8	0.02*	2.56	2.84
	Control	3.02	0.18				2.86	3.18
Day 14	Treated	1.80	0.16	-12.03	6	2e-05*	1.66	1.94
	Control	2.77	0.08				2.70	2.84
32 mg/L at 21°C for 5.5h								
Capsicum								
Day 1	Treated	1.52	0.11	0.19	5	0.86	1.42	1.62
	Control	1.51	0.04				1.47	1.55
Day 7	Treated	1.66	0.16	-0.07	8	0.95	1.52	1.80
	Control	1.67	0.12				1.56	1.78
Day 14	Treated	1.43	0.15	-0.11	8	0.92	1.30	1.56
	Control	1.44	0.14				1.32	1.56
Chilli								
Day 1	Treated	0.79	0.05	-0.06	8	0.95	0.74	0.83
	Control	0.79	0.05				0.74	0.83
Day 7	Treated	0.80	0.08	-0.28	7	0.79	0.73	0.87
	Control	0.81	0.05				0.76	0.86
Day 14	Treated	0.41	0.10	-0.51	7	0.62	0.32	0.37
	Control	0.44	0.07				0.49	0.50
Eggplant								
Day 1	Treated	3.18	0.39	-1.01	8	0.34	2.84	3.52

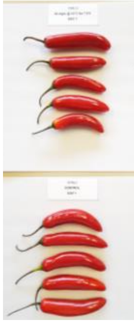
	Control	3.41	0.31				3.13	3.68
Day 7	Treated	2.32	0.35	-5.20	7	0.001*	2.01	2.63
	Control	3.28	0.22				3.09	3.47
Day 14	Treated	1.70	0.16	-9.10	8	1.7e-05*	1.55	1.84
	Control	2.75	0.20				2.56	2.93
32 mg/L at 13°C for 6h								
Capsicum								
Day 1	Treated	1.54	0.04	-0.75	8	0.47	1.50	1.58
	Control	1.56	0.04				1.52	1.60
Day 7	Treated	1.64	0.13	-0.19	8	0.85	1.53	1.76
	Control	1.66	0.13				1.54	1.78
Day 14	Treated	1.57	0.18	-0.27	8	0.79	1.41	1.73
	Control	1.60	0.19				1.43	1.77
Chilli								
Day 1	Treated	0.83	0.08	-0.08	8	0.94	0.76	0.90
	Control	0.83	0.08				0.76	0.90
Day 7	Treated	0.81	0.06	-0.11	8	0.92	0.76	0.87
	Control	0.82	0.05				0.77	0.86
Day 14	Treated	0.41	0.10	0.12	6	0.91	0.33	0.36
	Control	0.41	0.06				0.50	0.46
Eggplant								
Day 1	Treated	3.50	0.21	-0.24	8	0.81	3.29	3.71
	Control	3.54	0.30				3.28	3.80
Day 7	Treated	2.47	0.40	-3.58	6	0.01*	2.07	2.87
	Control	3.28	0.22				3.09	3.47
Day 14	Treated	1.83	0.42	-3.58	5	0.01*	1.40	2.25
	Control	2.65	0.18				2.50	2.81

§ Student's or Welch's t-tests. * Significant statistical difference with $p<0.05$.

Appendix 14. External appearance of fruit fumigated (top) with six low doses of methyl bromide and untreated fruit (bottom) on days 1, 7 and 14.

Days	Capsicum	Chilli	Eggplant
	24 mg/L at 21°C for 5.5h		
1			
7			
14			

Fruit fumigated with 24 mg/L at 21°C for 5.5h had no injury post-treatment at days 1, 7 and 14, except for eggplant. On day 14, eggplant displayed fungal growth on calyx, skin browning and few discoloured patches which was absent on control fruit.

Days	Capsicum	Chilli	Eggplant
	24 mg/L at 13°C for 7.5h		
1			
7			
14			

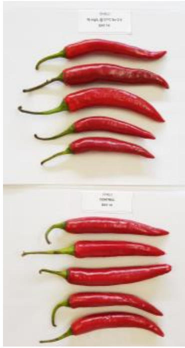
Fruit fumigated with 24 mg/L at 13°C for 7.5h had no injury post-treatment at days 1 and 7, except eggplant that showed slight browning of the calyx but none on its edible part. On day 14, while chilli showed no damage, treated and control capsicum displayed a few spots of fungal growth on calyx and flesh, and treated eggplant displayed skin browning and fungal growth on calyx.

Days	Capsicum	Chilli	Eggplant
	32 mg/L at 21°C for 5.5h		
1			
7			
14			

Fruit fumigated with 32 mg/L at 21°C for 5.5h had no injury post-treatment at days 1, 7 and 14 except eggplant which had injuries on day 7 and 14. On day 7, slight browning of the calyx was seen but there was none on its flesh. On day 14, eggplant displayed skin browning and fungal growth on calyx and flesh which was absent on control fruit.

Days	Capsicum	Chilli	Eggplant
	32 mg/L at 13°C for 6h		
1			
7			
14			

Fruit fumigated with 32 mg/L at 13°C for 6h had no fruit injury caused to any of the fruits post-treatment at days 1, 7 and 14 except eggplant which had injuries on day 7 and 14. On day 7, slight browning of the calyx was seen but not on its flesh whereas on day 14, treated eggplant displayed skin browning and fungal growth on calyx and flesh.

Fruit	Day 1	Day 7	Day 14
	16 mg/L at 21°C for 2h		
Capsicum			
Chilli			
Eggplant			
Tomato			

Fruit	Day 1	Day 7	Day 14
	24 mg/L at 21°C for 2h		
Capsicum			
Chilli			
Eggplant			
Tomato			

Appendix 15. Physicochemical parameters of untreated control and irradiated truss tomato.

Group	Mean (M)	Standard deviation (±SD)	$F_{(7,88)}$§	*p*	95% CI (Range) Lower	Upper
Truss tomato						
Hardness (kg/cm₂)						
Control	1.50	0.27	2.06	p>0.05	1.35	1.65
50 Gy	1.42	0.29			1.26	1.58
100 Gy	1.25	0.22			1.13	1.37
150 Gy	1.19	0.28			1.03	1.34
200 Gy	1.12	0.11			1.06	1.19
250 Gy	1.13	0.10			1.08	1.19
300 Gy	1.14	0.13			1.07	1.21
350 Gy	1.13	0.08			1.12	1.22
Weight (gm)						
Control	152.92	21.27	4.83	p<0.01**	140.88	164.95
50 Gy	133.09	8.91			128.08	138.13
100 Gy	151.59	18.81			140.95	162.23
150 Gy	134.64	18.38			124.24	145.04
200 Gy	136.21	26.25			121.36	151.06
250 Gy	131.54	9.54			126.14	136.94
300 Gy*	128.08	16.18			118.92	137.23
350 Gy*	121.18	11.22			114.83	127.52
°BRIX						
Control	4.04	0.36	2.06	p>0.05	3.84	4.24
50 Gy	4.04	0.46			3.78	4.30
100 Gy	3.87	0.28			3.71	4.03
150 Gy	4.15	0.46			3.89	4.41
200 Gy	3.71	0.34			3.51	3.90
250 Gy	3.83	0.25			3.69	3.97
300 Gy	3.92	0.27			3.77	4.07
350 Gy	3.84	0.27			3.69	4.00
TCI_f						
Control	44.23	2.73	1.64	p>0.05	42.68	45.78
50 Gy	41.90	2.07			40.73	43.08
100 Gy	42.26	6.46			38.61	45.91
150 Gy	43.01	1.44			42.19	43.83
200 Gy	42.26	6.46			38.61	45.91
250 Gy	40.55	3.27			38.70	42.40
300 Gy	39.25	4.47			36.72	41.78
350 Gy	41.25	2.74			39.70	42.80

§One-way ANOVA, *significant statistical difference with *p*<0.05.

Appendix 16. External appearance of irradiated and untreated fruit.

Dose	Irradiated	Control
50 Gy		
100 Gy		
150 Gy		
200 Gy		
250 Gy		
300 Gy		
350 Gy