

# Factors Affecting Ionizing Radiation Phytosanitary Treatments, and Implications for Research and Generic Treatments

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**ABSTRACT** Phytosanitary irradiation (PI) treatments are promising measures to overcome quarantine barriers to trade and are currently used in several countries. Although PI has advantages compared with other treatments one disadvantage bedevils research, approval, and application: organisms may remain alive after importation. Although this does not preclude their use as a phytosanitary treatment, it does leave the treatment without an independent verification of efficacy and places a greater burden for assuring quarantine security on the research supporting the treatment. This article analyses several factors that have been hypothesized to affect PI efficacy: low oxygen, pest stage, host, dose rate, and temperature. Of these factors, the first is known to affect efficacy, whereas host and dose rate probably need more research. The International Plant Protection Convention considered several PI treatments for its international standard on phytosanitary treatments and did not approve some at first because of perceived problems with the research or the presence of live adults after irradiation. Based on these concerns recommendations for research and dealing with the issue of live adults postirradiation are given. Generic PI treatments are suggested.

**KEY WORDS** quarantine, commodity treatment, irradiation, disinfestation

Ionizing radiation as a phytosanitary treatment is increasing in commercial use because it possesses some advantages over other treatments, such as applicability to packed commodities and broad tolerance by fresh fruit (Heather and Hallman 2008). A major disadvantage for plant protection organizations with phytosanitary irradiation (PI) compared with other treatments is that PI is the only commercially applied treatment that does not result in significant acute mortality, leaving phytosanitary inspectors with no independent verification of efficacy. This shortfall is important because major phytosanitary treatments based on heat, cold, and methyl bromide fumigation have apparently failed, and the only way this was known was by the discovery of live insects after treatment (Heather and Hallman 2008). These treatments were based on accepted research, and there is no reason to believe that irradiation could not fail as well except in this case the failure might not be known.

The measure of efficacy of PI is prevention of further development or successful reproduction (FAO 2003). If inspectors find live quarantine pests for virtually every other treatment the consignment is rejected or retreated regardless of certification of treatment. It is assumed that the treatment was not properly done or does not work as applied or that the shipment was contaminated with nontreated, infested

commodity or reinfested after treatment. Live pests are expected after irradiation, and finding them does not preclude entry of the consignment as long as treatment certification is verified.

The U.S. Animal and Plant Health Inspection Service (APHIS) has approved several PI treatments (Table 1). The International Plant Protection Convention (IPPC) adopted eight in 2009 (FAO 2009b) and recently adopted three more at the fifth meeting of the Commission on Phytosanitary Measures (CPM) (FAO 2010), making 11 adopted PI treatments in total (Table 1).

All of the treatments approved by APHIS by 2006 were considered for submission to the IPPC vetting process; two were not proposed by APHIS and six were eliminated during the process at different stages (FAO 2009c, 2010). The following section discusses the fate of these treatments in the submission and approval process.

APHIS did not propose the treatments for *Brevipalpus chilensis* Baker and *Sternonchetus mangiferae* (F.) (Table 1) because of insufficient numbers of organisms tested and poor performance of controls. The 300-gray (Gy) dose for *B. chilensis* was confirmed with a total of 8,042 irradiated adults, which is not considered sufficient for a mite that may be present in considerable numbers in regulated articles. Furthermore, controls laid a mean of only 1.0 egg per female with only 33.2% of the eggs hatching (Castro et al. 2004). Also, 56.6% of irradiated mites were still alive when the experiments were terminated. To demonstrate failure of reproduction after irradiation the ir-

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**Table 1.** Radiation phytosanitary treatments approved by APHIS (2008a, 2010a,b) and the IPPC (FAO 2009b,c, 2010)

Species or group	Order: Family	Dose (Gy)	
		APHIS	IPPC
<i>Anastrepha ludens</i> (Loew)	Diptera: Tephritidae	70	70
<i>Anastrepha obliqua</i> (Macquart)	Diptera: Tephritidae	70	70
<i>Anastrepha serpentina</i> (Wiedemann)	Diptera: Tephritidae	100	100
<i>Anastrepha suspensa</i> (Loew)	Diptera: Tephritidae	70	
<i>Aspidiotus destructor</i> Signoret <sup>a</sup>	Hemiptera: Diaspididae	150	
<i>Bactrocera jarvisi</i> (Tryon)	Diptera: Tephritidae	100	100
<i>Bactrocera tryoni</i> (Froggatt)	Diptera: Tephritidae	100	100
<i>Brevipalpus chilensis</i> Baker	Acari: Tenuipalpidae	300	
<i>Ceratitis capitata</i> (Wiedemann) <sup>a</sup>	Diptera: Tephritidae	100	
<i>Conotrachelus nenuphar</i> (Herbst)	Coleoptera: Curculionidae	92	92
<i>Copitarsia decolora</i> (Guenée) <sup>a</sup>	Lepidoptera: Noctuidae	100	
<i>Cryptophlebia ombrodelta</i> (Lower)	Lepidoptera: Tortricidae	250	
<i>Cryptophlebia illepidia</i> (Butler)	Lepidoptera: Tortricidae	250	
<i>Cylas formicarius</i> (F.)	Coleoptera: Brentidae	150	
<i>Cydia pomonella</i> (L.)	Lepidoptera: Tortricidae	200	200
<i>Euscepes postfasciatus</i> (Fairmaire)	Coleoptera: Curculionidae	150	
<i>Grapholita molesta</i> (Busck)	Lepidoptera: Tortricidae	200	232 <sup>b</sup>
<i>Omphisa anastomosalis</i> (Guenée)	Lepidoptera: Pyralidae	150	
<i>Pseudaulacaspis pentagona</i> (Targioni-Tozzetti) <sup>a</sup>	Hemiptera: Diaspididae	150	
<i>Rhagoletis pomonella</i> (Walsh)	Diptera: Tephritidae	60	60
<i>Sternochetus mangiferae</i> (F.)	Coleoptera: Curculionidae	300	
Tephritidae	Diptera: Tephritidae	150	150
Insecta except pupa and adult of Lepidoptera	Class Insecta	400	

<sup>a</sup> These pests were not considered for inclusion in the IPPC ISPM #28 in 2006 because they were not approved by APHIS until later.

<sup>b</sup> There are two treatments for *G. molesta*, in ambient and hypoxic atmospheres; although the dose is the same, the measures of efficacy are different.

radiated organisms should be kept alive under favorable conditions for reproduction until they eventually die. The APHIS-approved treatment for *B. chilensis* has not been used commercially.

After considering a dose of 100 Gy for *S. mangiferae*, APHIS set the dose at 300 Gy based on low confidence in research supporting 100 Gy (APHIS 2002). The dose of 300 Gy may be excessive and has not been used commercially.

The IPPC Technical Panel on Phytosanitary Treatments (TPPT) rejected the proposed treatments for *Anastrepha suspensa* (Loew), *Cryptophlebia ombrodelta* (Lower), and *C. illepidia* (Butler), the former species because the supporting research did not seem to use the most resistant stage (third instar) present in shipped fruit (Gould and von Windeguth 1991) and the latter two species because the research was done exclusively in diet without comparing efficacy on a plant host (Follett and Lower 2000).

Fifteen treatments were proposed for adoption by the TPPT, and eight survived the initial vetting process during member consultation where individual member countries (173 as of August 2010) and their plant protection organizations review and comment on the proposed standard. When there are no objections or unresolved issues, the standard is submitted to the IPPC Commission on Phytosanitary Measures for adoption. These eight treatments were adopted in 2009 (FAO 2009b,c). Three more were adopted after reexamination in March 2010 (FAO 2010).

A member country objected to setting the dose for *Grapholita molesta* (Busck) at 200 Gy because Hallman (2004a) recorded maximum absorbed doses in the large-scale tests confirming the treatment to be as

high as 232 Gy. This objection is a reasonable because the maximal dose recorded during research supporting a treatment should become the minimal dose required for commercial application.

Two treatments were proposed for *G. molesta*: one treatment for commodities stored in ambient atmospheres and one treatment for low oxygen storage. The effect of hypoxia on the efficacy of PI is discussed below. The measure of efficacy for PI in ambient atmosphere is prevention of emergence of the adult when stages no more advanced than larvae are irradiated. At the same dose in a low oxygen atmosphere, 5.3% of irradiated fifth-instar *G. molesta* emerged as normal-looking adults, although the females did not lay eggs and died within 8 d (Hallman 2004a). However, the fact that some adults emerged concerned some member countries because 1) no further metamorphosis is needed for the insects to reach the reproductive stage; and 2) live, although nonreproducing, individuals might be found in survey traps triggering restrictive and costly regulatory responses.

In March 2010, the CPM met and adopted both *G. molesta* treatments at 232 Gy (FAO 2010). The CPM concluded that although the treatment done in hypoxic atmospheres may result in the presence of live but irradiated adults, only a very small percentage of adults are likely to emerge after irradiation and that these adults are very unlikely to survive for >1 wk. Both factors greatly reduce the likelihood of adults being found in monitoring traps in importing countries.

A dose of 150 Gy was proposed for *Omphisa anastomosalis* (Guenée) based on research that allowed for adult emergence from irradiated late pupae but not

production of  $F_1$  adults (Follett 2006a). It is generally not possible to prevent adult emergence from late pupae of any insect at a dose tolerated by fresh commodities, so the most likely measure of efficacy will be prevention of reproduction. Member countries were concerned about adult *O. anastomosalis* being found in survey traps. In addition, the nonirradiated control did not reproduce as well as expected indicating that unknown factor(s) in addition to irradiation were repressing reproduction.

A dose of 92 Gy was proposed for *Conotrachelus nenuphar* (Herbst) based on research that allowed for  $F_1$  mid-instar to develop when adults were irradiated (Hallman 2003). In this case, adults were considered the most radiotolerant stage that could be present on transported fruit hosts and objection to the presence of live adults postirradiation was made. In March 2010, the CPM adopted the treatment concluding that, although the treatment may result in the presence of live but irradiated adults, they are rarely (if ever) present in shipped fruit and irradiated adults are very unlikely to survive for >1 wk (FAO 2010). Both factors greatly reduce the likelihood of adults being found in monitoring traps in importing countries.

Doses of 140 and 145 Gy, respectively, for *Cylas formicarius* (F.) and *Euscepes postfasciatus* (Fairmaire) were based on exposure of adults to radiation with no  $F_1$  adults resulting (Follett 2006a). Objection was made to the presence of live adults post irradiation and the uncertainty as to how far the  $F_1$  generation would develop after irradiation. Prevention of the  $F_1$  adult as the measure of efficacy of PI does not leave much of a margin of security. These two proposed treatments have been forwarded to the IPPC Standards Committee for further review.

PI treatments have been applied generically with regard to host and also pest to a very large extent. Irradiation treatments in the IPPC phytosanitary treatment standard (FAO 2009b) apply to all fruits and vegetables. APHIS (2010b) has approved irradiation as a phytosanitary treatment for all admissible fresh fruits and vegetables from all countries. The IPPC has approved a generic dose of 150 Gy for all Tephritidae (FAO 2009c), whereas APHIS (2010b) has approved the same dose for tephritids as well as a 400-Gy dose for Insecta except pupae and adults of Lepidoptera, which may require higher doses. The generic dose of 400 Gy did not survive the IPPC vetting process because it was considered excessive extrapolation for the data that had been accumulated so far. Also, the presence of live adults among most quarantine pests in that group (all Insecta except pupae and adults of Lepidoptera) was objectionable to some countries. Limiting phytosanitary irradiation treatments to cases where live adults will not be present after treatment would greatly restrict the applicability of this technology to instances where tephritids and some Lepidoptera were the only quarantine pests that could be present on transported commodities.

The objective of this review is to examine several factors related to PI, determine which have sufficient information to determine whether they affect efficacy

and which need further research in light of the fact that irradiation does not have an independent method of verifying efficacy. The impact of these factors on PI research and application to generic treatments is discussed.

### Phytosanitary Irradiation Variables

Several variables have been noted from the literature to possibly affect PI efficacy (Hallman 2000, 2001). The more compelling of these are examined in greater detail below.

**Effect of Low Oxygen on Efficacy.** Hypoxia during irradiation was observed to reduce phytosanitary efficacy for tephritids in vitro by Balock et al. (1963) and substantiated in a number of instances since then (Hallman and Hellmich 2010). Hallman (2004b) showed that the reduction is much less for tephritids in fruit compared with in vitro probably because in fruit the larvae are already in a low oxygen environment.

International standards prohibit phytosanitary radiation to commodities from low oxygen storage except for product at risk of *Rhagoletis pomonella* (Walsh), which was studied under hypoxic conditions (Hallman 2004b, FAO 2009b). However, APHIS (2010b) does not prohibit commercial irradiation of product stored in low oxygen.

More research is needed to decide under what circumstances low oxygen storage might be a detriment to phytosanitary radiation and how to regulate commodities stored as such. This is the most significant recognized factor affecting efficacy of PI after dose itself. Hypoxic conditions might occur under storage regimes that do not necessarily use low oxygen by design, such as packaging restrictive of gas flow. Low oxygen itself is a phytosanitary treatment but usually at levels that are lower than those in storage (Neven et al. 2009).

**Most Radiation Tolerant Stage.** A phytosanitary treatment should be demonstrated effective against the most tolerant stage present on the regulated article and it follows that it will be effective against the other stages that might be present. Although it is generally accepted that radiotolerance in insects increases as they develop and is frequently cited as such, this fact has not been robustly summarized for the measures of efficacy used in PI.

Table 2 presents relative radiotolerances of stages of organisms for measures of efficacy used in PI. We include all published studies where three or more stages can be compared, with stage being defined liberally as any specific period of development. The studies support the hypothesis that radiotolerance increases as insects develop. There are several exceptions; some may be dismissed but some cannot, given the information in the respective publication. Other studies not included in Table 2 report relative differences among only two stages or report differences in a way not easily summarized (e.g., Hallman 2003). Still, none of these other studies contradict the hy-

Table 2. Radiotolerances of different stages (where three or more stages can be compared) of arthropods to measures of efficacy that are used as objectives for phytosanitary irradiation treatments

Order: Family	Organism	Measure of efficacy; prevention of	Stages tested in order of development with least developed first (min. dose [Gy] to achieve near 100% efficacy) <sup>a</sup>	Reference
Acar: Acarididae	<i>Acarus siro</i> (L.)	Adult	Egg (>450); larva (450); Hypopus (1,000)	Burkholder et al. (1966)
Coleoptera: Anobiidae	<i>A. siro</i>	F <sub>1</sub> adult	Egg (250); larva (250); Hypopus (450); adult (<450)	Burkholder et al. (1966)
	<i>Lasioderma serricorne</i> (F.)	Adult	Egg 1 d (10), 2 d (15), 3–4 d (>20); larva 5 d (25), 10 d (25), 15 d (>25)	Harwalkar et al. (1995)
Coleoptera: Bostrichidae	<i>L. serricorne</i>	Adult	Egg 1–2 d (50), 5–6 d (100); larva (100); pupa (>200)	Inai et al. (2006)
	<i>Rhyzopertha dominica</i> (F.)	Adult	Egg 2–4 d (40); larva 14–16 d (80); pupa (>200)	Martin and Hooper (1974)
Coleoptera: Bruchidae	<i>R. dominica</i>	Reproduction	Egg 2–4 d (30); larva 14–16 d (40); pupa (140); adult 4–7 d (115)	Martin and Hooper (1974)
	<i>Callosobruchus maculatus</i> (F.)	Adult	Egg 0–1 d (5), 2 d (10), 3 d (15); larva 1–10 d (20), 11 d (100), 12 d (>150)	Dongre et al. (1997)
Coleoptera: Curculionidae	<i>C. maculatus</i>	Adult	Egg (20); larva early (20), middle (30), late (60); pupa (140)	Diop et al. (1997)
	<i>Anthonomus grandis</i> Boheman	Reproduction	Instar 3 (19); pupa early (22), middle (>28), late (>28); adult (45)	Flint et al. (1966)
Coleoptera: Tenebrionidae	<i>Sitophilus granarius</i> (L.)	Reproduction	Egg (30), larva (30), pupa (70), Young adult (100), Old adult (70)	Aldryhim and Adam (1999)
	<i>Palorus subdepressus</i> (Wollaston)	Adult	Egg (50); larva (100); pupa (1000)	Brower (1973a)
Diptera: Tephritidae	<i>Anastrepha ludens</i> (Lowe)	Adult emergence	Instar 3 feeding (16), postfeeding (16); Prepupa (14); pupa cryptocephalic (18), phanerocephalic 1 d (22), 2 d (25); adult pharate 1 d (35), 2 d (100), 3 d (250)	Hallman and Worley (1999)
	<i>Anastrepha obliqua</i> (Macquart)	Adult emergence	Instar 3 (50); puparium 1 d (20), 10 d (300)	Arthur et al. (1994)
	<i>A. obliqua</i>	Adult emergence	Instar 3 (14); Prepupa (14); pupa (14); adult pharate 1 d (20)	Hallman and Worley (1999)
	<i>Bactrocera cucurbitae</i> (Coquillett) <sup>b</sup>	Adult emergence	Egg (>33); larva 1 d (>46), 2 d (>45), 3 d (>50), 4 d (>45), 5 d (>41); puparium 1 d (>32), 2 d (>42), 3 d (>61), 4 d (>270), 5 d (>450), 6 d (>665), 7 d (>830), 8 d (>1170), 9 d (>1215)	Balock et al. (1963)
	<i>Bactrocera dorsalis</i> (Hendel) <sup>2</sup>	Puparia	Egg 1 d (>120); larva 1 d (>280), 2 d (>470); instar 3 (>930)	Balock et al. (1963)
	<i>B. dorsalis</i> <sup>b</sup>	Adult emergence	Egg (>26); larva 1 d (>27), 2 d (>25), 3 d (>32), 4 d (>34), 5 d (>35), 6 d (>33); puparium 1 d (>24), 2 d (>27), 3 d (>30), 4 d (>180), 5 d (>290), 6 d (>420), 7 d (>570), 8 d (>680), 9d (>1100), 10 d (>1400)	Balock et al. (1963)
	<i>B. dorsalis</i>	Adult emergence	Egg (25); instar 1 (25), 2 (25), 3 (>80)	Vijayasegaran et al. (1992)
	<i>Bactrocera trijoni</i> (Froggatt)	Adult emergence	Egg (<50); larva young (50), old (75)	Rigney and Willis 1983
	<i>B. trijoni</i>	Adult emergence	Egg (<75); larva young (75), old (75)	Jessup et al. (1992)
	<i>Ceratitis capitata</i> (Wiedemann)	Adult emergence	Egg 1–24 h (10), 48 h (20); larva 2–4 d (20), 8 d (40)	Masour and Franz (1996)
	<i>C. capitata</i> <sup>d</sup>	Adult emergence	Egg 1 d (>14), 2 d (>22); larva 1 d (>18), 2 d (>20), 3 d (>22), 4 d (>23), 5 d (>25), 6 d (>25); puparium 1 d (>17), 2 d (>16), 3 d (>23), 4 d (>150), 5 d (>255), 6 d (>370), 7 d (>470), 8 d (>560), 9d (>840), 10 d (>980)	Balock et al. (1963)
	<i>C. capitata</i>	Pupariation	Egg 2–4 h (10), 27–30 h (20), 45–49 h (240); larva 1 d (240), 3 d (360), 5 d (840), 7 d (1000)	Sheta (1983)
Hemiptera: Diaspididae	<i>Pseudaulacaspis pentagona</i> (Targioni-Tozzetti)	Gravid F <sub>1</sub> female	Instar 2 (90); adult female (60), gravid female (120)	Follett (2006c)
Hemiptera: Pentatomidae	<i>Nezara viridula</i> (L.)	Egg hatch	Egg 0 d (5), 1 d (10), 2 d (10), 3 d (>10), 4 d (50), 5 d (>50)	Mau et al. (1967)
Hemiptera: Pseudococcidae	<i>Planococcus minor</i> (Maskell)	F <sub>1</sub> egg hatch	Egg (150); Nymph (150); adult (150)	Raviwasa et al. (2009)
Lepidoptera: Gelechiidae	<i>Pluthecia operculella</i> Zeller	Adult	Larva 1–1.5 d (100), 6–6.5 d (100), 12–12.5 d (125)	Saour and Makee (2004)
	<i>P. operculella</i>	Adult	Instar 4 (120); pupa 1–3 d (400), 6–8 d (700)	Al-Taweel et al. (2007)
	<i>Sitotroga cerealella</i> (Olivier)	Adult	Egg (175); larva (175); pupa (>1000)	Cogburn et al. (1966)

Table 2. Continued

Lepidoptera: Noctuidae	<i>Heliothis virescens</i> (F.)	Adult	Larva 1 d (<100), 6 d (100), 9 d (>100), 15 d (>100)	El Sayed and Graves (1969)
Lepidoptera: Pyralidae	<i>Spodoptera litura</i> (Fabr.)	Pupation	Egg 3 d (<200), 4 d (200); instar 3 (200), 5 (400)	Dohlino et al. (1996a)
	<i>Anagasta kuehniella</i> (Zeller)	Adult	Egg (200); larva 7 d (200); instar 5 (250)	Ayvaz and Tunçbilek (2006)
	<i>A. kuehniella</i>	F <sub>1</sub> egg production	Egg (200); larva 7 d (200); instar 5 (150); pupa 5 d (>350); adult (>550)	Ayvaz and Tunçbilek (2006)
	<i>A. kuehniella</i>	F <sub>1</sub> egg hatch	Egg (150); instar 5 (250); pupa 5 d (300); adult (300)	Ayvaz and Tunçbilek (2006)
	<i>Cadra cautella</i> (Walker)	Adult	Egg 3 d (300), instar 5 (200), pupa 3–4 d (1000)	Cogburn et al. (1973)
	<i>Corcyra cephalonica</i> (Staunton)	Adult	Egg 0 d (10), 1 d (25), 2 d (75), 3 d (250), 4 d (250), instar 1 (100), 5 (200)	Huque (1971)
Lepidoptera: Tortricidae	<i>Plodia interpunctella</i> (Hbner)	Adult	Egg (175); larva (132); pupa (>1000)	Cogburn et al. (1966)
	<i>Anorbis emigratella</i> Busck	Adult	Instar 1 (90), 2–3 (120), 4–5 (>150); pupa (>150)	Follett (2008)
	<i>Cryptophlebia illepidia</i> (Butler)	Adult	Egg 3 d (125); instar 1 (<250), 2–3 (125), 4–5 (>250); pupa (>250)	Follett and Lower (2000)
	<i>C. illepidia</i>	Reproductive females	Egg 3 d (62.5); instar 1 (125), 2–3 (62.5), 4–5 (125); pupa 1–5 d (125), 7–8 d (>250)	Follett and Lower (2000)
	<i>C. illepidia</i>	Oviposition	Instar 1 (125), 2–3 (62.5), 4–5 (125); pupa 1–5 d (125), 7–8 d (>250)	Follett and Lower (2000)
	<i>Ctenopseustis obliquana</i> (Walker) <sup>c</sup>	Pupation	Egg 1 d (81), 5 d (159); instar 1 (193), 3 (192), 5 (215)	Lester and Barrington (1997)
	<i>C. obliquana</i> <sup>d</sup>	Adult	Egg 1 d (42), 5 d (111); instar 1 (150), 3 (126), 5 (117)	Lester and Barrington (1997)
	<i>C. obliquana</i> <sup>d</sup>	Oviposition	Egg 5 d (36); instar 1 (55), 3 (65), 5 (70)	Lester and Barrington (1997)
	<i>Cydia pomonella</i> (L.)	F <sub>1</sub> egg hatch	Instar 1 (93); pupa early (>70), mid (>233), late (>400); adult (400)	Proverbs and Newton (1962)
Thysanoptera: Thripidae	<i>Retithrips syriacus</i> (Mayet)	F <sub>1</sub> adult	Egg (100); larvae (100); pupae (150); adult (150)	Bhuiya et al. (1999)
	<i>R. syriacus</i>	Adult	Egg (25); larvae (50); pre- and pupae (500)	Majumder (2001)
	<i>R. syriacus</i>	Fertility <sup>d</sup>	Egg (25); larvae (25); pre- and pupae (50); adult (50)	Majumder (2001)
	<i>R. syriacus</i>	Oviposition	Larvae (25); pre- and pupae (50); adult (>500)	Majumder (2001)
	<i>Thrips palmi</i> Karny	Reproduction	Egg (100); instar 2 (100); adult (>200)	Dohlino et al. (1996b)
	<i>Thrips tabaci</i> Lindeman	Reproduction	Egg (100); instar 2 (100); adult (<400)	Dohlino et al. (1996b)

<sup>a</sup> Doses are for relative comparisons within rows because they are nominal (dosimetry sometimes not reported) and other differences among studies caution against direct comparisons.

<sup>b</sup> Estimates of 95 percentile (hence, "greater than" added to achieve 100%); actual data not given.

<sup>c</sup> Estimates of 99 percentile; actual data not given.

<sup>d</sup> Reported as "sterility" without identifying what was measured.



pothesis that insects increase in radiotolerance as they develop for measures of efficacy used in PI.

Several apparent exemptions to the rule that radiotolerance increases as insects develop are discussed below. In a daily analysis of radiotolerance of two tephritids Balock et al. (1963) found that radiotolerance did not increase steadily with increasing development, but increased in a somewhat zigzag manner. Results are estimates for the 95th percentile and not the actual data. Taken as distinct developmental stages, though, the flies increase in radiotolerance as they develop.

A reason Arthur et al. (1994) found the *Anastrepha obliqua* (Macquart) prepupa to be more susceptible than the third instar (Table 2) may be that because they were irradiated in sealed tubes the more active larvae depleted the oxygen in the tube to a greater extent than the prepupae, increasing radiotolerance of the former. Hallman and Worley (1999) found no difference in radiotolerance in vitro between third instar and prepupal *A. obliqua*.

Lester and Barrington (1997) found that larval *Ctenopseustis obliquana* (Walker) (Tortricidae) decreased in radiotolerance as they developed when measured as prevention of adult emergence but not when measured as prevention of pupation or oviposition (Table 2). These observations are 99th percentile estimates and not the actual data, and there is some overlap in 95% confidence intervals. Follett and Lower (2000) found that mid-instar *Cryptophlebia illepidia* (Butler) were more susceptible than first instars regardless of the measure of efficacy (Table 2).

Nymphal *Pseudaulacaspis pentagona* (Targioni-Tozzetti) were more radiotolerant than adults for prevention of gravid  $F_1$  (Follett 2006c). Sixty gray applied to second-stage nymphs ( $n = 172$ ) and pregravid adults ( $n = 358$ ) resulted in 237 and 0 gravid  $F_1$ , respectively.

We found four exceptions with stored product pests. A higher dose was required to prevent reproduction in pupal *Rhyzopertha dominica* (F.) than adult (Matin and Hooper 1974). However, adults were irradiated with a different source (an X-ray therapy unit) than immatures (gamma from cobalt 60); it is not clear that the same doses were being absorbed by adults versus immatures. A higher dose was required to prevent egg stage *Cadra cautella* (Walker) from reaching the adult stage than fifth instars (Cogburn et al. 1973). However, this is probably an anomaly as only one egg reached the adult stage at 200 Gy, whereas at the next lowest dose (100 Gy), 0.8 and 10.9% eggs and larvae, respectively, reached the adult stage, showing the fifth instar to be more tolerant than the egg stage. The fifth (final) instar *Anagasta kuehniella* (Zeller) was more susceptible than a younger (7-d-old) larva when measured as  $F_1$  egg production at a series of doses from 50 to 150 Gy (Ayvaz and Tuncbilek 2006). Fate of the eggs laid by irradiated 7-d-old larvae is not reported. When measured as adult emergence, the fifth instar was more tolerant than 7-d-old larvae. When measured as development to the adult stage,

2–3-d-old *Plodia interpunctella* (Hübner) eggs were more tolerant than last instars (Cogburn et al. 1966).

Of these exceptions in Table 2 to the axiom that radiotolerance in insects increases as they develop, three that are not easily dismissed are *C. illepidia*, *P. pentagona*, and *P. interpunctella*. However, in the first two cases the most developed stages that could be present on transported commodity, fifth instar and gravid female, respectively, are more tolerant than any of the other stages present, resulting in no increased risk to quarantine security if they were truly exceptions (Follett and Lower 2000, Follett 2006c). *P. interpunctella* is not a quarantine pest; however, as an example of a pyralid, the measure of efficacy may be prevention of reproduction by late pupae (pyralids often pupate in shipped commodity), which required a higher dose for pupae than eggs or larvae (Cogburn et al. 1966).

Some research demonstrates that, although radiotolerance may increase as an insect develops, old adults may be more susceptible than young adults (e.g., Aldryhim and Adam 1999), indicating that actively reproducing adults should be used in phytosanitary research and not adults past their prime reproductive age.

Cryptically-feeding Curculionidae that occur as immatures inside the host plant represent a potentially special case regarding most radiotolerant stage. Immatures may be in hypoxic atmospheres, possibly increasing their radiotolerance in the same manner as occurs with tephritids (Hallman and Loaharanu 2002). It could be reasonable to hypothesize that adults treated in vitro could be more susceptible. However, Hallman (2003) found that third-instar *Conotrachelus nenuphar* (Herbst) cryptically infesting immature apples (*Malus* spp.) were more susceptible than adults outside of apples.

**Effect of Host on Efficacy.** Another characteristic that has been largely accepted in radiation treatments but that has not been adequately analyzed is that host does not affect efficacy. International standards state that radiation treatments are valid for all fruits and vegetables that are hosts for the given pests (FAO 2009b, 2010). APHIS (2005) states that “Specific characteristics of the fruits and vegetables being treated . . . are irrelevant to the effectiveness of irradiation as long as the required minimum dose is absorbed.”

Gould and von Windeguth (1991) concluded that three different hosts of *Anastrepha suspensa* (Loew) required different doses (50–150 Gy) to achieve the same level of quarantine security. Hallman and Loaharanu (2002) successfully argued that variations in the research allegedly supporting different doses among these hosts do not in fact support different doses and that 70 Gy is sufficient (Table 1).

Apparent differences in dose among various studies of *Ceratitis capitata* (Wiedemann) that could be hypothesized at least in part as due to host are not consistent (Torres-Rivera and Hallman 2007). For example, doses for orange range from 40 to >200 Gy and doses for papaya range from 100 to 225 Gy. The wide variation in doses for *C. capitata* can more logically be

explained by irregularities in some of the research, and a dose of 100 Gy should suffice (APHIS 2010a).

A few studies directly compare hosts for measures of efficacy used in PI (acute mortality is not considered). When corrected for control emergence, adult emergence from *Bactrocera tryoni* (Froggatt) larvae irradiated with 40 Gy in meridic diet, orange, *Citrus sinensis* L., and apple, *Malus domestica* Borkh., was 0, 0.34, and 0.15%, respectively (Macfarlane 1966). *Bactrocera tryoni* third instars infesting oranges and avocados, *Persea americana* Mill, responded to radiation dose equally (Rigney and Wills 1983). Jessup et al. (1992) report on disinfestation of six fruits of *B. tryoni* third instars and found that 50 Gy prevented 98.6–100% adult emergence except for mangoes, *Mangifera indica* L. (87.1% adult emergence at 50 Gy). However, although these hosts were reported in the same article, it was a compilation of several years of research that may not have been done under the same circumstances. Regardless, all were disinfested at 75 Gy, although sample size for mangoes was small (504 larvae versus 2,891–220,328 for the others), yielding a low level of confidence that 75 Gy would provide complete control in that fruit. Heather et al. (1991) demonstrated a high level of quarantine security against *B. tryoni* in mangoes with a dose of 74–101 Gy. The target dose was 75 Gy, but dosimetry showed some doses to be as high as 101 Gy. It is possible that dose reports of 75 Gy in Jessup et al. (1992) were targets and that both studies used the same irradiation facility, thus demonstrating that *B. tryoni* in mangoes are controlled with a dose not far from other fruits studied by Jessup et al. (1992).

There was no significant difference in completion of development for irradiated eggs and larvae of *Tribolium confusum* Jacquelin du Val reared on wheat (*Triticum* sp.); barley, *Hordeum vulgare* L.; and corn, *Zea mays* L., flour (Tunçbilek and Kansu 1966). Mansour and Franz (1996) found no difference between *C. capitata* larvae reared and irradiated in orange (*Citrus* sp.) and peach, *Prunus persica* (L.) Batsch.

Apparent differences between studies can often be explained by deficiencies in research, although it may not be possible to prove. Tephritid larvae treated in open diet are controlled with lower doses than in fruit (Macfarlane 1966, Hallman and Loaharanu 2002). These differences may be due in part to differences in atmospheric content in vitro versus in fruit. Some tephritid research has been conducted by rearing third instars in diet and inserting them in fruit  $\approx 1$  d before irradiation without comparing the results of this technique to more natural infestation (Mansour and Franz 1996, Follett and Armstrong 2004). Such a comparison should be made before an unnatural infestation technique is used to develop a phytosanitary treatment (Hallman and Thomas 2010).

Differences in efficacy for diet versus a plant host were not found for the lepidopterans *G. molesta* or *Ostrinia nubilalis* (Hübner) and insects reared on diet were then used to develop treatments (Hallman 2004a, Hallman and Hellmich 2009).

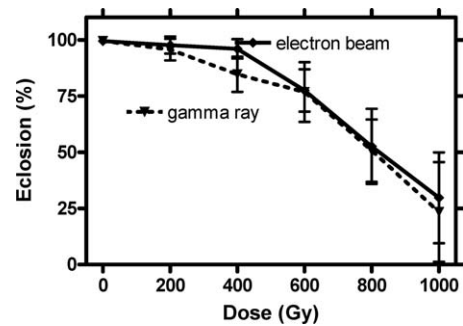


Fig. 1. Eclosion of *T. urticae* eggs irradiated when 4 d old (after Dohino et al. 1994). Dose rate for electron beam was several hundred times that for gamma ray.

Host does not seem to affect efficacy for the measures of efficacy used for PI treatments (prevention of development or reproduction). However, direct comparisons among hosts are few and comparisons of different studies sometimes yield quite variable results which may or may not have anything to do with host. Also, there is considerable difference in efficacy of irradiation of tephritids in diet versus fruit, and the reason for that difference should be examined as it may relate to host.

**Effect of Dose Rate on Efficacy.** The possible effect of dose rate on efficacy and commodity quality has not been considered when approving PI treatments. Hallman (2000) reviewed some articles on this subject which indicated that dose rate may be directly related to damage to pests and commodities. Results from other articles follow. Bletchly (1961) reported no difference between two dose rates (0.5 and 11.5 Gy/min) at doses (60–80 Gy) that prevented adult emergence from irradiated *Anobium punctatum* (De Geer) middle-aged larvae. Gonen and Calderon (1973) found that dose rate was directly related to male reproductive sterility of *E. cautella* when the dose–rate ratio was as little as 3:1; egg hatch was 15 and 1% for the lower and higher dose rates, respectively.

Dohino et al. (1994) compared electron beam to gamma ray (cobalt 60) for effect on eclosion of *Tetranychus urticae* Koch eggs and found no significant difference (Fig. 1). This example is an extreme example of dose rate differences because the entire radiation dose via electron beam is applied to a given spider mite egg in a fraction of a second, whereas it takes several minutes to apply the same dose via cobalt 60. Given that a spider mite egg is  $\approx 0.14$  mm in diameter, and the speed of the electron beam conveyor is 3.0 m/min, any given egg was irradiated in  $\approx 0.17$  s via electron beam, whereas cobalt required 15 min to deliver 1 kGy to any given egg. Therefore, at the largest dose (1 kGy), which resulted in  $\approx 30\%$  eclosion, the dose rate for electron beam was  $15.0 \text{ min} (60 \text{ s/min}) / 0.17 = \approx 5,300$  times the dose rate for cobalt 60. However, the beam is not as narrow as the diameter of a spider mite egg, so the dose via electron beam was probably delivered only several hundred times faster than the dose via cobalt 60.

Development of *Callosobruchus maculatus* (F.) adults from irradiated larvae and pupae was completely prevented with 25 Gy applied at a rate of 23.6 Gy/min, whereas 100 Gy was required at 0.9 Gy/min (Fontes et al. 2003).

Hatch of eggs laid by irradiated (50 Gy) *Tribolium castaneum* (Herbst) adults was 1.1 and 0% at 0.5 and 23.3 Gy/min, respectively (Nair and Subramanyam 1963).

Until recently, most phytosanitary radiation research was conducted using isotopic sources (cobalt 60 and cesium 137) with slow to modest dose rates that are usually exceeded by commercial application. In that case, efficacy of a commercial application may exceed the efficacy level used in the research. However, in recent years research has been performed with machine sources with very high dose rates. If this research is applied using slower commercial isotopic sources, there is reason to hypothesize that the level of efficacy might be inferior in the isotopic commercial application. Therefore, the effect of dose rate on efficacy should be tested.

**Effect of Temperature on Efficacy.** Cold may reduce the efficacy of sanitary applications of radiation (Dickson 2001); however, that generally occurs when the product is frozen, which is never the case for phytosanitary applications. Few and inconclusive studies addressed the effect of temperature on efficacy of phytosanitary radiation when it was reviewed by Hallman (2000). Research done since then found that temperature did not affect efficacy for a tephritid and a crambid within the range used for cold storage of fresh commodities and ambient temperatures (Hallman 2004b, Hallman and Hellmich 2009). A few more studies are warranted before a definite conclusion should be made.

**Variability for Radiosusceptibility Among Populations.** In general, phytosanitary treatments have been considered efficacious against all populations of an approved species, and irradiation is no exception. However, because radiation treatments have no independent verification of efficacy it would be more crucial to know whether differences among populations exist for irradiation. Taken at face value, the literature seems to indicate differences in radiotolerance among populations of the same insects. Torres-Rivera and Hallman (2007) examine studies that suggest control doses for different populations of *C. capitata* infesting the same fruit range from 40 to 200 Gy. However, because these studies were done by different researchers, factors other than genotype may be responsible for apparent differences.

Several studies directly compared known populations of the same species for radiotolerance. Because acute mortality is not a measure of efficacy for phytosanitary radiation, we ignore studies comparing mortality and instead report studies comparing metamorphosis or reproduction, measures of efficacy for phytosanitary radiation.

Eggs laid by a population of *T. castaneum* selected for increased pupal weight (2.5× normal) were significantly more radiosusceptible than the source popu-

lation when irradiated as adults (Bartlett and Bell 1962). At 50 Gy 2.3 and 10.4%, respectively, of eggs of the large and normal-size populations hatched.

Although Cornwell (1966) noted marked differences in mortality among 35 irradiated strains of *Sitophilus granarius* (L.), only insignificant differences were found for fertility.

Reproduction of two malathion-resistant and one susceptible strain of *P. interpunctella* was reduced similarly (≈44%) when irradiated with 50 Gy (Brower 1973b). Likewise a strain of *T. castaneum* resistant to DDT and malathion and a susceptible strain were both reduced to ≈31% of reproduction when irradiated with 20 Gy (Brower 1974). Phosphine-resistant *T. castaneum* was significantly more tolerant than a susceptible strain when measured as adult emergence from irradiated larvae (Nakakita et al. 1985); there was no difference between the two strains regarding reproduction of irradiated adults.

Chung et al. (1971) reported a difference in reproduction between field and laboratory-sourced chafers *Rhizotrogus majalis* (Razoumowsky), although both sources were collected close together in time in the same place and were most likely the same genotype, indicating other reasons than genetics for the difference. They suggest that the irradiated adults of both sources were of different ages when irradiated, which could account for the difference.

Hallman (2003) found that reproduction of diapausing northern strain of *Conotrachelus nenuphar* (Herbst) was prevented with half the dose required for the nondiapausing southern strain. However, the two strains are somewhat reproductively incompatible and possibly should be treated as different species. Also, insects in diapause may be more susceptible to radiation than ones not in diapause (Hallman 2000).

Twenty-five gray applied to cage (laboratory) and field-infested peaches, *P. persica*, resulted in 5.0 and 4.4%, respectively, *C. capitata* adult emergence in two separate studies done during the same time period with the same methodology (Arthur et al. 1993a,b). Adult emergence for the cage-infested flies should probably be slightly lower as the percentage emerged is based on the number of puparia forming and not larvae irradiated, which is not given; this pair of studies shows no difference between wild and laboratory *C. capitata*. Follett and Armstrong (2004) found laboratory and wild strains of three species of tephritids (including *C. capitata*) to respond the same when measured as adult emergence from irradiated third instars.

The aggregate of studies where direct comparisons are made between genotypes of the same species does not support significant differences in response to radiation. A few more studies may be warranted because those done do not represent a broad range of taxa (mainly tephritids and stored product pests from Coleoptera and Lepidoptera).

Although increased tolerance to radiation has been bred in the laboratory (e.g., Enfield et al. 1983), this increase would not likely happen for phytosanitary applications in nature because the dose used should



leave essentially no survivors to reproduce and, even if it did, they would usually be exported and thus removed from the population. Also, the degree of induced increase in radioresistance achieved has been small compared with resistance to pesticides.

Our conclusion is that variations among populations for resistance to radiation, if they exist, are probably not significant. However, it may always be prudent to use wild material when researching phytosanitary treatments, including irradiation. Populations that show distinct biological differences, such as northern and southern strains of *C. nenuphar* (Hallman 2003), possibly should be treated as distinct species for phytosanitary research purposes.

### Generic Treatments

The generic phytosanitary treatment concept is that one specific treatment is used for a group of quarantine pests and/or commodities, although not all were tested for efficacy. Although it has been applied to various treatments, such as cold storage and fumigation, it is applied to a much broader degree with irradiation (Heather and Hallman 2008). A generic treatment should contain a margin of error to cover untested pests that might require higher doses than those for pests in the group that were tested. The generic dose of 150 Gy for all Tephritidae was accepted by APHIS in 2006 and the IPPC in 2009. Also, in 2006, APHIS accepted a generic dose of 400 Gy for all Insecta minus pupae and adults of Lepidoptera.

Generic treatments should be developed for groups of pests for which it is feasible that they will be commercially used. The generic dose of 150 Gy for Tephritidae is used for mangoes and citrus fruit exported from Mexico to the United States. In several other cases (guavas from Mexico to the United States, mangoes from India to the United States, dragon fruit, *Hylocereus undatus* (Haworth) Britton & Rose, from Vietnam to the United States, and several fruit from Thailand to the United States) the APHIS-approved generic treatment of 400 Gy is used because insects besides fruit flies may be present. Australia sends mangoes and litchi to New Zealand using a generic treatment of 250 Gy for Insecta (MAF 2009).

Virtually any small organism feeding on the part of a commodity that is exported can be a quarantine pest to any part of the world that does not have that organism and where it could become established. Additional organisms may be found on commodities as 'contaminating pests' that may be carried by a commodity but do not feed on it (FAO 2009a). Two common examples intercepted in the United States are the khapra beetle, *Trogoderma granarium* Everts, with brass items from Asia and terrestrial Gastropoda with ceramic tiles from the European Union.

Although a single generic dose for all invertebrate quarantine pests would be useful, it would necessarily be set at the minimum absorbed dose required for the most tolerant organism within that group, which could require at least 500 Gy (Hallman and Phillips 2008). Although many commodities tolerate 500 Gy they may

not tolerate the maximum dose that will be absorbed by the commodity when a minimum dose of 500 Gy is sought, which could be at least twice that dose (Heather and Hallman 2008). Therefore, viable generic doses for groups of quarantine pests that would be <500 Gy should be sought.

A reduction in dose also can lead to savings in application time and costs. For example, Follett (2009) reports that a reduction in treatment dose for Hawaiian sweet potato, *Ipomoea batatas* (L.) Lam., from 400 to 150 Gy resulted in a 60% reduction in cost of treatment.

Weevils. Cryptic feeders cannot be easily found and culled upon inspection without damaging the fruit. Therefore they will usually require a treatment if there is more than a negligible risk of their presence. Curculionidae and Brentidae form such a group of cryptic quarantine pests. Fresh commodity-infesting weevils usually have narrow host ranges, thus most commodities are not hosts in most of their growing ranges, so they are not as omnipresent as Tephritidae in their hosts. A generic treatment for these weevils could be as low as 150 Gy (Heather and Hallman 2008).

Three weevils discussed above (*C. nenuphar*, *C. formicaries*, and *E. postfasciatus*) have been studied to the degree required for phytosanitary treatments, but only one weevil survived IPPC vetting so far. Before a generic treatment for these weevils can be seriously considered more species must be studied with large numbers (tens of thousands) of individuals. Good candidate species for research are those infesting fruits for which irradiation at 400 Gy is presently applied: *Conotrachelus psidii* Marshall and *Conotrachelus dimidiatus* Champion in guava from Mexico, and *Sternonchetus frigidus* (F.), *Sternonchetus mangiferae* (F.), and *Sternonchetus olivieri* (Faust) in mango from India and Thailand.

Lepidoptera Larvae. A generic treatment for Lepidoptera that infest regulated articles only in the egg or larval stages would be useful. Although the larvae leave feeding holes visible on the outside of a commodity in which they bore, they may not be effectively eliminated by culling; thus, postharvest phytosanitary treatments are often required. This group would include the families Crambidae, Gracillariidae, Lycaenidae, Noctuidae, Pyralidae, and Tortricidae. The measure of efficacy could be the same as for Tephritidae: prevention of emergence of normal-looking adults from irradiated eggs and larvae. The final instar is the most radiotolerant stage. Candidate species for research are the following quarantine pests on commodities already using PI at a dose of 400 Gy: *Deanolis sublimbalis* Snellen (Pyralidae) in mango from India; *Gymnandrosoma aurantianum* Lima (Tortricidae) in guava from Mexico; *Conopomorpha sinensis* (Bradley) (Gracillariidae), *Cryptophlebia ombrodelta* (Lower) (Tortricidae) and *Deudorix epijarbas* (Moore) (Lycaenidae) in longan (*Dimocarpus longan* Lour.) and lychee (*Litchi chinensis* Sonn.) from Thailand; and *Conopomorpha cramerella* (Snellen) (Gracillariidae) in rambutan (*Nephelium lappaceum* L.) from Thailand. A reasonable target for a generic dose for Lep-

idoptera eggs and larvae is 250 Gy (Heather and Hallman 2008).

Those Lepidoptera that pupate in the transported commodity will not be included in this generic dose and may require higher doses. For example, >300 Gy was required to prevent F<sub>1</sub> egg hatch from irradiated *Ostrinia nubilalis* (Hübner) late pupae (Hallman and Hellmich 2009). However, a lower dose could be used if efficacy were measured later than F<sub>1</sub> egg hatch. In the above-mentioned example prevention of F<sub>1</sub> pupation was accomplished with <250 Gy.

**Sternorrhyncha or Coccoidea.** Hemipterans of the families Aleyrodidae, Coccidae, Diaspididae, and Pseudococcidae are external feeders that may not be readily removed from commodities during the packing process and may require postharvest phytosanitary treatments. A generic treatment comprising at least these four families might be ≈250 Gy, although Heather and Hallman (2008) suggest a dose for some non-Coccoidea in the Sternorrhyncha (Aleyrodidae and Aphididae) might be as low as 100 Gy. Many aleyrodids, coccids, diaspids, and pseudococcids are quarantine pests of commodities currently treated by the APHIS generic dose of 400 Gy and would be prime candidates for research aimed at lowering the doses applied to host commodities (APHIS 2006, 2007, 2008b,c; BA 2008). The presence of live adults postirradiation must be broadly accepted for a generic treatment for this group to be viable.

**Acari.** Plant-infesting Acari of the families Eriophyidae, Tarsonemidae, Tenuipalpidae, and Tetranychidae are quarantine pests for which postharvest phytosanitary treatments are frequently required. The dose for these pests could be at least 350 Gy (Heather and Hallman 2008); therefore, a generic treatment including Acari would probably suffice for all Insecta as well except for certain adults of Lepidoptera (Hallman and Phillips 2008). Adult Lepidoptera may rarely be of quarantine importance in the normal pathways for agricultural commodities.

### Phytosanitary Research

The experience with the IPPC vetting of PI treatments provides some lessons for research. Even if techniques for identifying irradiated organisms were available, they would not, of course, identify instances where the research supporting the treatment was inadequate or other factors had changed, thus reducing efficacy of the treatment. Therefore, PI research bears a greater burden of providing quarantine security than does research with all other commercial phytosanitary treatments that have an independent method of verifying efficacy. Furthermore, PI research requires more expertise in rearing and organism-handling techniques than other treatments because organisms must be held under favorable conditions and monitored for efficacy until they die, and controls must perform within expected norms. The IPPC vetting process has identified areas where increased attention to research is recommended.

**Dosimetry.** Although the IPPC accepted treatments for which dosimetry was not reported, it was noted that not only should dosimetry be done but the results should be reported. Dosimeters should be placed in areas of the load where extreme values are shown to occur via prior dose mapping (Heather and Hallman 2008). The highest doses absorbed during the large-scale testing confirming dose efficacy become the minimum absorbed doses required for commercial use.

**Live Adults Postirradiation.** Rejection of the presence of live adults threatens broad application of PI because most quarantine pests will be present as adults and they will not be killed rapidly by irradiation. This problem was faced years ago by APHIS (1996) when they stated that it was desirable that live irradiated quarantine pests could not emerge from the commodity unless they could be demonstrated to have been irradiated and that live pests for which treatments were approved will presume to have been effectively treated unless "evidence exists that the integrity of the treatment was inadequate." ISPM #18 also states that it is preferable that live pests not emerge from the commodity unless proof of irradiation was available (FAO 2003). APHIS has not objected to live adults via a comprehensive process of validation and certification of treatment facilities with monitoring of dosimetry and dose application during preclearance programs and safeguarding of regulated articles posttreatment.

Although the presence of live parent generation adults cannot reasonably be prohibited, postirradiation restrictions on development of the F<sub>1</sub> generation should help instill confidence that the risk of an infestation by progeny of irradiated adults is negligible. A treatment for *Aspidiotus destructor* Signoret allows for up to F<sub>1</sub> adult development after irradiation (Follett 2006b) and represents an extreme measure of efficacy for an accepted treatment. Objectives of treatments should ideally prevent significant F<sub>1</sub> development. Eggs may be laid, hatch, and early instars appear, but they should be documented to stop developing well before the F<sub>1</sub> adult could be present.

The use of specific measures of efficacy that do not leave significant unknown periods in the developmental process can help ensure that any progeny die well before reaching the F<sub>1</sub> adult. Almost all tephritid PI research has been done without observing development during the lengthy puparial stage where the insect develops from a larva to a pharate adult. Exceptions are some research with diapausing tephritids and *A. ludens* that found that most development ceases before the irradiated third instar reaches the phanerocephalic pupal stage, leaving a considerable margin of security between that stage and the emerged adult (Hallman 2004b, Hallman and Thomas 2010).

Research that allows for live adults to be present at least for a few days postirradiation should monitor the behavior of those adults until they die to estimate the likelihood of their detection by survey programs in importing countries. If it was determined that the pests could be found by the trapping systems used, the PI

treatment might not be permitted unless it could be determined that any adults found in survey traps were adequately irradiated.

Several techniques have been developed to identify irradiated organisms. They may not be very precise or easy to apply and largely depend on holding the organism for a while to track abnormal development (Heather and Hallman 2008). Thus, they are not generally useful at points of inspection. Although having reliable techniques that could be quickly used at points of inspection for validating treatment efficacy would be useful, the development of such techniques may not be generally feasible and should not delay use of irradiation as a phytosanitary treatment. The United States and New Zealand (the only countries currently importing commodities irradiated for phytosanitary purposes), the countries exporting these commodities, and several other interested countries have accepted this concept, but resistance by other countries has delayed or hindered the IPPC approval of some treatments.

**Infestation Techniques.** Some PI treatments were developed using artificial infestation techniques without comparing the results with natural infestation. It has been known for years that tephritids may be controlled with much lower doses in vitro than in fruit, so any infestation technique that strays from the natural state should be tested before being used to develop treatments. Although Hallman and Thomas (2010) noted in PI studies with *C. capitata* by using insertion of third instars into fruit that adult emergence was prevented with slightly lower doses than studies using infestation via oviposition, they found no statistically significant difference between insertion of diet-reared third instars in fruit 24 h before irradiation versus infestation by oviposition for *A. ludens*. In two studies with Lepidoptera, diet-reared insects were no easier to control than host-reared ones meaning that diet-reared ones could be used to develop PI treatments (Hallman 2004a, Hallman and Hellmich 2009).

**Controls.** Some PI treatments were approved although the controls did not perform within the range of expectation, meaning that experimental conditions were not favorable for the organisms. Both control and irradiated organisms should be held under conditions favorable for development and reproduction until all irradiated organisms die.

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