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LOW-DOSE IRRADIATION PHYTOSANITARY TREATMENT
AGAINST MEDITERRANEAN FRUIT FLY (DIPTERA: TEPHRITIDAE)ZOILA TORRES-RIVERA¹ AND GUY J. HALLMAN²¹San Borja, Lima, Peru²USDA, ARS, Weslaco, TX 78596

ABSTRACT

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is one of the most important quarantine pests in the world. Host commodities shipped from infested parts of the world to non-infested parts that might be susceptible to infestation should undergo a phytosanitary measure to render negligible the risk of shipping viable flies. Ionizing irradiation is a promising phytosanitary treatment that is tolerated by the great majority of hosts of the Mediterranean fruit fly. The current dose in the US is 150 Gy. This research conducted with cage-infested 'Haden' mangoes in Peru showed that 100 Gy is sufficient to provide a high level of quarantine security against this important pest. That dose did not affect pupation when applied to late 3rd instars, but it did prevent any from emerging as adults. A dose of 100 Gy might allow for irradiation of avocados, one of the few fruits that does not tolerate more than 100-200 Gy.

Key Words: *Ceratitis capitata*, ionizing irradiation, quarantine treatment, disinfestation

RESUMEN

La mosca mediterránea de la fruta, *Ceratitis capitata* (Wiedemann), es una de las plagas cuarentenarias más importantes en el mundo. Las materias primas hospederos embarcadas desde regiones del mundo infestadas con destino a zonas no infestadas que podrían ser susceptibles de infestación deberían ser sometidas a una medida fitosanitaria que permita eliminar el riesgo de estos embarques. La radiación ionizante es un tratamiento fitosanitario prometedor y tolerable por la gran mayoría de hospederos de la mosca mediterránea de la fruta. La dosis corriente en los Estados Unidos es 150 Gy. La presente investigación conducida con mangos infestados en jaulas en Perú mostró que 100 Gy son suficientes para proporcionar un nivel alto de seguridad cuarentenaria contra esta importante plaga. La mencionada dosis no afectó el proceso de empupar cuando se aplicó en larvas del tercer instar, pero sí evitó la emergencia a adultos. Una dosis de 100 Gy podría adecuarse para la irradiación de aguacates, una de las pocas frutas que no tolera más de 100-200 Gy.

Translation provided by the authors.

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), may be considered the premier quarantine pest in the world. It occurs in much of Africa and southern Europe, most of Latin America from southern Mexico to Argentina, parts of Australia and the Middle East, and Hawaii. Non-infested countries from the tropics into temperate regions place quarantines on a broad range of fresh fruit hosts from infested regions. The host list includes most commercial sweet tree-fruits, avocados, tomatoes, peppers, cotton bolls, walnut fruits, and coffee berries. Over 250 hosts have been listed. Even poor hosts are quarantined because they may carry enough Mediterranean fruit fly individuals for the pest to reproduce and become established.

Much effort and money are spent preventing, managing, and eradicating the Mediterranean fruit fly throughout the world. Millions of sterile males per week are released in Florida, California,

and other areas as a preventative measure. Thousands of survey traps are maintained in many countries. The pest has been found and eradicated 7 times in Florida and individuals are found almost every year in California. The United States (US), Mexico, and Guatemala collaborate in trying to eradicate the fly from Mexico and parts of Guatemala. The Mediterranean fruit fly was officially eradicated from Chile in 1995 and cooperation with Peru aims to prevent its reintroduction into Chile as well as achieve eradication in southern Peru.

Phytosanitary treatments may be required to export hosts from Mediterranean fruit fly-infested areas to non-infested areas that could support establishment of the pest. Several treatment options are available. Holding at 1.1-2.2°C for 14-18 d is used to disinfect tangerines shipped from Spain to the US. Methyl bromide fumigation is used for various fruits. Immersion of mangoes in water at 46.1°C for 65-110 min (depending on

shape, mass, and origin of the mangoes) is used to facilitate shipment of mangoes from Latin America to several countries. Heated air is used to facilitate shipment of papayas from Hawaii to Japan and the mainland US. Ionizing irradiation is used for shipment of several fruits from Hawaii to the mainland US.

From the standpoint of fruit quality, irradiation is the most broadly applicable commercial treatment at the doses for tephritid fruit flies (Hallman 2007). It also has advantages over other treatments; e.g., very few external variables affect treatment efficacy and it can be applied after packing and palletizing.

The chief disadvantage of irradiation is that, unlike all other commercially-applied treatments, it does not cause acute mortality but renders insects unable to complete development and/or reproduce. Although preventing development or reproduction is sufficient to prevent the establishment of invasive species, it does not provide inspectors with a simple and reliable independent verification of treatment efficacy, i.e., dead insects. Correct and complete conduction of the research supporting the treatment, robust certification that treatments are done adequately, and careful protection of the treated lots from re-infestation are necessary to ensure commercial viability of phytosanitary irradiation treatments. Irradiation has been used commercially since 1995 for interstate disinfestation of perishable commodities of several pests including tephritids within the US and since 2004 to disinfest mangoes shipped from Australia to New Zealand of tephritids without insurmountable incident.

In the US a minimum absorbed dose of 150 Gy is allowed for any fruit against any tephritid fruit fly (APHIS 2006). Very few fruits do not tolerate that dose applied on a commercial scale, which could be up to 2.5 times the minimum absorbed dose (Hallman & Loaharanu 2002). The reason commercial doses may be up to 2.5 times the minimum prescribed dose is that commercial irradiation facilities may treat pallet-loads and irradiation diminishes as the distance from the source increases. In order to get the minimum absorbed dose required to the farthest fruit in a pallet-load, the nearest fruit may receive a much higher dose.

Because it is such a significant quarantine pest Mediterranean fruit fly has received much phytosanitary attention. For example in the book, *Invasive Arthropods in Agriculture: Problems and Solutions* (Hallman & Schwalbe 2002), Mediterranean fruit fly is mentioned more than any other arthropod. It is also the most studied pest regarding irradiation phytosanitary treatments. Thirteen studies provide sufficient data to estimate doses required for quarantine security with varying levels of security (Table 1), the most for any quarantine pest. Reasoning for many of the doses listed in Table 1 is given in Hallman (1999).

TABLE 1 MINIMUM IONIZING RADIATION DOSE TO PREVENT ADULT EMERGENCE FROM MEDITERRANEAN FRUIT FLY THIRD INSTARS IN FRUIT ACCORDING TO VARIOUS STUDIES LISTED IN CHRONOLOGICAL ORDER

Dose (Gy)	Fruit	Reference
225	Papaya	Seo et al. (1973)
>200	Orange	Fésüs et al. (1981)
~80	Mango	Potenza et al. (1989)
~80	Mango	Raga (1990)
~80	Peach	Arthur et al. (1993a,b)
~70	Grapefruit	Raga (1996)
~200	Orange	Adamo et al. (1996)
40 ¹	Peach, orange	Mansour & Franz (1996)
150	Mango	Bustos et al. (2004)
100 ¹	Papaya	Follett & Armstrong (2004)

¹Fruit infestation involved rearing larvae in diet and inserting them into fruit 24-30 h before treatment.

The literature suggests 2 conflicting peak doses for providing quarantine security against Mediterranean fruit fly, one at 70-100 Gy and the other at 200-225 Gy (Table 1). Hallman & Loaharanu (2002) argue that the upper peak is not well supported and could be dismissed. The currently accepted dose for this pest in the US is 150 Gy (APHIS 2006), but this may not be the minimum absorbed dose that could prevent adult emergence of 3rd instar Mediterranean fruit fly in fruit as evidenced by several studies in Table 1.

Two large-scale studies used Mediterranean fruit fly 3rd instars reared in diet and then inserted in fruit 24-30 h before treatment. Mansour & Franz (1996) obtained no adult emergence when >100,000 3rd instars were reared in diet and then placed in peaches and oranges 30 h prior to irradiation with 40 Gy. Follett and Armstrong (2004) found no adult emergence when 31,920 3rd instars were reared in diet and placed in papayas 24 h before irradiation with 100 Gy. Follett and Armstrong obtained 0.47% emergence of normal-looking adults when 3rd instars were irradiated with 40 Gy and 0.07% at 50 Gy, doses that provided complete prevention of adult emergence in the similar study by Mansour & Franz (1996).

For any phytosanitary treatment, infestation that differs significantly from the natural situation should be tested for relative tolerance to the natural situation. If the semi-artificial technique results in increases in pest tolerance, it would not be of phytosanitary concern, although the treatment may be harsher on the commodity than it need be. But if the semi-artificial infestation increases susceptibility, phytosanitary security will be at risk. Hypoxia reduces radiosusceptibility of organisms (Hallman & Hellmich 2007), and tephritid immatures inside the hypoxic atmosphere of fruit seem to show increased tolerance (Hall-

man & Worley 1999). Lack of hypoxic protection may explain why 40 Gy prevented Mediterranean fruit fly adult emergence in >100,000 third instars reared in diet and placed in peaches and oranges 30 h before irradiation (Mansour & Franz 1996). A higher dose was required using the same techniques with papayas (Follett & Armstrong 2004). Perhaps a hypoxic atmosphere was easier to achieve and maintain in papayas after artificial infestation compared with peaches and oranges making work with papayas more akin to natural conditions, at least in this case.

The most rigorous standard used for confirming the efficacy of a phytosanitary treatment is "probit 9" at the 95% confidence level (Hallman & Loaharanu 2002). Probit 9 represents the effective dose (ED) to achieve a result at the 99.9968 percentile ($ED_{99.9968}$). This entails treating 93,600 individuals with no survivors when done to a confidence level of 95% (Couey & Chew 1986). Efficacy of an irradiation phytosanitary treatment against tephritids is measured by the prevention of the emergence of adults capable of flight when irradiated as 3rd instars inside fruit (Hallman & Loaharanu 2002).

Although Follett & Armstrong (2004) found that 100 Gy would probably control Mediterranean fruit fly in the system that they studied (diet-reared larvae placed in papaya a day before irradiation), they did not do a "probit 9"-level study because their goal was to find a single dose that would control all quarantined tephritid fruit flies in Hawaii. They did not try to make the dose for all fruit flies in Hawaii lower than 150 Gy because their studies indicated that one tephritid, melon fly, *Bactrocera cucurbitae* (Coquillett), would not be controlled to the "probit 9" level with <150 Gy.

To save resources and reduce the potential negative effect of a phytosanitary treatment on commodities the effective dose for any treatment should be made as low as possible. In the case of ionizing irradiation and the Mediterranean fruit fly Hallman & Loaharanu (2002) observed that doses to control all *Anastrepha* fruit flies studied were similar and about 70 Gy achieved a high level of control. They argued that enough phytosanitary research had been done with the neotropical genus to permit a dose of 70 Gy for all *Anastrepha*. Four studies that included both *Anastrepha* spp. and Mediterranean fruit fly showed the latter to require about 1.4× the dose to achieve the same effect against *Anastrepha*. Therefore, if 70 Gy is sufficient for *Anastrepha* then 70 Gy × 1.4 or ~100 Gy should provide a high level of control of Mediterranean fruit fly.

The objective of the research was to determine the dose at the 95% confidence level, beginning at 100 Gy that would prevent the emergence of adults capable of flight when irradiated as 3rd instar Mediterranean fruit flies inside fruit.

MATERIALS AND METHODS

The flies used in this research were from the colony in the sterile insect release program in La Molina, Peru. About 16,500 Mediterranean fruit fly adults (sex ratio about 1:1) were maintained in each of 3 cages ($0.6 \times 2 \times 0.35$ m). They were fed a mixture of sugar, protein, and water. About 60-80 mature green, freshly picked 'Haden' mangoes (0.3-0.5 kg) were placed in each cage for 24 h at about 27°C with constant lighting. The resulting mean infestation rate was about 45 larvae/fruit. Upon removal from the infestation cages mangoes were kept at 27°C until the flies developed to late 3rd instars (9-10 days); mangoes were periodically opened to observe fly development. The test was repeated until at least 93,600 third instars were irradiated at 100 Gy, which would satisfy "probit 9" at the 95% confidence level (Couey & Chew 1986).

When most Mediterranean fruit flies had developed to the 3rd instars 90% of the infested mangoes were irradiated with a cobalt-60 source (model Gammabeam 127, Nordion, Kanata, Canada) that was delivering a dose rate of 2.8 Gy/minute. Dosimetry was done with the Fricke system (ASTM 2006). Timing of irradiation was set so that the maximum dose measured did not exceed the target dose. At that timing the minimum absorbed dose was about 87 Gy when the maximum was 100 Gy. The 10% non-irradiated mangoes were held as controls. After irradiation the mangoes were held at 25°C on a bed of moist sawdust to absorb fluids leaking from the fruit and serve as a burrowing and pupariation medium for emerging larvae. After 5-6 d the mangoes were examined for remaining larvae, both dead and alive. All larvae and puparia were collected, counted, and held at 25°C until after adults emerged. Tests continued until a minimum of 96,400 third instars were irradiated with no adults emerging. If adults capable of flight (fully extended wings) were found the dose would be raised depending on the failure rate and the testing begun anew.

It is expected that Mediterranean fruit fly 3rd instars will largely pupariate at 100 Gy, although adult emergence should be extremely low. Statistical significance of pupariation between irradiated and control 3rd instars was tested via a two-tailed, paired *t*-test (Prism 4, GraphPad Software, San Diego, CA).

RESULTS AND DISCUSSION

After 9 tests consisting of 7-12 thousand irradiated 3rd instars each, a total of 99,562 Mediterranean fruit fly 3rd instars were irradiated in mangoes with no adults emerging; 88.5% of these 3rd instars pupariated. Pupariation rate of the control was 90.7%, but it was not significantly different from the irradiated 3rd instars ($t = 0.98$, *df*

= 8, $P = 0.36$) showing that 100 Gy did not affect the ability of Mediterranean fruit fly late 3rd instars to pupariate. Adult emergence from control puparia was 86.9%.

This research shows that phytosanitary irradiation at an absorbed minimum dose of 100 Gy provides quarantine security against Mediterranean fruit fly to the highest degree demanded of a commercial phytosanitary treatment, ED_{99.9968} at the 95% confidence level. Almost all hosts of the pest would tolerate this treatment applied on a commercial scale. Even avocado, which has low tolerance to any phytosanitary treatment, such as those based on fumigation, heat, or cold (McDonald & Miller 1994), might have a viable treatment against Mediterranean fruit fly with 100 Gy. Avocado tolerates about 100-200 Gy (Thomas 2001), and the dose uniformity ratio (maximum absorbed dose divided by minimum absorbed dose) expected in commercial irradiation facilities can vary anywhere from 1.2-2.5, depending on the source and arrangement of irradiated product.

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