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Review

Ionizing radiation quarantine treatments against tephritid fruit flies

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Abstract

Fruit flies of the family Tephritidae are considered the most important insect pest risk carried by exported fruits worldwide. Fruits suspected of harboring fruit fly eggs and larvae must be treated to control virtually 100% of any tephritids present. Irradiation is unique among quarantine treatments in that it is the only treatment used which does not cause acute mortality; instead, insects are prevented from maturing or are sterilized. Tephritids have been the most studied group of quarantined pests as far as irradiation; minimum absorbed doses confirmed with large-scale testing to provide control to the probit 9 level (99.9968%) have ranged from 50 to 250 Gy. Considerable work has been done with the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), one of the most important quarantined pests worldwide, and doses suggested to provide quarantine security have varied widely. The fact that insects are still alive for some time after irradiation has been one of the major obstacles to its use. Irradiation may be the most widely applicable quarantine treatment from the standpoint of fruit quality. However, some important fruits shipped across quarantine barriers (mangoes, *Mangifera indica* L., and citrus) may suffer from doses as low as 150 Gy when applied on a commercial scale where much of the fruit load may receive >300 Gy. Fortunately, some of the important tephritids attacking these fruits, such as *Anastrepha* spp., can be controlled with lower doses. Mainland USA has begun to use irradiation as a quarantine treatment for some fruits imported from Hawaii since April 1995 and remains the only country using irradiation as a quarantine treatment, although on a very limited basis. Irradiation offers some additional risk abatement advantages over other quarantine treatments. © 1999 Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Post harvest quarantine treatments of fresh agricultural commodities are devised to prevent migration of potentially damaging organisms to

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new areas. Traditional treatments, which most commonly involved chemical fumigants, such as ethylene dibromide and methyl bromide, and both hot (43–48°C) and cold (0–3°C) temperatures, work by killing essentially 100% of all stages of quarantined pests which might be present in the commodity. Fruit flies of the family Tephritidae have been considered the most important group of quarantined insects for their ability to render unmarketable significant portions of fruit harvests and for the extra burden placed on fruit production by detection, monitoring, and control programs which must be in place often despite the pests not actually being present to any significant degree. When exotic fruit flies are discovered in new areas large sums of money are spent to eradicate the fledgling populations. For example, in Florida US$25 million was spent in 1997 and even more in 1998 to eradicate outbreaks of Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann).

Alternative quarantine treatments for fresh commodities are needed because fumigants are being lost due to health and environmental problems, heat damages many commodities, such as stone and pome fruits and avocados, *Persea americana* Mill., and cold treatment requires ≥ 12 days. Irradiation has been studied as a quarantine treatment for over 40 years but has rarely been used to ship fruit across quarantine barriers. In 1986 one shipment of mangoes, *Mangifera indica* L., from Puerto Rico and in 1987 another shipment of papayas, *Carica papaya* L., from Hawaii were exported to the continental USA and sold in commercial markets after irradiation for quarantine control of fruit flies. The objective was a commercial test of irradiation as a replacement for ethylene dibromide which was banned as a suspected carcinogen. Both irradiated fruits were clearly labeled as such and were well received by consumers. However, heat treatments were developed for mangoes and papayas, and irradiation was not used on a commercial scale again until April of 1995 when growers in Hawaii began to ship small amounts of papayas and later carambolas, *Averrhoa carambola* L.; lychees, *Litchi sinensis* Sonn., atemoyas, *Annona squamosa* L. X *A. cherimola* Mill., and rambutans, *Nephelium lappaceum* L., to an Isomedix facility near Chicago, IL for irradiation with a minimum absorbed dose of 250 Gy against fruit flies or 400 Gy when mealybugs were present followed by distribution to markets in several states (Wong, 1999). Importation of Florida guavas, *Psidium guajava* L., irradiated with a minimum absorbed dose of 150 Gy for disinfestation of Caribbean fruit fly, *Anastrepha suspensa* (Loew), has been approved by the states of Texas and California, and approval for other fruits is being sought. Plans for the construction of an irradiator in Hawaii would enable irradiation of produce before it is shipped, thus reducing costs and increasing potential output.

Following is an analytical review of research into irradiation as a quarantine treatment for fruit flies of the family Tephritidae with recommendations for future research. Terminology follows American Society for Testing and Materials (ASTM, 1998b).

2. The irradiation process

Irradiation dose is measured in gray (Gy), which is the energy absorbed in J kg^{-1} of material. (The discontinued unit is the rad, which equals 0.01 Gy.) Absorbed dose, the amount of ionizing radiation energy imparted on the material being irradiated, is what is measured and reported. There are four sources of ionizing radiation for use on food: γ irradiation with the isotope of (1) cobalt-60 or (2) cesium-137, (3) electron beam (β particles), or (4) X-ray (bremsstrahlung) radiation produced when an electron beam strikes a converter, which can be any of a number of high density materials. The energy efficiency of this process is low; only about 5% of the energy in the β particles is converted to bremsstrahlung. Bremsstrahlung radiation is very similar to γ ray from isotopes; both can penetrate pallet-loads of produce. A major difference is that bremsstrahlung radiation is concentrated in the same direction as the electron beam while γ rays from isotopes are isotropic (emitted in all directions uniformly). Electron beam radiation only penetrates a few centimeters and, thus, is limited

to small products, such as shallow boxes of berries, passing the irradiation source on a conveyor line.

Two different logistical strategies have been developed for commercial γ irradiation: (1) A chamber is loaded with the materials to be irradiated and the radioactive source is raised into the chamber for the appropriate amount of time to achieve the required absorbed dose. (2) A conveyor system carries the materials to be irradiated past the exposed source at a speed which will give the required absorbed dose. The unidirectional nature of electron beam and bremsstrahlung radiation require that they be applied in a conveyor system.

3. Uniqueness of irradiation quarantine treatments

Unlike traditional treatments, the objective of an irradiation quarantine treatment is not acute mortality. Doses necessary to kill fruit fly larvae before they might be encountered by inspectors or emerge from the fruit would damage most fresh produce. For example, after 0.8 kGy was applied to Mediterranean fruit fly third instars (last larval stage) in oranges, *Citrus sinensis* (L.) Osbeck, some larvae lived for up to 8 days (Fésüs et al., 1981). However, much lower doses will prevent fruit fly immatures from reaching the adult stage, and this has been recommended as the objective of irradiation quarantine treatments against tephritid fruit flies.

When developing a quarantine treatment the most tolerant stage (or stages) of the pest which might be present in the shipped commodity must be identified and work directed primarily toward proving efficacy against that stage. With irradiation, the most tolerant stage is almost invariably that present which is most developed, although there may be a 'ratcheting' effect whereby the insect immediately preceding developmental milestones (egg hatch and pupation) is less tolerant of radiation than the insect immediately succeeding that milestone (Fig. 1). After the third instar (last larval stage) fruit fly has fully developed it leaves the fruit to pupariate in the soil. Therefore, with fruit flies, the fully developed third instar is the most radiation-tolerant stage that is present in the fruit. However, a radiation quarantine treatment will most likely be applied to fruits that are already packed, and some packed fruits, such as citrus, may sit at ambient temperatures for a few days after packing. This time would allow for emergence of larvae from the fruit and formation of post-larval stages inside the packaging which would be more tolerant of radiation than third instars (Balock et al., 1963; Hallman and Worley, 1999). Although this may be a theoretical problem, it is not known whether post-larval stages would be present in irradiated packages of fruit in sufficient numbers to result in a failure of a quarantine treatment. Also, when oxygen levels are taken into account, post-larval stages in packaging (ambient oxygen levels) do not seem to have as much tolerance to irradiation as third instars inside fruits (lower than ambient oxygen levels). Hallman and Worley (1999) found that although outside fruit, third instar Mexican fruit fly, *Anastrepha ludens* (Loew), were prevented from emerging as adults with 16 Gy; inside fruit required >50 Gy. This situation could change if fruits stored under controlled atmospheres are disinfested with irradiation, perhaps then post-larval stages in packaging under low oxygen conditions would indeed be more tolerant of irradiation than third instars in fruit.

There has been some variation in the precise definition of efficacy of irradiation as a quaran-

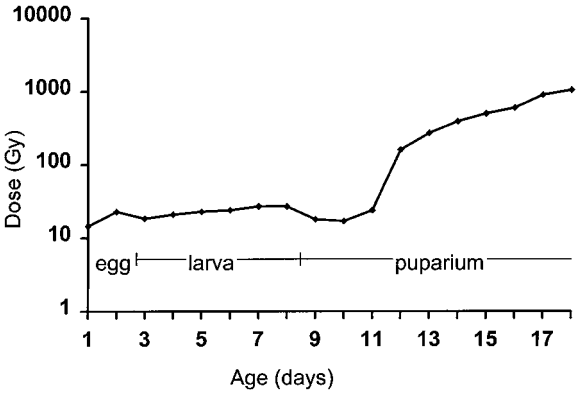

Fig. 1. Estimated doses required to prevent Mediterranean fruit fly adult emergence at different stages (Balock et al., 1963).

tine treatment against fruit flies. Efficacy with irradiation has been defined as prevention of adult emergence (Ohta et al., 1985), prevention of emergence of flies capable of flight (APHIS, 1987), and prevention of flies capable of reproducing (APHIS, 1989). These seemingly minor discrepancies may result in different doses required. Of the above three criteria, prevention of reproduction may be achieved with lower doses than prevention of adult emergence with prevention of adults capable of flight requiring a dose between those two. Currently APHIS bases efficacy on prevention of adults capable of flight in order to prevent any flies surviving a quarantine treatment from reaching detection traps and needlessly triggering eradication programs (APHIS, 1996).

Another unique property of irradiation is that, when applied on a commercial scale to pallet-loads of produce, most of the commodity will be treated with doses well in excess of (up to $3\times$) the minimum absorbed dose required to achieve quarantine security. This is due to attenuation because of (1) distance between radiation source and product to be irradiated and (2) density of product. The higher the density of the product the more likely each γ photon will hit a subatomic particle, thus the lesser the penetration. Fruits are mostly water, and, thus, have a density of about 1 g/cm^3. Packed fruits, however, have much air space which reduces the overall density of a pallet-load of fruit. With some commercial irradiation facilities, the dose uniformity (max/min) ratio can be as much as three to one. Therefore, to be safe, fruits must be able to tolerate about three times the minimum absorbed dose needed to control the insect.

4. Doses to prevent adult emergence from irradiated immatures

The International Consultative Group on Food Irradiation recommended a generic dose of 150 Gy for prevention of adult emergence from tephritid fruit fly eggs and larvae in the absence of data to support a lower dose for any species (Burditt, 1994). This dose was set by the US Department of Agriculture, Animal and Plant Health Inspection Service (APHIS) as the minimum dose required for disinfesting Hawaiian papayas of three fruit flies (APHIS, 1989). Although this treatment was never used on a routine commercial basis, its authorization was considered a valuable exercise to the establishment of irradiation policy and its application as a viable phytosanitary treatment in the USA (APHIS, 1996). Subsequent review of the literature led to the establishment of doses as high as 250 Gy for fruit flies (Table 1). Therefore, fruits treated with irradiation for export from Hawaii must be treated with a minimum absorbed dose of 250 Gy, the highest dose required for any fruit fly in Hawaii.

At the same time that doses were proposed for Hawaiian fruit flies, a minimum dose of 150 Gy was proposed for six other species which do not exist in Hawaii (Table 1). Subsequently another Hawaiian fruit fly, *Bactrocera latifrons* (Hendel), was added at 150 Gy. As of the time this article went to press final APHIS approval for the use of irradiation against fruit flies outside of Hawaii had not been given.

APHIS has stated that "All doses are subject to adjustment based on the scientific evidence supporting a different dose" (APHIS, 1996). Scientific evidence, including large-scale confirmatory tests, supports lowering doses for all of the species listed at 150 Gy except *B. latifrons* and possibly *A. suspensa*. Bustos et al. (1992) treated $>100\,000$ third instars of each of three species of *Anastrepha: ludens*, *obliqua*, and *serpentina*, in mangoes with 100 Gy without any adults developing. Adult emergence in the untreated controls was $\geq 83.5\%$. I am currently doing a large-scale confirmatory test with third instars of *A. ludens* in grapefruits, *Citrus paradisi* Macf., at 60–68 Gy; a previous test at 50–57 Gy was terminated when one adult emerged after $>52\,000$ third instars were treated. Rigney and Wills (1985) found that at 75 Gy no adult *B. tryoni* emerged from a total of 24 700 third instars in oranges and avocados. Over one-half million third instar *B. tryoni* in five different fruit-hosts were irradiated with 75 Gy, and none survived to the adult stage (Jessup et al., 1992). The large number treated with no adult survivors demonstrates that 75 Gy was excessive and the minimum dose to achieve quarantine

Table 1
Minimum absorbed doses suggested by USDA-APHIS for quarantine security of fruit flies (APHIS, 1996)

Species	Common name	Dose (Gy)	Geographical distribution
Anastrepha ludens (Loew)	Mexican fruit fly	150	Extreme southern Texas to Guatemala
A. obliqua (Macquart)	West Indian fruit fly	150	Caribbean islands, Mexico to Brazil
A. serpentina (Wiedemann)	Zapote fruit fly	150	Mexico to Argentina
A. suspensa (Loew)	Caribbean fruit fly	150	Florida, Caribbean islands
Bactrocera cucurbitae (Coquillett)	Melon fly	210	Asia, parts of eastern Africa, Hawaii
B. dorsalis (Hendel)	Oriental fruit fly	250	India to southern China, Hawaii, N. Mariana Islands
B. jarvisi (Tyron)		150	Northern Australia
B. latifrons (Hendel)	Malaysian fruit fly	150	India to China, Laos, and Singapore; Hawaii
B. tryoni (Froggatt)	Queensland fruit fly	150	Australia, New Guinea, New Caledonia, Austral Islands, Society Islands
Ceratitis capitata (Wiedemann)	Mediterranean fruit fly	225	Mediterranean, Africa, Central and South America, Middle East, Hawaii, Western Australia, N. Mariana Islands

security based on probit 9 (99.9968% control) is less than 75 Gy. Unfortunately, without further testing it cannot be known how much less the dose can be. But, the results of that study give 99.99999% confidence that 75 Gy will offer quarantine security at the probit 9 level (calculation of confidence following Couey and Chew, 1986). Heather et al. (1991) irradiated 153 800 third instar *B. jarvisi* in mangoes at 74–100 Gy with no adult survivors. This, too, was more than needed to prove probit 9 security, yielding a confidence level of 99.3% that a minimum dose of 100 Gy achieved probit 9 quarantine security. With no survivors, probit 9 security at the 95% confidence level is proven with 93 600 individuals tested. Although adult emergence of *A. suspensa* in carambolas was prevented in $>100\,000$ eggs and larvae with 50 Gy (Gould and von Windeguth, 1991), few of the insects were probably in the most tolerant stage (fully developed third instars) when they were irradiated 0–9 days after oviposition. Balock et al. (1963) found that adult emergence from 1-day-old eggs and middle-aged larvae of the Mediterranean fruit fly could be stopped with 54 and 85%, respectively, of the dose required for third instars. Fruit flies reared in most fruits take much longer to mature than those reared in a meridic diet. For example, the larval stage of Mexican fruit fly at 28°C lasts 10 days in meridic diet and peach, 22 days in mango, 27 days in grapefruit, and 30 days in orange (Leyva et al., 1991). Although no adults emerged from $>$ 100 000 *B. latifrons* in artificially infested green peppers, *Capsicum annuum* L., irradiated with 150 Gy, data from lower doses appear to support an effective dose of <100 Gy (Phillips, unpublished data).

Without further study it is not possible to determine if doses for three flies found in Hawaii: melon fly, oriental fruit fly, and Mediterranean fruit fly, can be lowered from their respective 210, 250, and 225 Gy. Doses for the first two flies are based on studies done in Hawaii 25–30 years ago, while recommended doses for Mediterranean fruit fly in the literature range from 40 to >300 Gy.

Haque and Ahmad (1967) found that the lowest dose they used, 55 Gy (37 Gy/min), completely prevented adult emergence of about 800 third instar peach fruit fly, *Bactrocera zonata* (Saunders), infesting guavas. Mean emergence in the control was 95%.

4.1. Oriental fruit fly

Irradiation quarantine treatment research with the oriental fruit fly has been reported from other parts of the world besides Hawaii. A mean of 2.25% adults emerged from larvae irradiated 7 days post-infestation with 50 Gy (0.1 Gy/s) in mangoes in the Philippines (Manoto et al., 1992). In a large-scale test 100 Gy prevented adult emergence from > 131 000 larvae 5–6 days post-infestation in mangoes. These were not the most developed larvae possible; thus, they would be easier to control than fully grown third instars which would not be present until at the very least 8 days post-infestation. Population density was high: a mean of 117 larvae per 250– 300 g mango. It is not known whether competition from the high density might have made the larvae more susceptible to irradiation. At a dose of 150 Gy, one adult emerged from > 173 000 larvae 6 days post-infestation in mangoes in Thailand (Komson et al., 1992). Again, these larvae were not the most developed possible, and population density was high: 115 puparia recovered per mango. At a dose of 80 Gy (1.3 Gy/s) 0.07% of larvae 5 days post-infestation (at 27°C) in carambolas emerged as adults (Vijaysegaran et al., 1992). In a study in India showing what appears to be extreme tolerance to irradiation for a tephritid, 31.5% of third instars which were still 7 days away from pupariation when they were irradiated with 200 Gy (at the rate of 1.7 Gy/s) in vitro emerged as adults (Prasad and Sethi, 1980).

Drew and Hancock (1994) divided what was considered *Bactrocera dorsalis* into 41 species and limited the range of the true *B. dorsalis* to India, Sri Lanka, Nepal, Bhutan, Myanmar, Thailand, Laos, Vietnam, Cambodia, southern China, Taiwan, Hawaii, and the Mariana Islands. Therefore, if that classification is followed, research reported with *B. dorsalis* in Malaysia and the Philippines was actually done with other species, most probably the newly named *papayae*, *carambolae*, or *philippinensis* Drew and Hancock. Also, research reported with *B. dorsalis* in Thailand could have been done with *papayae* or *carambolae*, which, along with *dorsalis*, are all reported attacking economically important fruits in that country. Nevertheless, the response of these closely related species to irradiation might be expected to be similar, as it is with the four species of *Anastrepha* mentioned earlier which have been studied to a considerable extent. Unfortunately, because the studies with members of the oriental fruit fly complex cited in the previous paragraph were all done with larvae which were not fully developed (fully developed being the most tolerant stage present in fruit), conclusions on lowering minimum doses necessary to prevent adult emergence of this important group of fruit flies below the 250 Gy established in the protocol for importation of Hawaiian fruits cannot be made without further large-scale studies.

4.2. Mediterranean fruit fly

There is considerable variation in the literature as to the dose needed to provide quarantine security against the Mediterranean fruit fly. One of the first estimates was by Seo et al. (1973), who irradiated 1608 papayas infested with third instars at a minimum dose of 218–291 Gy. Two of 110 800 larvae survived to the adult stage when irradiated with a minimum absorbed dose of 225 Gy; none survived from 70 400 irradiated with 218 Gy nor 30 500 irradiated with a minimum of 244–246 Gy. From these data it appears that 225 Gy comes close to satisfying 99.9968% control requirements at the 95% confidence level, which does not allow for any survivors of 93 600 tested.

Fésüs et al. (1981) cite an unpublished study where 200 Gy applied to infested oranges in Hungary (number of insects not given) resulted in the emergence of one adult. Shehata (1983) reported that when 10 apples, *Malus domestica* Borkhausen, infested with 7-day-old larvae in Egypt were irradiated with 840 Gy (0.6 Gy/s) six puparia were formed and four adults emerged (number of larvae not given). These four adults were 'very weak, undersized and died on the next day'. Adult emergence from third instars irradiated in diet with 25 and 26 Gy was 1.1 and 0%, respectively. The fact that four adults emerged from only six puparia which were formed from larvae in a small amount of apples irradiated with 840 Gy is incredible and not supported by any

other references; if it were true it would mean that the dose required to prevent adult emergence would be >1000 Gy! The pupariation rate of irradiated third instars was similar to other studies: about 1–1.4 kGy needed to prevent pupariation (Balock et al., 1963; Mansour and Franz, 1996), but the fact that several adults emerged after doses comprising several hundred gray is not found in other literature, especially when it is considered that very few larvae pupariated. Usually the gap between percentage pupariation and adult emergence is large except at doses <20 Gy where many adults will emerge. For example, Mansour and Franz (1996) found that 40 Gy applied to third instars did not significantly prevent pupariation although no adults emerged.

Four larvae emerged as adults from third instars (numbers not given) irradiated in papayas and persimmons, *Diospyros kaki* L., in Brazil with 389–400 Gy (Suplicy Filho et al., 1987), indicating that the dose required to completely prevent adult emergence could be ≥ 500 Gy. However, this dose is considerably higher than found from other irradiation disinfestation studies done in Brazil. The estimated dose for achieving probit 9 security (99.9968% control) of Mediterranean fruit fly immatures in papayas was 108 Gy (Faria, 1989); I have only seen the summary of this master's thesis. Raga (1990, 1996) reared larvae in diet and then placed them in mangoes, oranges, and grapefruits for irradiation. At doses of 30–40 Gy (1.8 or 12 Gy/min) no larvae emerged as adults, while 92–98% of the control did. Rearing larvae in diet for insertion in fruit before irradiation may not adequately simulate field conditions, and the validity of this technique should be verified before being used to develop a quarantine treatment. It may be more akin to irradiation in vitro; lower doses of radiation prevent fruit fly adult emergence in vitro than in fruits (Hallman and Worley, 1999). When the same method was used against 7-day-old larvae of *Anastrepha fraterculus* (Wiedemann) and *A. obliqua* reared on diet and inserted into mangoes for irradiation, no adults emerged from larvae subjected to 17.5 Gy (Raga, 1990). As discussed before, the dose needed to provide quarantine security against *A. obliqua* in fruit is considerably higher (Bustos et al., 1992).

Potenza et al. (1989) (as cited by Arthur et al., 1993a) also found that 40 Gy applied to mangoes infested with 7-day-old larvae prevented adult emergence. Adult emergence of third instars irradiated (1 Gy/s) in peach, *Prunus persica* (L.) Batsch, was 83% for the control, 5% at 25 Gy, and 0% at ≥ 50 Gy; percentages are based on puparia formed (a total of 90 in the control) as number of larvae treated was not given (Arthur et al., 1993b.) At 0.016 Gy/s adult emergence based on an estimate of 45 total larvae per dose in the same type of experiment was 73% for the control, 4% at 25 Gy, and 0% at ≥ 50 Gy (Arthur et al., 1993a).

Five of 106 100 third instars in mangoes irradiated with 150 Gy (0.23 Gy/s) survived to the adult stage in Mexico (Bustos et al., 1992). However, the five survivors all occurred within the first 5300 larvae tested (the 'laboratory test'); none occurred in the rest of the $>100\,000$ larvae irradiated (the 'confirmatory test'). This result indicates that the two tests may have been handled differently, with the laboratory test favoring survival. Also, data from dose-response testing (laboratory test) showed that the level of adult emergence remained stable at 0.09–0.16% from 80–150 Gy instead of gradually declining as the dose increased. This type of relationship between dose and response is indicative of a mixed population where some of the individuals are significantly more tolerant of irradiation than the rest.

Mansour and Franz (1996) reported that no adults emerged from $>100\,000$ third instars in Austria irradiated in air with 40 Gy (0.07 Gy/s). Fruit fly larvae irradiated in air may be less tolerant than those irradiated in fruits because of a lower oxygen level inside the fruits and possibly other factors (Hallman and Worley, 1999). When 6-day-old larvae were inserted into oranges and peaches and allowed to feed for ~ 30 h before irradiation, adult emergence was also stopped with 40 Gy (Mansour and Franz, 1996). Adult emergence at 20 Gy was 1.9 ± 1.6 and $2.5 \pm 2.9\%$ for third instars irradiated in air and inside fruits, respectively. Adult emergence in the controls was 89% in air and 49% from larvae placed in fruits for ~ 30 h. Therefore, we can calculate that adult survival for larvae irradiated with 20 Gy in fruits

would have been 89% [control air]/49% [control fruits] × 2.5%[fruits at 20 Gy] = 4.5% if the larvae in the fruits had responded the same as larvae in air. This data would agree with other studies which show larvae in air to be significantly less tolerant of irradiation than larvae in fruits. Further speculation would suggest that a dose of 4.5%/1.9% [air at 20 Gy] × 40 Gy [dose which prevented emergence in air after > 100 000 treated] would yield a dose of ~ 95 Gy to prevent adult emergence at the probit 9 level with 95% confidence from third instar Mediterranean fruit flies placed in fruits for ~ 30 h before irradiation. In any case, a study with much mortality in the control cannot be given great credence in developing quarantine treatments against tephritids which have been adapted to laboratory rearing because the insects do not usually suffer much mortality under laboratory conditions. I would suggest a control mortality limit of 20% for tephritids from established colonies before a particular replicate is discarded.

In a final paper Adamo et al. (1996) in Italy found that 0.4% of third instars (based on a mean of 128.5 larvae per replicate) irradiated in oranges with 150 Gy (0.3 Gy/s) emerged as adults. No adults emerged from oranges irradiated with 400 Gy, the next highest dose.

The wide range of reported reactions of Mediterranean fruit fly third instars to irradiation, which could be interpreted to mean that quarantine security, when based on prevention of adult emergence at levels near probit 9, might be achieved with doses as low as 40 Gy to doses as high as 1000 Gy, makes it difficult to suggest a universal minimum absorbed dose for use against this important quarantined pest. If the two studies that reported adult emergence from third instars treated with ≥ 400 Gy are discounted, the remaining studies fall into two groups: (1) those that yield a quarantine security dose of ≥ 200 Gy, and (2) those that imply a dose of ≤ 100 Gy (Table 2). The doses in Table 2 are my educated guesses as to minimum doses needed for quarantine security based on the information given in each study. I do not assume a long tail in the dose-response data such as was found by Bustos et al. (1992). Many of the studies which found no adult emergence from third instars irradiated in fruits at 30–50 Gy were done with small numbers of insects (dozens to hundreds). Large scale testing needed to confirm a dose could almost double these doses found to achieve 100% control with low numbers of insects. In any case Faria (1989), Raga (1990), Bustos et al. (1992), and Duarte et al. (1993) found that the Mediterranean fruit fly was more tolerant of irradiation than *Anastrepha* spp.

It is probably not reasonable to suppose that the Mediterranean fruit fly could be so variable in its response to irradiation, although that possibility cannot be categorically eliminated, either. Rather, because it is a pest of such singular quarantine significance many researchers have studied it, and the widely different results may be due to differences in technique, dosimetry, interpretation, or degree of care taken in the experimentation. For example, accidental post-treatment re-infestation is suggested as a reason for reports of some adults at doses higher than other doses which resulted in no adults (Burditt, 1994). Because irradiated tephritid larvae must be kept long enough to determine lack of adult emergence, this allows extra time for errors to occur. Hypoxia has been known for some time

Table 2
Speculative doses for achieving quarantine security of Mediterranean fruit fly third instars according to various studies

Reference	Country	Fruit	Dose (Gy)
Seo et al. (1973)	USA (Hawaii)	Papaya	~225
Fésüs et al. (1981)	Hungary	Orange	>200
Potenza et al. (1989)	Brazil	Mango	~80
Raga (1990)	Brazil	Mango	~80
Bustos et al. (1992)	Mexico	Mango	~200
Arthur et al. (1993a,b)	Brazil	Peach	~80
Raga (1996)	Brazil	Grapefruit	~70
Adamo et al. (1996)	Italy	Orange	~200
Mansour and Franz (1996)	Austria	Peach, orange	~95

to increase tolerance of fruit flies to irradiation, maybe by 50 or at most 100%, but nowhere near the degree seen in the studies previously cited. Farrar (1999) argues that many published reports on radiation quarantine treatment research are of little value because of insufficient information about the dosimetry and uncertainties in measuring dose. In some published papers the dose reported is often the minimum dose to which insects were exposed. This is because researchers will use the centerline dose as the true dose ignoring the higher dose received by insects closer to the γ source rods. Dose-attenuation by the fruit may not be measured. This experience with the Mediterranean fruit fly clearly demonstrates the need for careful dose measurement and experimental methodology in general. Too often insufficient information is given to be able to fully interpret results or reproduce research, but that is not a fault unique to irradiation work. In any case, radiation treatment researchers should provide basic methods and materials which are covered in different documents (ICGFI, 1991; APHIS, 1996; NAPPO, 1996; ASTM, 1998a,b; Farrar, 1999). Unfortunately, some of these documents are in the draft stage and taken together they still do not provide a comprehensive guide to conducting radiation treatment research. The International Atomic Energy Agency is developing a guide for radiation quarantine treatment research which should help standardize what and how information is reported and allow for more informed interpretation of results.

4.3. Diapausing tephritids

Working with temperate fruit flies, such as *Rhagoletis*, presents the additional problem of diapause. Using the standard measure of efficacy, prevention of adult emergence, on fruit flies that diapause requires that diapause be terminated in order to evaluate the response. This involves a lengthy process of several months cold treatment which may cause significant mortality to diapausing insects, whether irradiated or not. Also, not all tephritids in diapause emerge after one winter, further complicating efforts to evaluate efficacy. This problem was solved by measuring efficacy based on the insects achieving the diapausing stage: the phanerocephalic pupa (Hallman and Thomas, 1997). This technique involves more work in that the puparium must be opened in order to observe the pupa. The technique is even more conservative than irradiation of non-diapausing fruit flies (see Section 5) in that risk of fruit fly introduction is further reduced because (1) doses to prevent formation of the phanerocephalic stage are higher than doses to prevent adult emergence (Table 3), and (2) diapausing insects would have additional obstacles to overcome involving diapausing successfully before they could become established in a new area.

Table 3
Percentage pupation (phanerocephalic) and adult emergence of apple maggot irradiated as third instars in 'Red Delicious' apples

Dose (Gy)	% Pupation	% Adult emergence
0	86	80
10	47	6.0
15	11	0.7
20	3.7	0.0
25	0.32	0.0

5. Increases to quarantine security by irradiation

A number of factors combine to provide greater security than that initially apparent from the development of irradiation quarantine treatments. Although some of these and other factors may be present with other types of treatments, they are not as beneficial to the same degree as with irradiation. (1) Tolerance to irradiation increases markedly as an insect develops (Fig. 1). Other treatments do not show such a marked increase in tolerance as the insect develops nor such great differences between stages. Most fruit flies occurring in marketed fruit are arguably in the earlier stages of development meaning that irradiation doses designed to stop third instars would be more than adequate. (2) Because of the large dose uniformity ratio, when irradiation is done on a commercial scale, a vast majority of the fruit will receive significantly greater than the minimum

absorbed dose required for quarantine security, much of it two times or more. Other treatments do not experience such a large dose uniformity ratio. (3) Even if adults emerged from irradiated third instars they would most likely be sterile and not live very long, a claim which cannot be made for survivors of other treatments (Mangan and Hallman, 1998).

6. Identification of irradiated products

The ability to recognize insects or fruits that had been irradiated would allow for independent confirmation of irradiation. A number of physical, chemical, and biological techniques have been developed to determine if products have been irradiated (Haire et al., 1997; Delincée, 1998). Some suffer from specificity, practicality, and dose determination uncertainties. Many detection procedures are not effective at the relatively low radiation doses used for disinfestation treatments. Chemical methods are generally not very promising because processes besides irradiation give the same results, and low dose radiation quarantine treatments cause extremely minute chemical changes. Some approaches rely on lack of insect development measured several days post-irradiation. One novel technique for detecting irradiated fruit flies is suppression of phenoloxidase production in late instar larvae irradiated as early instars (Nation et al., 1995). A simple spot test was developed for identifying these larvae. It is of little practical use because it works only for early instar larvae irradiated before significant phenoloxidase is produced and then tested only after significant phenoloxidase production would have occurred. In any case these larvae would not be expected to reach the third instar because radiation doses designed to stop third instars from emerging as adults would also stop first instars from reaching the third instar. Furthermore, suppression of phenoloxidase production is achieved at doses as low as 20 Gy which do not provide quarantine security against third instars. It would concern me if I found a live third instar that did not show phenoloxidase activity in an irradiated fruit; that would signify that, although the fruit was irradiated, some of the fruit load did not receive the minimum required dose for quarantine security against insects that were third instars when irradiated.

Electron spin resonance (ESR) spectroscopy, which detects free radicals formed radiolytically, may be suited to radiation dose determination of some irradiated fruits that have solid, relatively dry parts, such as the endocarp of drupes. Perhaps ESR might also identify heavily sclerotized insect mouthparts that have been irradiated. ESR responses have proven quite variable, however, when applied to complex crustacean exoskeletons (Delincée, 1998). Because DNA is such a large target for ionizing radiation, several approaches involve detecting DNA fragmentation, both nuclear and mitochondrial. Other useful techniques might be derived from the effect of radiation on impeding cell division and germination of fruit seeds or on the actively dividing insect midgut.

7. Market quality of irradiated fruits

Irradiation may be the most broadly applicable quarantine treatment against fruit flies from the standpoint of fruit quality. At the doses needed to achieve quarantine security of most fruit flies, especially if doses were below 150 Gy for some species, many fruits can tolerate doses needed for quarantine security even if those doses were tripled as could occur in some commercial situations. Burditt (1994) and Morris and Jessup (1994) report on the damages caused by irradiation to some fruits and tolerance of various fruits to different radiation doses. Sometimes there is a discrepancy between what researchers find and what occurs on a commercial scale regarding fruit quality after a quarantine treatment. This may be due to several reasons: (1) research not applied across the whole growing region, all cultivars and crop management systems, nor sufficient seasons, (2) relatively small numbers of fruits treated in research compared with tons treated commercially, and (3) inability of commercial systems to follow careful research protocols. Nonetheless, significant quantities of papayas, rambutans, lychees, atemoyas, and carambolas have been

shipped from Hawaii for treatment with a minimum dose of 250 Gy (and some with a minimum dose of 400 Gy) with no significant quality problems resulting. One benefit of irradiation over other treatments, such as hot air of papayas, is that fruits can be harvested riper for irradiation, thus resulting in a higher quality product.

8. Irradiation quarantine treatment for mangoes

Irradiation could be a viable alternative to the 46.1°C water immersion quarantine treatment for mangoes ≤ 0.7 kg, which is sometimes detrimental to fruit quality, and a treatment for mangoes > 0.7 kg, which cannot currently be treated with hot water due to lack of efficacy and commodity tolerance data for the longer treatments which would be necessary. Mangoes are picked, treated, and shipped mature green. Akamine and Moy (1983) found that 250 Gy scalded mature green mangoes. McLauchlan et al. (1990) felt that a dose of ≥ 300 Gy applied to mature green but preclimacteric 'Kensington Pride' mangoes would not be advised because of the degree of lenticel damage caused. Spalding and von Windeguth (1988) recommended that mature green mangoes (cvs. Tommy Atkins and Keitt) not be irradiated with ≥ 250 Gy because of scald and increased internal breakdown; 'Keitt' mangoes suffered more injury than 'Tommy Atkins'. In general, the riper the mango, the more tolerant it is of irradiation, and ripe mangoes could tolerate a fairly high dose of radiation (Akamine and Moy, 1983; Boag et al., 1990). However, ripe mangoes have a short shelf life not amenable to marketing. Because of the damage observed to mature green mangoes with moderate doses of radiation, it would be desirable to use as low a dose as possible and to irradiate the mangoes as late as is feasible for marketing purposes. The four species of *Anastrepha* that have been studied with large-scale tests show remarkable similarity in response to irradiation. A minimum dose of 100 Gy is adequately supported by research; the dose could probably be somewhat lower. Tests with *A. fraterculus* in mangoes and other fruits indicate that its response is no different (Arthur et al., 1989; Raga, 1990; Arthur and Wiendl, 1996). The only mango-infesting fruit fly in the mango-producing regions of the Western Hemisphere whose control would be in doubt when exposed to 100 Gy is the Mediterranean fruit fly. A minimum absorbed dose of 100 Gy could be used in the neotropical mango-growing regions which do not have Mediterranean fruit fly, and 225 Gy for those areas which do have this fly.

9. Irradiation quarantine treatment for citrus

Citrus are treated with methyl bromide fumigation and cold storage quarantine treatments. Alternatives are sought because these treatments may sometimes result in unacceptable levels of damage to the fruits as well as the fact that methyl bromide may not be available in the future. Irradiation could be a viable alternative; however, citrus are less tolerant of irradiation than many fruits. Hatton et al. (1984) found that percentage rind injury of grapefruits irradiated with 600, 300, 150, and 0 (control) Gy was 20, 9, 4, and 1%, respectively. Miller and McDonald (1996) felt that 300 Gy was about the maximum that grapefruits could tolerate without significant loss due to peel pitting. Current recommendations of 150 Gy for several species of fruit flies which infest grapefruits, such as *A. ludens* and *A. suspensa* (Table 1), would result in significant damage to irradiated grapefruits when applied on a commercial scale because the dose uniformity ratio is usually $> 2:1$. Fortunately data support minimum absorbed effective doses of ≤ 100 Gy for *A. ludens*, and other species, which would cause minimal damage to citrus when applied on a commercial scale. Unfortunately, data do not yet support a dose < 225 Gy for Mediterranean fruit fly nor < 250 Gy for oriental fruit fly; hence, irradiation could not be used against citrus infested with those important pests unless the fruits were irradiated in a fashion, which would be slow and costly, that would allow for a very small dose uniformity ratio.

10. Conclusions and future directions

Based on over 4 years' experience in shipping fruits from Hawaii to the mainland USA for irradiation for quarantine purposes, the process has been demonstrated to be a viable quarantine treatment and a replacement for methyl bromide in many cases. Many fruits can tolerate doses necessary for quarantine treatment against tephritid fruit flies when applied on a commercial scale, which would mean that much of the fruits would receive up to three times the minimum dose required for quarantine security. Irradiation is probably the most widely applicable quarantine treatment from the standpoint of fruit quality. However some important fruits which are shipped in large quantities using quarantine treatments, such as mangoes and citrus, may, under certain circumstances, suffer unacceptable levels of damage at doses as low as 250 Gy. Quarantine treatment doses should be as low as is possible, provided that sufficient substantiating data support efficacy, in order to reduce fruit damage and speed treatment. For several species of *Anastrepha* and some species of *Bactrocera*, probit 9 efficacy can be achieved with 60–100 Gy.

More research is recommended to ascertain whether the Mediterranean fruit fly and the oriental fruit fly can be controlled with <250 Gy. Studies with the former, if taken at face value, suggest that it shows two distinct responses to irradiation: efficacy at 80–100 and ≥ 200 Gy. Some research would even suggest that the dose needed for quarantine security is much higher. The confusion with studies of the oriental fruit fly is a clear demonstration of the importance of storing voucher specimens for future reference.

The importance of accurate dosimetry cannot be overemphasized. There is some question as to the accuracy of the doses reported in previous research. In many cases reported doses were simply estimated centerline doses and the true dose would need to be raised; in other cases it may be impossible to know what the true dose might have been. Dosimetry must be traceable to recognized international standards.

ICGFI has proposed a generic dose of 150 Gy for fruit flies (Burditt, 1994). Although current data with several species of fruit flies demand doses >150 Gy (Table 1), perhaps a generic dose of 250 Gy for this important family of major quarantined pests, Tephritidae, would not be unwarranted. I think the validity of those few tests which indicate doses >250 Gy for tephritids can be questioned. Of course, when adequate, reliable data suggest lower doses for certain species, that lower dose should be used for those species. The acceptance of a generic dose of 250 Gy would help solve the problem of conducting research on a myriad of fruit fly species such as those described by Drew and Hancock (1994) and offer a viable alternative to methyl bromide fumigation.

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