RATIONALE FOR A GENERIC PHYTOSANITARY IRRADIATION DOSE OF 70 GY FOR THE GENUS ANASTREPHA (DIPTERA: TEPHRITIDAE)

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ABSTRACT

The phytosanitary irradiation (PI) literature relating to the genus Anastrepha was analyzed to determine if it was sufficient to support a generic dose < 150 Gy (the accepted generic dose for all of Tephritidae) that could be used on fruit in areas of the tropical and subtropical Americas where only species of the genus are quarantine pests. Although Anastrepha contains > 230 species only 7 have been consistently of quarantine significance, and PI research has been reported on all but one of those. The measure of efficacy for PI of Tephritidae is prevention of adult emergence when eggs or larvae are irradiated in fruit; the 3rd instar is the most radio-tolerant stage. Large-scale testing where ~100,000 third instars have been treated at one dose with no adults emerging has been successfully conducted at up to 100 Gy with 4 species. However, a rationale for a generic dose of 70 Gy is given based on the apparent homogeneity in response to radiation within the genus and the fact that the International Plant Protection Convention has approved a dose of 70 Gy for 2 key species.

Key Words: radiation, quarantine treatment, Anastrepha ludens, Anastrepha suspensa

RESUMEN

La literatura sobre irradiación fitosanitaria (IF) del género Anastrepha fue analizada para determinar la posibilidad de usar una dosis genérica de < 150 Gy (la dosis aceptada para toda la familia Tephritidae) para desinfectar frutas en las áreas del trópico y subtropical americano donde únicamente especies del género estén presentes como plagas cuarentenarias. Aunque el género Anastrepha contiene > 230 especies, en la actualidad solo siete son de importancia cuarentenaria, y se ha investigado la IF en seis de las siete especies. La medida de eficacia para la IF en la familia Tephritidae es la prevención de la emergencia de adultos cuando los huevos o larvas son irradiadas en el fruto; el tercer instar es el estadio más tolerante a la IF. Se han realizado experimentos exitosos a gran escala (~100,000 insectos de cuatro especies) con dosis entre 69 y 100 Gy sin emergencia alguna de adultos. Sin embargo, se propone una dosis genérica de 70 Gy basada en la aparente homogeneidad de respuesta a esta dosis de radiación por los huevos o larvas del género Anastrepha y al hecho de que la Convención Internacional de Protección Fitosanitaria ha aprobado esta dosis para dos especies claves.

Palabras Clave: irradiación fitosanitaria, tratamiento cuarentenario, radiación
Anastrepha is the most diverse and economically important genus of tephritid fruit flies in the American tropics and subtropics, where it is native, with more than 250 described species (Norrbom & Korytkowski 2011). Major tephritid pests are listed in Table 1. Bactrocera carambolae Drew & Hancock was introduced and is presently found in a restricted area of the region. The primarily monophagous nature and limited distribution of the 2 species of native Rhagoletis in the tropics and subtropics restrict the probability that treatments will be used against them. There are a number of other native species of Rhagoletis that, although some have been recorded on economic fruits (Foote 1981), have not been considered quarantine pests. Most feed on wild species of Solanaceae in the region. The native Toxotrypana curvicauda Gerstaecker, papaya fruit fly, is only found commonly on papaya. The introduced Ceratitis capitata (Wiedemann), Mediterranean fruit fly, is widely distributed in the region, and a PI dose of 100 Gy based primarily on research done in Peru (Torres & Hallman 2007) has been accepted (IPPC 2011). In most cases with fruit exported from the tropical and subtropical Americas only C. capitata and species of Anastrepha need be considered regarding Tephritidae. In some cases only species of Anastrepha are of concern.

The objective of this study was to examine the phytosanitary irradiation (PI) literature of Anastrepha spp. for a generic dose that would be efficacious for the entire genus.

**Materials and Methods**

A search for all PI treatment literature for any species of Anastrepha was conducted and the resulting literature evaluated for a number of criteria that would adhere to what is commonly referred to as ‘good laboratory practice’, or a set of standards by which studies are planned, conducted, monitored, recorded, reported, and interpreted. Issues concerning PI research have been dosimetry, definition of efficacy, most radiotolerant stage, infestation technique, low oxygen levels during irradiation, and proper response of non-irradiated control organisms. Guidance for PI research is available (Hallman 2001; Hallman et al. 2010; Heather & Hallman 2008; IPPC 2003).

**Measure of Efficacy**

The measure of efficacy used is the same as that commonly used for all PI treatments with Tephritidae: prevention of adult emergence when the 3rd instar, the most radiotolerant stage found in fruit (Hallman et al. 2010), is irradiated. Only studies that address adult emergence following irradiation of 3rd instars are included. Although prevention of reproduction of emerging adults could be achieved with lower doses than those required for prevention of adult emergence (Bustos et al. 2004; Hallman & Loaharanu 2002), basing efficacy on the former carries the risk that flies could be found in survey traps triggering expensive regulatory reactions. Allowing for adult tephritids to be found in importing countries is generally not acceptable to plant protection organizations. However, the fact that any adults that might emerge following a moderate dose of irradiation as third instars would not reproduce lends an extra margin of security to any dose recommended to prevent adult emergence.

**Dosimetry**

The ability to accurately quantify the dose received by the target organism is fundamental to any phytosanitary treatment. Unfortunately, many irradiation studies, even those published today, do not report dose measurement but simply report the dose that was sought. Ideally only those studies reporting the results of dosimetry should be included. All of the studies found for this analysis used isotopic sources, mostly cobalt-60 with one study using cesium-137. The quantity of gamma radiation emitted and the decay rate of these isotopes is extremely predictable, and the machines in which they are imbedded in the last 30 yr during which the research was done restrict the distance between the isotope and the material being irradiated to a relatively narrow field. Where results of dosimetry are not given an assumption is made for the purposes of this study that absorbed doses do not differ greatly from the target doses reported in the literature. Personal experience with 2 irradiators (Gammacell 220 and Husman), which were used or are similar to others machines used for some of the research reported herein indicates that absorbed doses may be up to 20% greater than target doses. Therefore, if dosimetry is not reported doses required for quarantine security in each study should be increased by 20%. A generic treatment provides a margin of security to compensate for uncertainties such as lack of dosimetry. The generic dose of 150 Gy for Tephritidae was used in some studies that did not report dosimetry (IPPC 2009d).

**Infestation Technique**

Some PI studies used artificial infestation techniques; larvae reared on diet were inserted into holes made in fruit or placed in vials which were inserted into fruit. Insertion of diet-reared larvae has theoretical advantages over rearing of larvae in the fruit, such as ability to precisely con-
Table 1. Tephritid species in the American tropics and subtropics that frequently occur in commercially traded fruits.

| Species           | Major fruits infested                                           | Geographic range in America                                      |
|-------------------|-----------------------------------------------------------------|------------------------------------------------------------------|
| *Anastrepha fraterculus* | Guava, *Psidium guajaba* L. (Myrtales: Myrtaceae) peach, *Prunus persica* L. (Rosales: Rosaceae) mango, *Mangifera indica* L. (Sapindales: Anacardiaceae) citrus, *Citrus* spp. (Sapindales: Rutaceae) | Trinidad, Tobago, continental Latin America, except Chile        |
| *A. grandis*     | Cucurbitaceae                                                    | Panama to southern Brazil                                          |
| *A. ludens*      | Citrus, *Citrus* spp. (Sapindales: Rutaceae) mango, *Mangifera indica* L. (Sapindales: Anacardiaceae) guava, *Psidium guajaba* L. (Myrtales: Myrtaceae) | Costa Rica to southern Texas                                      |
| *A. obliqua*     | *Spondias* spp. (Sapindales: Anacardiaceae) mango, *Mangifera indica* L. (Sapindales: Anacardiaceae) guava, *Psidium guajaba* L. (Myrtales: Myrtaceae) | Mexico and Caribbean to Ecuador                                   |
| *A. serpentina*  | Sapotaceae, mango, *Mangifera indica* L. (Sapindales: Anacardiaceae) citrus, *Citrus* spp. (Sapindales: Rutaceae) | Northern Mexico to northern Argentina, Trinidad, Tobago, Curacao  |
| *A. striata*     | Guava, *Psidium guajaba* L. (Myrtales: Myrtaceae) mango, *Mangifera indica* L. (Sapindales: Anacardiaceae) | Northern Mexico to Brazil                                         |
| *A. suspensa*    | Guava, *Psidium guajaba* L. (Myrtales: Myrtaceae) mango, *Mangifera indica* L. (Sapindales: Anacardiaceae) citrus, *Citrus* spp. (Sapindales: Rutaceae) mango, *Mangifera indica* L. (Sapindales: Anacardiaceae) | Greater Antilles, Bahamas, Florida                               |
| *Bactrocera carambola* | Caromibia, *Averrhoa carambola* L. (Oxalidales: Oxalidaceae) mango, *Mangifera indica* L. (Sapindales: Anacardiaceae) | Suriname, French Guiana, Amapá (northern Brazil)                 |
| *Ceratitis capitata* | Citrus, *Citrus* spp. (Sapindales: Rutaceae) persimmon, *Diospyros kaki* Thunb. (Ericales: Ebenaceae) apple, *Malus domestica* Borkh. ‘Gala’ (Rosales: Rosaceae) mango, *Mangifera indica* L. (Sapindales: Anacardiaceae) guava, *Psidium guajaba* L. (Myrtales: Myrtaceae) | Jamaica, Puerto Rico, Central America to Uruguay, except Chile  |
| *Rhagoletis pomonella* | Apple, *Malus domestica* Borkh. ‘Gala’ (Rosales: Rosaceae) | Mexican central highlands                                         |
| *R. tomatis*     | Tomato, *Solanum lycopersicum* L. (Solanales: Solanaceae)        | Southwestern Peru, northern Chile                                 |
| *Toxotrypana curvicauda* | Papaya, *Carica papaya* L. (Brassicaceae: Caricaceae) | Tropics, Florida                                                  |
trol insect numbers and stage of development and prevention of fruit degradation caused by insect feeding and time required to rear them to the 3rd instar. However, because the technique is unnatural its possible effect on insect radio-tolerance should be tested by comparison with infestation via oviposition. This comparison was not done for any of the studies that used artificial infestation.

Hallman & Thomas (2010) did not find a difference in prevention of adult emergence of irradiated 3rd instar *Anastrepha ludens* (Loew) reared inside grapefruit vs. reared on diet and inserted in grapefruit. However, they discuss a tendency in the literature of *C. capitata* for insertion of diet-reared larvae to result in lower doses compared with larvae reared from the egg in fruit. These observations may be made with *Anastrepha* as well. For example, Faria (1989) placed diet-reared 3rd instar *A. fraterculus* (Wiedemann) into holes bored to the center of papaya and sealed with the papaya plug and tape. At 24 and 28 Gy, respectively, 0.25 and 0% of 1,200 larvae emerged as adults (non-irradiated control emergence was 75%).

Most Radio-tolerant Stage

Of all of the life stages of Tephritidae that may occur in shipped fruit the most radio-tolerant is uniformly the 3rd instar (Hallman et al. 2010). Therefore, PI research should be done with fruit infested with that stage. Tephritids will frequently require more time to develop to the 3rd instar in fruit than in diet. For example, Leyva et al. (1991) found that although *A. ludens* reared in diet and peaches required similar time periods, those reared in mangoes and citrus fruit required over twice as much time to reach the 3rd instar.

RESULTS AND DISCUSSION

Results of PI studies are presented below by species in alphabetical order.

*Anastrepha fraterculus* (Wiedemann)

Arthur et al. (1989) subjected field collected fruit of Surinam cherry, *Eugenia uvalha* Cambess. (Myrtales: Myrtaceae), infested with *A. fraterculus* to 25-500 Gy and obtained no adult emergence at 50 Gy (n = 48) or higher doses (Table 2). At the next lowest dose (25 Gy) adult emergence was 39.6%. All adults emerged from the 50 puparia obtained in the control.

A dose of 50 Gy prevented adult emergence of 3rd instar *A. fraterculus* reared in mangoes (n = 100 as estimated by larval emergence from the control); no lower dose was attempted (Arthur et al. 1991). Adult emergence from larvae found in the control was 65%.

### Table 2. Radiation doses for 6 species of *Anastrepha* that prevented adult emergence when 3rd instars were reared and irradiated in fruit.

| Fruit host | Dose (Gy) | Number 3rd instars treated | References |
|------------|-----------|-----------------------------|------------|
| *Eugenia uvalha* | 50 | 48 | Arthur et al. (1989) |
| *Mangifera indica* | 100 | 100 | Arthur et al. (1991) |
| *Malus domestica* | 25 | 70 | Arthur et al. (1996) |
| *Citrus paradisi* | 69 | 94,400 | Hallman & Martinez (2001) |
| *Mangifera indica* | 60 | 5,513 | Bustos et al. (1992, 2004) |
| *Citrus x sinensis* | 60 | 1,716 | Toledo et al. (2001) |
| *Psidium guajaba* | 50 | 176 | Arthur et al. (1993) |
| *Averrhoa carambola* | 50 | 88 | Arthur & Wiendl (1994) |
| *Mangifera indica* | 60 | 4,194 | Bustos et al. (1992, 2004) |
| *Averrhoa carambola* | > 100,000 | Gould & von Windeguth (1991) |
| *Psidium guajaba* | 40 | 1,834 | Toledo et al. (2003) |
Arthur et al. (1996) irradiated apples (Malus domestica Borkh. ‘Gala’; Rosales: Rosaceae), 5-8 days after oviposition (held at 21-24 °C) and found that the lowest dose used (25 Gy) prevented adult emergence from 70 puparia (control emergence was 77% and the number of larvae irradiated not given). When irradiated the larvae were probably a mixture of mid and late instar.

Anastrepha ludens (Loew)

Wolfenbarger & Guenthner (1998) report that 250 Gy (the highest dose used) is insufficient to prevent adult emergence from 3rd instar A. ludens reared and irradiated in grapefruit. However, prevention of adult emergence seemed to level off at 60 Gy and then fluctuated in a zigzag fashion; for example, it was 100% at 180 Gy and 82% at 200 Gy. I conclude that there was a source of contamination in the research, which is apparently not unusual for phytosanitary treatment research (Heather & Hallman 2008). Even at lower doses there was considerable zigzagging of results: 29, 13, 21, and 14% prevention of adult emergence at 10, 20, 30, and 32.5 Gy, respectively. A further demonstration of the huge variation in results are the estimates to achieve 99% mortality of larval and puparial stages of 407,317 and 38,039 Gy, respectively! This study should not be considered in devising a generic PI treatment for Anastrepha.

Hallman & Martinez (2001) reported large-scale confirmatory testing for A. ludens; at a maximum absorbed dose of 69 Gy (target dose was 60 Gy) no adults emerged from an estimated total of 94,400 third instars resulting from oviposition in grapefruits. An estimated total of 52,000 larvae were treated at a maximum absorbed dose of 58 Gy (target dose 50 Gy) before one adult emerged. This study is the basis for the dose of 70 Gy for this insect in ISPM #28 (IPPC 2009a). Heather and Hallman (2008) note that many countries accept a confirmatory test size of 30,000 insects tested; under that scheme a dose of 58 Gy for A. ludens would be justified.

A large study with 4 species of Tephritidae, including A. ludens, in mangoes was variously reported (Bustos et al. 1992, 1993, 2004). The first report gives all of the essential details of the testing that the others lack (e.g., dosimetry and numbers tested in preliminary tests) while the last report is peer-reviewed. Sixty Gy (n = 5,513) prevented adult emergence of 3rd instars reared and irradiated in mangoes; the next lowest dose, 40 Gy (n = 5517), resulted in 0.1% adult emergence (Bustos et al. 1992, 2004). Adult emergence in the control was 86%. With the machine used (Model JS-7400, Nordion, Ottawa) the dose uniformity ratio (DUR) was always within 1.01, meaning essentially that the target dose was the absorbed dose (Bustos et al. 1992).

For the large-scale confirmatory testing a total of 101,794 3rd instars was irradiated at 100 Gy in mangoes with no adults emerged (Bustos et al. 2004). It is quite possible that < 100 Gy would have sufficed because Hallman & Martinez (2001) achieved the same result with a putative dose of 60 Gy as Bustos et al. (2004) at that dose, and the results of dose-response testing between the 2 studies were similar. Researchers are urged to initiate large-scale confirmatory testing for all phytosanitary treatments at the lowest dose feasible to provide commerce with the lowest possible final dose.

Toledo et al. (2001) found that 60 Gy to 3rd instar A. ludens (n = 1,716) reared and irradiated in oranges prevented adult emergence; the next lowest dose, 40 Gy, resulted in 1.5% emergence. Large-scale confirmatory testing at 100 Gy prevented adult emergence from a total of 20,359 third instars. Again, it is possible that < 100 Gy would have been found sufficient to prevent adult emergence had large-scale testing been undertaken at a lower dose. The same machine and techniques, including dosimetry, were used as in Bustos et al. (1992, 2004), so it might be assumed that although results of dosimetry were not given they might be similar; i.e., the target doses were essentially the absorbed doses.

Anastrepha obliqua (Macquart)

Fifty Gy, the lowest dose tried, prevented adult emergence of 3rd instar A. obliqua (n = 176) oviposited in guava (Arthur et al. 1993). Adult emergence in the control was 66% of total 3rd instars. Field-infested carambolas were collected and held until A. obliqua in them reached the 3rd instar before being irradiated (Arthur & Wiendl 1994). At 50 Gy (the lowest dose used) adult emergence was prevented (n = 88). Emergence in the control was 65% of total 3rd instars.

Bustos et al. (1992, 2004) reared and irradiated A. obliqua in mangoes. Adult emergence was 0.1 (n = 4872) and 0% (n = 4194) at 40 and 60 Gy, respectively. Large-scale confirmatory testing was done at 100 Gy and a total of 100,400 third instars were irradiated with no adults emerging. Emergence in the control was 83%. Because the dose uniformity ratio (DUR) was so low (Bustos et al. 1992), the absorbed doses were essentially the target doses. As with their work with A. ludens it is possible that < 100 Gy could have prevented adult emergence in large-scale testing.

A dose of 70 Gy for A. obliqua was accepted by the IPPC based on comparison with A. ludens (IPPC 2009b). It was concluded that A. obliqua was more radio-susceptible than A. ludens based on Bustos et al. (1992, 2004) and Hallman & Worley (1999). Therefore, a dose that controlled the latter (IPPC 2009a) would control the former.
Anastrepha serpentina (Wiedemann)

Bustos et al. (1992, 2004) reared and irradiated A. serpentina in mangoes to the 3rd instar and found that 40 (n = 5,537) and 60 (n = 4,025) Gy resulted in 0.2 and 0% adult emergence while emergence in the control was 79%. As with the other species of Anastrepha they studied, the authors did large-scale confirmatory testing at 100 Gy and irradiated 105,252 third instars with no adults emerging. Because the DUR was so low (Bustos et al. 1992) the absorbed doses were essentially the target doses. Again, it is possible that complete control with large numbers of 3rd instars irradiated could be achieved with < 100 Gy. The dose of 100 Gy in ISPM #28 (IPPC 2009c) was supported by Bustos et al. (1992, 2004).

Anastrepha suspensa (Loew)

Burditt et al. (1981) irradiated (25-300 Gy) grapefruits infested with Anastrepha suspensa via oviposition 7-14 days previously. From 25 to 50 Gy (n = 2,421) no adults emerged, at 75 Gy (n = 325) 3 adults emerged, and at 100 Gy (n = 831) and above no adults emerged. Although it is possible for 3 adults to emerge at 75 Gy while none emerged from greater numbers irradiated at lower doses, it seems improbable given that only 10 of 325 larvae pupariated at 75 Gy and at 25 Gy essentially all larvae pupariated (1,285) while none emerged as adults.

von Windeguth (1982) infested grapefruits via oviposition with A. suspensa and transported them by truck 3140 km to a pilot plant sewage sludge treatment irradiator and back for irradiation with 154-948 Gy, as measured by dosimetry. Non-irradiated controls were also transported. In one treatment of 4,840 larvae at 302 Gy only one puparium was formed and from it emerged an adult. In another treatment of 3,368 larvae at 172 Gy 107 puparia were formed and one adult emerged. It is unexpected that adult emergence would occur from such a low rate of pupariation, especially in the case of the irradiation at 302 Gy. In general if adults emerge from puparia (irradiated as larvae) the rate of pupariation is very high (Hallman et al. 2010). Cross contamination with the transported controls is suspected because of measures taken to prevent cross contamination in a similar later experiment (von Windeguth & Ismail 1987).

von Windeguth (1986) obtained one adult A. suspensa from oviposition-infested mangoes irradiated with 55 Gy and none at 30 Gy (estimated number of late larvae at both doses was 2437). Grapefruits infested via oviposition were transported by truck 1,740 km and back to be irradiated with absorbed maximum doses (as measured by dosimetry) of 52-533 Gy (von Windeguth & Ismail 1987). No adults emerged; the number of insects tested at the lowest dose (52 Gy) was 2877.

Gould & von Windeguth (1991) reported 0.1% adult emergence when carambola infested via oviposition were irradiated at 25 Gy. The A. suspensa population consisted of all stages because some fruit were irradiated after exposure to oviposition for 3-5 days (longer exposure time for cooler days) and some were held an additional 4 days for larval development. Large scale confirmatory testing was done at 50 Gy with > 100,000 mixed-age eggs and larvae treated with no adult emergence. The IPPC did not accept a dose of 50 Gy for A. suspensa because there was concern that insufficient insects of the most tolerant stage (3rd instar) were present (Hallman et al. 2010). As mentioned above, tephritids reared on fruit often require longer development times than those reared on diet. As an example with the same fruit, Hallman & Sharp (1990) used a minimum of 10 days to obtain 3rd instar A. suspensa in carambola infested via oviposition.

Anastrepha striata Schiner

Field-infested guavas were held until 3rd instar Anastrepha striata Schiner was present and then irradiated (Toledo et al. 2003). Results of dosimetry are provided, and at narrow absorbed dose ranges of 29.7-30.3 and 39.7-40.3 Gy, respectively, adult emergence was 0.1 (n = 1,750) and 0% (n = 1,834). Medium-scale confirmatory testing at 100 Gy resulted in no adult emergence from 13,094 third instars irradiated. As with other species of the genus, A. striata could probably be controlled with < 100 Gy.

Generic Dose for Anastrepha

A PI generic dose for the genus Anastrepha is supported at 100 Gy by large-scale confirmatory testing done at or below this dose for several species. Because the dose for C. capitata is 100 Gy and possibly cannot be lowered significantly (Torres & Hallman 2007) it might seem appropriate to propose a dose for Anastrepha no less than 100 Gy. However, there are areas where C. capitata is not found in the region and it is being controlled in other areas, so the opportunity to use a lower dose is considerable. Bustos et al. (1992, 2004) concluded that A. ludens was the most radio-tolerant of the 3 species they studied, although it is doubtful that differences among the species would be found via statistical comparisons. Nevertheless, the raw data support the opinion that a dose that controls A. ludens would control A. obliqua and A. serpentina, and the IPPC agreed concerning A. obliqua (IPPC 2009b). Small-scale testing with A. fraterculus in 3 studies with 3 different fruits reported above found that 25-50 Gy prevented adult emergence (Table 2). Because no dosimetry was reported, increasing the maximum
dose in that range by 20% gives 60 Gy. Medium-scale testing in one study with A. striata found that 40 Gy sufficed. Large-scale testing for both of these species may increase the dose to >50 Gy. The data for A. suspensa are more variable but if the apparent problems in methodology are ignored the overall results align with the other species of Anastrepha studied. The studies in Table 2 show considerable uniformity within the genus if problematic studies with A. ludens and A. suspensa described above are excluded. Taking this evidence into consideration a generic PI dose for the genus Anastrepha could be 70 Gy. This dose would satisfy the maximum degree of quarantine security that may be required by plant protection organizations of 99.9968% efficacy (probit 9) at the 95% level of confidence level if the study by Hallman & Martinez (2001) is considered representative of the genus.

One reviewer suggested that because A. grandis (for which I found no PI data) may infest large fruits of the family Cucurbitaceae perhaps a higher dose would be required to achieve adequate penetration and control for that species. However, PI doses are based on minimum dose absorbed by the entire load, not dose reaching the outside of the load. Therefore, any dose set for PI would ensure that the entire load absorbs at least the minimum dose required.

Because Anastrepha is reported to be more radio-tolerant than Rhagoletis (Hallman and Thompson 1999, Hallman and Loaharanu 2002; Hallman 2004) including the latter under a generic dose of 70 Gy might be justifiable as well.

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