Irradiation of Melon Fly, *Zeugodacus cucurbitae* (Diptera: Tephritidae), in Artificially versus Naturally Infested Papayas

Peter A. Follett
USDA-ARS, Daniel K. Inouye U.S. Pacific Basin Agricultural Research Center,
64 Nowelo Street, Hilo, Hawai‘i. Correspondence: peter.follett@usda.gov

Abstract. Many fruits are naturally rarely infested by their quarantine pests or are poor hosts for larval development and may require manipulation during quarantine treatment development to achieve infestation. This study compared the effects of artificial versus natural infestation on melon fly, *Zeugodacus cucurbitae* (Cocquillet) (Diptera: Tephritdae), survival in papaya after irradiation. Papayas were infested either by inserting third instar larvae through a bore hole into the center of the papaya (artificial infestation) or by exposing fruit to adult oviposition in small cages and raising larvae to third instar (natural infestation). Infested fruit were then irradiated at 40 Gy and held for adult emergence. Mean adult emergence was higher in artificially (7.6%) than naturally (0.6%) infested papayas after irradiation, but the difference was non-significant. The advantages to artificially infesting fruit during quarantine treatment studies are discussed.

A complication in quarantine studies with tephritid fruit flies is the poor hosts status of certain fruits. Many commodities are naturally rarely infested by their quarantine pests or are poor hosts for larval development. For example, the natural infestation rate (insects per fruit) of lychee (*Litchi chinensis*) and avocado (*Persea americana*) by Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), is 0.0056 and 0.021, respectively (Follett and McQuate 2001, Follett et al. 2021). In finger lime (*Citrus australasica*), melon fly, *Zeugodacus cucurbitae* Cocquillet (Diptera: Tephritidae), readily oviposited into ripe finger lime fruits in no-choice tests in laboratory cages, but their survival from egg to pupa and egg to adult was estimated as 0.0009% and 0.00022%, respectively (Follett et al. 2022). Low infestation rates may be due to physical or chemical properties of the fruit that provide it with insect resistance (Aluja and Mangan 2008).

During quarantine treatment testing, a poor host fruit must be manipulated so that it can more readily become infested by the fruit fly species of interest. Often infestation can be achieved by puncturing or otherwise damaging the fruit to facilitate oviposition. This method often helps equalize the numbers of eggs deposited and can result in a more even number of larvae within the fruit (Heather et al. 1991). Alternatively, a hole can be bored in the fruit so that the desired stage and number of larvae can be inserted. Several studies have used this infestation method in developing phytosanitary irradiation treatments for tephritid fruit flies (e.g., Follett and Armstrong 2004, Follett 2006). This type of artificial infestation during dose–response testing with irradiation has several advantages over natural infestation: (1) placing a known number of insects into fruit before treatment allows for accurate estimates of control and treatment mortality, (2) test insects can be placed in the center of fruit

Figure 1. Artificial infestation involved insertion of third instar melon flies, *Zeugodacus cucurbitae*, into papayas

where they are hardest to kill to simulate the worst-case infestation scenario, and (3) dosimeters can be placed alongside the insects inside the fruit to better estimate the irradiation dose received by the test insects. Knowing the number of insects used in the test also simplifies statistical analysis and increases precision for comparisons between life stages and species. For natural infestation, the host fruit is typically exposed to gravid adult fruit flies at a susceptible maturity stage in cages under semi-natural conditions, then the fruit is held for fruit fly development to the desired life stage (Follett et al. 2021).

Although there are advantages to using artificially infested fruit for testing, the FAO/IPPC standard ISPM No. 18 (Guidelines for the use of irradiation as a phytosanitary measure, Appendix 2) (IPPC 2019) recommends that testing be carried out using naturally infested fruit. Irradiation studies that have compared dose response with insects in artificially versus naturally infested fruit are rare. Studies are needed to establish the suitability of using artificial infestation rather than the natural infestation during quarantine treatment development using irradiation. We conducted a study to compare the effects of artificial versus natural infestation on melon fly survival in papaya after irradiation.

Materials and Methods

Melon flies used in the experiment came from a colony maintained at the USDA-ARS Daniel K. Inouye Pacific Basin Agricultural Research Center, Hilo, HI, for approximately 400 generations in the laboratory following a standard rearing protocol (Vargas 1989). Late third instar melon flies were irradiated in papayas with 40 Gy after either artificial or natural infestation. The third instar is considered the most radiation tolerant life stage occurring in fruit for melon fly and other tephritid fruit flies (Follett and Armstrong 2004). Third instar larvae can be distinguished from other instars by their relative size, and third instars are characterized as late when a few individuals have started "popping." After irradiation, infested fruits were held on sand in ventilated containers for insect pupation and adult emergence. Artificial infestation was carried out by inserting late third instars (previously raised on artificial diet) into the center of the 3/4-ripe 'Rainbow' papayas (*Carica papaya*) (Fig. 1) and holding the infested fruits for 24 h before treatment. A sublethal irradiation dose was applied to allow for a significant number of survivors so that treatment comparisons could be made. Natural infestation was achieved by exposing 3/4 -ripe 'Rainbow' papayas to 50 gravid melon flies in small (25 x 25 x 25 cm) cages for 4 hours for oviposition and then holding infested fruits for insect development to the late third instar stage in fruit. Dosimeters (Optichromic detectors, FWT-70-83M, Far West Technologies, Goleta, CA) were placed in the center of several uninfested fruits during irradiation to confirm the dose. Artificially infested (n=8) and naturally infested (n=10) papayas were irradiated at Hawaii Pride LLC (Kea'au, HI) using a linear accelerator producing high energy (5 MeV) X-rays. Papayas moved on a conveyor belt in front of the beam one at a time (rate was 1.2 m^{-min}), and therefore each papaya served as a replicate. An equal number of unirradiated control fruit from each infestation treatment (artificial and natural) were held under the same conditions to estimate the natural survival rate from the third instar to adult. After irradiation, infested fruits were held individually on sand in ventilated containers for insect pupation and adult emergence. Data on percentage pupal eclosion (number adults/number puparia) were arcsin transformed and subjected to a two-factor (infestation method, irradiation dose) analysis of variance using the standard least squares model (SAS 2021).

Results

The percentage of pupae eclosing was not significant for the effect of infestation type (F = 1.1, df = 1,1, P = 0.30), was significant for the effect of dose (F = 123.8, df = 1,1, P <0.0001), and was marginally nonsignificant for the infestation by dose interaction (F = 3.8, df = 1,1, P = 0.06). Papayas in the natural infestation treatment were about twice as heavily infested as the artificially infested fruit as evidenced by the number of puparia (Table 1). Pupal eclosion from unirradiated (control) fruits was 39% for rhe artificial infestation treatment and 46% for the natural infestation treatment, which was not significant. In the irradiation treatment (40 Gy), pupal eclosion was higher as in the artificially infested fruit (7.6 + 4.5 %) than the naturally infested fruit (0.6 + 0.16 %) but due to variation this was only marginally significant. The high variation in the irradiated artificial infestation treatment (Table 1) was due to relatively high survivorship in one fruit (27%).

Discussion

Melon flies showed equal adult emergence from unirradiated control fruit in the artificially infested and naturally infested treatments. The higher survivorship in irradiated melon flies inserted into fruits artificially as third instars may have been due to their greater concentration in the center of the fruit near where they were placed because they would have received a lower average dose than the naturally infested flies, which were likely more evenly

Table 1. Mean percentage (+SE) pupal eclosion (adult emergence) from irradiated and unirradiated melon fly third instars after artificial or natural infestation of papayas.

Infestation type	Irradiation dose (Gy)	No. infesting third instars	No. puparia	No. adults	% pupal eclosion[1]
Artificial	0	200	72.4 (13.6)	28.4 (5.9)	39.0 (3.1)a
	40	200	93.3 (11.8)	4.6 (3.3)	7.6 (4.5)b
Natural	0	--	184.0 (27.0)	92.8 (19.3)	46.6 (5.6)a
	40	--	161.9 (26.7)	1.1 (0.4)	0.6 (0.15)b

[1] Values in a column followed by different letters are significantly different at $P<0.05$

distributed throughout the fruit. In the latter case any larvae near the fruit surface would have received a higher dose. For example, Follett (2024) reported that the irradiation dose at the center of a papaya is 25% lower than the dose at the surface of the fruit at an equal distance from the radiation source due to attenuation as X-rays pass through the fruit. In addition, larval development in naturally infested fruits may have had a wider range of ages at the time of irradiation, with younger third instars being more susceptible to irradiation than late third instars (Follett and Armstrong 2004). Such variation in effective radiation dose (due to differences in the spatial distribution of larvae in the fruit) or a wider range of ages complicates interpretation of the results in this type of experiment. Larvae reared on artificial diets are also usually larger and so possibly healthier than those reared on fruit (personal observation) which may have increased their radiation tolerance. Hallman and Thomas (2010) irradiated Mexican fruit fly, *Anastrepha ludens* Loew, as late third instars in grapefruit with 15 to 30 Gy using natural and artificial infestation methods and found no significant difference in adult emergence. Therefore, based on our results and the results reported in Hallman and Thomas (2010) the method of infesting fruit is probably not an important variable in phytosanitary irradiation treatment development for tephritid fruit flies. The FAO/IPPC guidelines in ISPM 28 (Phytosanitary treatment for regulated pests) (IPPC 2007) (different from ISPM 18 mentioned above) allow the use of efficacy data in support of the phytosanitary treatment from either artificially or naturally infested fruit, provided the treatment is confirmed under operational conditions.

Other types of host manipulation have been used to obtain data when using poor hosts. In certain cases, insects may be removed from the test fruit after irradiation and placed on diet to maximize chances for survival and isolate the effects of irradiation from any adverse host plant effects (Heather et al. 1991, Hallman and Thomas 2010, Follett et al. 2011).

When the host fruit is small, or only one insect typically develops in a fruit, insects may be irradiated on artificial diet to expedite treatment development using larger numbers of test subjects (Follett and Lower 2000), but the validity of using artificial diet rather than the natural fruit should be established (Follett and Armstrong 2004, Hofmeyr et al. 2016). Macfarlane (1966) observed that Queensland fruit fly, *Bactrocera tryoni*, larvae developed more slowly and less uniformly in oranges or lemons than in an artificial carrot-based diet. In many studies, dose–response tests are conducted in vitro or in artificial diet, then for large-scale confirmatory testing, insects are irradiated under natural conditions in fruit (e.g., Heather et al. 1991, Follett and Armstrong 2004).

For artificial infestation tests with a known number of test subjects, untreated control insects are always included along with irradiated insects so that mortality can be adjusted for natural variation and to guard against changes in experimental conditions over the course of testing that may cause higher than normal mortality. Although control mortality ≤20% is desirable, higher mortality may be normal when using wild insects and naturally infested commodities, particularly for poor hosts. The FAO/IPPC standard ISPM No. 18 (Guidelines for the use of irradiation as a phytosanitary measure, Appendix 2) (IPPC 2019) recommends that control or check mortality during a phytosanitary treatment should not exceed 10% and suggests that higher mortality indicates that test insects were held under suboptimal conditions. But this expectation may be too stringent and unrealistic for insects, including fruit flies (Follett et al. 2021) that infest poor hosts where natural survivorship is expected to be low.

Literature Cited

Aluja, M., and **R.L. Mangan.** 2008. Fruit fly (Diptera: Tephritidae) host status determination: critical conceptual, methodological, and regulatory considerations. Annu. Rev. Entomol. 53:473–502.

Follett, P.A. 2006. Irradiation as a methyl bromide alternative for postharvest control of *Omphisa anastomosalis* (Lepidoptera: Pyralidae) and *Euscepes postfasciatus* and *Cylas formicarius elegantulus* (Coleoptera: Curculionidae) in sweet potatoes. J. Econ. Entomol. 99:32–37.

Follett, P.A. 2024. Phytosanitary irradiation of tephritid fruit flies. *In* F.R.M Garcia (ed), Management of Fruit Flies in the Americas. Springer Nature. 607 pp. ISBN-13: 9783031486074.

Follett, P.A., and **R. Lower.** 2000. Irradiation to ensure quarantine security for *Cryptophlebia* spp. (Lepidoptera: Tortricidae) in sapindaceous fruits from Hawaii. J. Econ. Entomol. 93: 1848–1854.

Follett P.A., and **G.T. McQuate.** 2001. Accelerated development of quarantine treatments for insects on poor hosts. J. Econ. Entomol. 94:1005–1011.

Follett, P.A., and **J.W. Armstrong.** 2004. Revised irradiation doses to control melon fly, Mediterranean fruit fly and Oriental fruit fly (Diptera: Tephritidae) and a generic dose for tephritid fruit flies. J. Econ. Entomol. 97:1254–1262.

Follett, P.A., F.E.M. Haynes, and **B.C. Dominiak.** 2021. Host suitability index for polyphagous tephritid fruit flies. J. Econ. Entomol. 114(3):1021–1034.

Hallman, G.J., and **D.B. Thomas.** 2010. Ionizing radiation as a phytosanitary treatment against fruit flies (Díptera: Tephritidae): efficacy in naturally vs. artificially infested fruit. J. Econ. Entomol. 103:1129–1134.

Heather, N.W., R.J. Corcoran, and **C. Banos**. 1991. Disinfestation of mangoes with gamma irradiation against two Australian fruit flies (Diptera: Tephritidae). J. Econ. Entomol. 84:1304–1307.

Hofmeyr, H., M. van der Rijst, H. Hofmeyr, and **K. Slabbert**. 2016. Postharvest phytosanitary disinfestation of *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) in citrus fruit: comparative tolerance of larvae reared in synthetic diet and oranges to ionizing radiation. Florida Entomol. 99:43–47.

IPPC (International Plant Protection Convention). 2019. International standards for phytosanitary measures (ISPM) No. 18, Guidelines for the use of Irradiation as a phytosanitary measure. Appendix 2. Food and Agricultural Organization, Rome.

IPPC (International Plant Protection Convention). 2007. International standards for phytosanitary measures (ISPM) No. 28, Phytosanitary treatments for regulated pests. Irradiation treatments annexed 2009. Food and Agricultural Organization, Rome.

Macfarlane, J. J. 1966. Control of Queensland fruit fly by gamma irradiation. J. Econ. Entomol. 59(4):884–889.

SAS Institute. 2021. JMP Pro User's Guide. SAS Institute, Cary, NC.

Vargas, R.I. 1989. Mass production of tephritid fruit flies, pp 141–151 *In* Robinson, A.S., Hooper, G. (eds), World crop pests. Vol. 3B. Fruit flies, their biology, natural enemies and control. Elsevier, New York.