Generic Phytosanitary Irradiation Treatment for "True Weevils" (Coleoptera: Curculionidae) Infesting Fresh Commodities

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Abstract

Some species of weevils (Coleoptera: Curculionoidea) are quarantine pests that affect a large variety of fruits and vegetables. Many of these fresh commodities require phytosanitary treatment as a biosecurity measure before being exported to geographical areas where the weevil species do not exist but could become established and negatively affect agricultural production or the environment. There are phytosanitary irradiation (PI) doses that are already accepted as commercial PI treatments for some weevil species, but a generic dose that would suffice for all weevils would be useful. This paper examined the weevil PI literature for support of a generic dose. This literature review identified PI studies involving 14 species in the Brentidae and Curculionidae—which are 2 of 7 weevil families in the superfamily Curculionoidea—and all but 1 of these 14 species are in the family Curculionidae. A generic PI dose of 150 Gy for the Curculionidae that would prevent development beyond the hatching of eggs in the F_1 generation is supported by the available literature.

Key Words: generic dose; quarantine treatment; Curculionoidea; *Cylas formicarius*; radiation

Resumen

Algunas especies de gorgojos (Coleoptera: Curculionoidea) son plagas cuarentenarias que afectan a una gran variedad de frutas y verduras. Muchos de estos productos frescos requieren un tratamiento fitosanitario como medida de bioseguridad antes de ser exportados a áreas geográficas donde no existen los gorgojos, pero que podrían llegar a ser establecidos y negativamente afectar la producción agrícola o el medio ambiente. Hay dosis de irradiación fitosanitarias (IF) que ya se aceptan como un tratamiento IF comercial para algunas especies de gorgojos, pero una dosis genérica que sería suficiente para todos los gorgojos sería útil. Este artículo examina la literatura cerca de la IF de picudos para llegar a una dosis genérica. Esta revisión de la literatura identificó estudios IF que involucran 14 especies de Brentidae y Curculionidae - que son 2 de las 7 familias de gorgojos del Curculinoidea - y todos menos 1 de estas 14 especies se encuentran en la familia Curculionidae. Se respalda en la literatura disponible la dosis PI genérica de 150 Gy para los Curculionidae que impida el desarrollo más allá de la eclosión de los huevos en la generación F_1.

Palabras Clave: dosis genérica; tratamiento de cuarentena; Curculionoidea; *Cylas formicarius*; radiación

Generic treatments (a single dose that applies to a group of regulated pests) are used for most of the commercial applications of phytosanitary irradiation (PI) (Hallman 2012). Further progress in the development of generic PI treatments focuses on reducing currently accepted doses by providing additional data to more accurately determine an appropriate treatment dose to develop generic PI dose treatments that can be accepted as commercial treatments for many species or broader groups of quarantine pests. For example, a generic dose of 400 Gy is approved by the United States of America (USA) for all imported fresh commodities against all insect species—except the pupae and adults of lepidopteran species—and New Zealand has approved a 400 Gy dose for all insect species—except pupae and adults of lepidopteran species and species that vector plant diseases—but only on mango (*Mangifera indica* L.; Sapindales: Anacardiaceae), lychee (*Litchi sinensis* Sonn.; Sapindales: Sapindaceae), tomato *Solanum lycopersicum* L.; Solanales: Solanaceae), and capsicum (*Capsicum* spp.; Solanales: Solanaceae).

Radiation studies have been reported for several weevil species infesting fruit and root crops, and sufficient data may exist to propose a generic dose for this taxonomic group. The objective of this study was to analyze the available data on weevils infesting fruits and vegetables and recommend a generic dose where feasible.

WEEVIL CLASSIFICATION

The taxonomic classification to the family level in this paper follows Oberprieler et al. (2007) who recognized 7 families. Weevils for which there are relevant PI data are in the families Brentidae and Curculionidae. Brentidae with 6 subfamilies and ~4,000 known species include the quarantine pest, the sweetpotato weevil, *Cylas formicarius* (F.) (Coleoptera: Brentidae: Clyadinae). "True weevils" belong to Curculionidae, one of the largest insect families with 10 subfamilies and ~51,000 known species, and > 80% of all weevil species are curculionids. Included in this family are the vast majority of the weevil species that are currently quarantine pests throughout the world.

IMPORTANCE OF A GENERIC PI DOSE FOR WEEVILS

Hallman (2012) considered weevils as fifth in phytosanitary importance for fresh commodities (after fruit flies, mealybugs, lepidopteran

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larvae and scale insects), and 25% of the pest risk analyses examined required a phytosanitary treatment for at least 1 weevil species. Plant families for which weevils of the family Curculionidae are quarantine pests include Anacardiaceae (e.g., mango, *Mangifera indica* L.; Sapindales: Anacardiaceae), Convolvulaceae (e.g., sweetpotato, *Ipomoea batatas* L. [Lam.]; Solanales: Convolvulaceae), Myrtaceae (e.g., guava, *Psidium guajava* L.; Myrtales: Myrtaceae), and Rosaceae (e.g., apple, *Malus domestica* Borkh.; Rosales: Rosaceae; peach, *Prunus persica* (L.) Batsch; Rosales: Rosaceae; cherry, *Prunus avium* [L.] L.; Rosales: Rosaceae). These fresh commodities are traded in large quantities and, therefore, a generic PI treatment against weevil pest species generally would be advantageous.

RADIATION BIOLOGY OF THE CURCULIONIDAE

The radiation biology of the cotton boll weevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae) was investigated quite intensively during the 1960s when the cotton industry of the USA and the US Department of Agriculture agreed that a concerted effort should be made to eradicate this alien pest, which had entered the country in 1894 and which required the use of large quantities of insecticides for its control. A concerted effort was made to develop the use of the sterile insect technique for this purpose. However difficulties in providing a competitive sterile male became evident when Davich & Lindquist (1962) found that both longevity and fecundity of reproducing boll weevils irradiated with 50 Gy or more were drastically reduced, while induction of complete sterility in males required their irradiation with doses of 100–150 Gy, which were debilitating and lethal. Flint et al. (1966) confirmed that all life stages of the boll weevil are very sensitive to ionizing radiation as measured by survival 3 wk after irradiation. High mortality of irradiated adult weevils occurred mostly during 7–14 d after treatment, and the death rate did not increase when doses were increased in increments from 32 to 150 Gy. Riemann & Flint (1967) established that irradiation caused death of the weevils by the destruction of dividing cells clustered in nidi of the midgut epithelium. These dividing cells are needed to the continuously replace the cells that produce digestive enzymes and which break open to release their contents into the lumen of the weevil's midgut.

Damage to the spermatogonia was evident at 10 Gy, extensive at 20–30 Gy, and complete at 40 Gy. Spermatogonia that survived 20–30 Gy were found to repopulate the germarium of the testis in some individuals and restore the spermatogenic cycle, and fertility. The dividing cells of the midgut proved to be only slightly more radio-resistant than the spermatogonia (Reineke et al. 1969), and the sperm were the most radio-resistant cells involved in spermatogenesis. Therefore, the application of 100–150 Gy, which was needed to induce complete sterility—i.e., prevent the hatch of F_1 eggs—inevitably sickened and drastically shortened the life spans of both male and female boll weevils. Further, even by irradiating boll weevils with either thermal or fast neutrons substantial mortality was induced at much lower doses than required to induce a satisfactory level of sterility in both males and females (Klassen et el. 1969).

Mature eggs in female insects are pre-meiotic and they usually develop to metaphase I and then wait until after oviposition to complete meiosis with the production of 1 pronucleus and 3 polar bodies. As noted by Robinson (2005), mature eggs in the female are much more radioresistant than earlier cell types involved in oogenesis including the oogonia. Consequently a dose of irradiation that induces full sterility of the eggs also causes complete but delayed infecundity. Evidence that this general scheme holds for female boll weevils can be seen in the data of Davich & Lindquist (1962), Flint et al. (1967) and Riemann & Flint (1987).

In only 1 of 6 other species of Curculionidae was longevity of adults irradiated with efficacious doses, such as those presented in Table 2, not markedly shorter than the longevity of non-irradiated adults, further supporting the contention that efficacious doses are highly debilitating to curculionids. The highest dose studied by Ferrer & Hower (1975) against *Hypera postica* (Gyllenhal), 45 Gy, prevented hatch of eggs in the F_1 generation but did not reduce longevity of adults. At 50 Gy, which prevented hatch of eggs in the F_1 generation, longevity of *Pissodes strobe* (Peck) female adults mated before irradiation was halved (Jaynes & Godwin 1957). Longevity of *Sternochetus frigidus* (Fabr.) was reduced by > 60 and 80%, respectively, when irradiated at 100 and 150 Gy (Obra et al. 2013), doses that achieved 2 different measures of efficacy against that weevil species (Table 2). All adult *Sitophilus granarius* (L.) irradiated at 100 Gy, which completely prevented production of progeny, were dead by 4 wk, whereas < 4% of non-irradiated adults had died (Aldryhim & Adam 1999). Brown et al. (1972) found similar responses in the cases of *S. granarius* and *S. zeamais* Motschulsky. Adult *Conotrachelus nenuphar* (Herbst) irradiated at a dose found to prevent F_1 generation larvae (80 Gy) died by 2 wk while > 90 % of non-irradiated adults were still alive (Hallman 2003).

EXISTING PHYTOSANITARY IRRADIATION TREATMENTS AGAINST WEEVILS

Approved Phytosanitary Treatments

The first approved PI treatment against a weevil was by the California Department of Food & Agriculture effective 1 Apr 2000 against *C. formicarius* (Table 1) (Hallman 2001). By late May 2000, PI was being used to treat sweetpotato obtained from Florida. This was also the first use of PI against an adult insect, which was a milestone in phytosanitation because it was the first time that live adults were permitted after a phytosanitary treatment. Acceptance of live adults after irradiation was at the time considered especially risky by some, because if the treatment was not effective the fertile adults could immediately reproduce. However, live adult mango seed weevils (*Sternochetus mangiferae* [F.];

Table 1. Phytosanitary irradiation treatments approved for use against weevils.

Weevil	Minimum dose (Gy)	Approving plant protection organization[1]	Has been used commercially?
Conotrachelus nenuphar	92	APHIS, IPPC	Yes
Cylas formicarius	150	APHIS	Yes
Cylas formicarius	165	CDFA, IPPC	Yes
Euscepes postfasciatus	150	APHIS, IPPC	Yes
Sternochetus mangiferae	300	APHIS, CPPQ	Yes

[1]APHIS = Animal & Plant Health Inspection Service (United States); CDFA = California Department of Food & Agriculture; CPPQ = Crop Protection & Plant Quarantine (Malaysia); IPPC = International Plant Protection Convention.

Coleoptera: Curculionidae) have been found in irradiated mango fruits received in New Zealand from Australia (El-Gamel 2015), but because the treatment is effective at the dose used and the treatment schedule allows for live adults no further regulatory action was taken.

The dose of 300 Gy for *S. mangiferae* is probably excessive, as discussed by Hallman (2012). Originally a PI dose of 100 Gy was proposed, but when the supporting research was re-examined at the time of adoption, it was decided that a dose of about 300 Gy was necessary "to effectively control the weevil".

There are 2 similar doses for *C. formicarius* (Table 1): a 165 Gy dose based on Hallman (2001) was adopted by the California Department of Food & Agriculture before APHIS adopted the 150 Gy dose based on Follett (2006). The criterion of efficacy of both doses is the prevention of F_1 generation adults. It is not clear why the higher dose of 165 Gy was adopted by the International Plant Protection Convention (IPPC 2011) given that the originally proposed effective PI dose was 140 Gy (Hallman et al. 2010). Regardless of the reason, both of those doses were based on the prevention of F_1 generation adults, which is a measure of efficacy that might be too loose for broad acceptance. Hallman et al. (2010) recommended that PI doses against adults be based on measurements of efficacy that do not allow much development of the F_1 life stages, because moderate variations in response among populations of the same pest increase the risk of F_1 adults being formed when the measure of efficacy is so close to that result (F_1 adult formation), with ensuing reproduction a credible concern. In this review an appropriate measure of efficacy for a generic weevil PI treatment is prevention of development beyond hatch of the eggs of the F_1 generation.

POSSIBLE GENERIC PI DOSE FOR WEEVILS

Table 2 summarizes the various irradiation studies on weevils that supply measurements of efficacy that might be used for phytosanitary purposes. Fourteen weevil species in 2 families are represented. Determinations of efficacy ranged from a very lax measure, i.e., prevention of F_1 generation adults, to a very strict measure, i.e., prevention of oviposition by irradiated females. Only 1 species is from the family Brentidae, and the measure of efficacy used was prevention of F_1 generation adults, which is much more lax than the measures used for any of the curculionid species listed in Table 2. Therefore, the Brentidae are excluded from a generic dose for weevils.

The remaining 13 species reported in the literature are all Curculionidae. The study with *Sitophilus granarius* (L.) uses a vague measure of efficacy, i.e., "prevention of progeny". The study for another species, *Diaprepes abbreviatus* (L.), used a well-defined, restrictive measure of efficacy (prevention of hatch of eggs in the F_1 generation), but irradiated adults were only observed for 6 days after which most of them were still alive. Female insects are not known to recover fertility after exposure to a reproductively sterilizing dose of radiation; however, ideally, pests should be held for observation until all have died to be certain that they did not reproduce. Measures of efficacy for the remaining studies were quite precise, ranging from prevention of oviposition to the observation that host material does not show feeding damage, which strongly indicates no development of F_1 generation larvae.

Phytosanitary treatments should be supported by confirmatory testing with large numbers of insects, i.e., tens of thousands. Confirmation is not supported by research involving small numbers of insects because statistical extrapolations at the levels of control required of phytosanitary treatments carry inadequate statistical confidence (West & Hallman 2013). Three studies used large numbers of insects, i.e., 25,000–60,000. Although another used >4,500 insects, it employed a very restrictive measure of efficacy (prevention of oviposition), and resulted in the largest treatment dose in Table 2, i.e., 164 Gy. The slightly less precise measure of efficacy (prevention of hatch of eggs in the F_1 generation) in the same study resulted in a dose of 109 Gy confirmed with fewer insects (1,480).

Hallman (2012) suggested that a generic dose for weevils would be > 165 because of studies done with *C. formicarius* and *Sternochetus frigidus* (F.). However, if Brentidae (*C. formicarius*) are removed from consideration and the very precise measure of efficacy for an irradiation treatment (prevention of oviposition) for *S. frigidus* is replaced by prevention of hatch of eggs in the F_1 generation—accomplished with 109 Gy—then the highest dose in Table 2 is 150 Gy for prevention of egg hatch in the F_1 generation of *Orchidophilus aterrimus* (Waterhouse). However, it should be noted that 150 Gy was the lowest dose used in that study (Manoto et al. 1999), and the actual dose to prevent egg hatch in the F_1 generation may be less than this. The next highest dose to prevent egg hatch in the F_1 generation in Table 2 is 145 Gy for no egg larval development (no frass production) of *Euscepes postfasciatus* (Fairmaire) (Coleoptera: Curculionidae). When irradiated with 100 Gy a mean of 1.5 F_1 generation adults of *E. postfasciatus* developed from 100 irradiated adults and frass production was 0.011% of the control, indicating extremely little feeding by F_1 generation larvae at that dose. Efficacious doses for all of the other species range from 45 to 120 Gy for the prevention of significant development in the F_1 generation. Based on these studies a generic PI dose for Curculionidae of 150 Gy as suggested by Heather & Hallman (2008) is plausible. Clearly the literature provides evidence for a generic PI dose of 150 Gy for the Curculionidae with the measure of efficacy being the prevention of development beyond hatching of eggs in the F_1 generation.

Seven curculionid subfamilies are represented by the studies evaluated here. Although the number and definition of subfamilies in the Curculionidae is controversial (Oberprieler et al. 2007), PI studies have been done over a rather broad taxonomic range within the family. Two subfamilies not represented are the wood-boring weevils, Platypodinae and Scolytinae, which have been considered separate families (Jordal et al. 2014). Because these 2 subfamilies do not infest fresh commodities they could be excluded from a generic dose for curculionids infesting fresh commodities. Data are lacking in 2 subfamilies that infest fresh commodities, i.e., the Brachycerinae and the Cyclominae. Some Brachycerinae feed on foliage of legume as adults while their larvae bore into the roots, and others bore into onion (*Allium cepa* L.; Asperagales: Amaryllidaceae), garlic (Allium sativum L.; Asperagales: Amaryllidaceae), and flower bulbs. Many Cyclominae infest a variety of vegetables, including root crops. Phytosanitary irradiation research with species of the Brachycerinae and the Cyclominae is recommended before a generic dose for Curculionidae is expanded to include them.

A generic dose against weevils would exclude commodities stored in hypoxic atmospheres, as is currently the case for most PI treatments (e.g., IPPC 2011). Hallman (2005) concluded that a dose to prevent reproduction of adult *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae) irradiated in an atmosphere containing very low concentrations of oxygen could be twice the dose required in ambient atmospheres.

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Table 2. Phytosanitary irradiation measures of efficacy and doses for weevils by family and subfamily.

Family Subfamily	Genus species	Measure of efficacy	Dose to achieve efficacy (Gy)	Numbers treated at efficacious dose	Reference
Brentidae					
Clyadinae	*Cylas formicarius*	Prevention of F_1 generation adults	165	30,655	Hallman 2001
Clyadinae	*C. formicarius*	Prevention of F_1 generation adults	140	62,783	Follett 2006
Curculionidae					
Baridinae	*Orchidophilus aterrimus*	Prevention of hatch of eggs in the F_1 generation	150	10	Manoto et al. 1999
Cryptorhynchinae	*Euscepes postfasciatus*	No frass produced in sweetpotato roots exposed to irradiated adults, indicating no development of F_1 generation larvae	145	60,400	Follett 2006
Cryptorhynchinae	*Sternochetus frigidus*	Prevention of oviposition	164	4,549	Obra et al. 2014
Cryptorhynchinae	*S. frigidus*	Prevention of hatch of eggs in the F_1 generation	109	1,480	Obra et al. 2014
Cryptorhynchinae	*Sternochetus mangiferae*	Prevention of oviposition	105	~80	Follett 2001
Curculioninae	*Anthonomus grandis*	Prevention of hatch of eggs in the F_1 generation	100	23-29	Davich & Lindquist 1962
Dryophthorinae	*Sitophilus granarius*	Prevention of the production of progeny	100	80	Aldryhim & Adam 1999
Dryophthorinae	*S. oryzae*	No loss of rice weight after infestation with irradiated adults, indicating negligible development of F_1 generation larvae	120	38,025	Follett et al. 2013
Dryophthorinae	*S. zeamais*	Prevention of F_1 generation adults	100	90	Brown et al. 1972
Entiminae	*Diaprepes abbreviatus*	Prevention of hatch of eggs in the F_1 generation; adults observed for only 6 d but many survived much longer	50	220	Gould & Hallman 2004
Entiminae	*Phlyctinus callosus*	Prevention of hatch of eggs in the F_1 generation	80	400	Duvenhage & Johnson 2014
Hyperinae	*Hypera postica*	Prevention of hatch of eggs in the F_1 generation	45	18	Ferrer & Hower 1975
Molytinae	*Conotrachelus nenuphar*	Lack of tunneling in apples exposed to irradiated adults, indicating no development of F_1 generation larvae	92	25,000	Hallman 2003
Molytinae	*Pissodes strobi*	Prevention of hatch of eggs in the F_1 generation	50	40	Jaynes & Godwin 1957

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