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# Irradiation as a Quarantine Treatment for Mango Seed Weevil

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**Abstract:** The mango seed weevil, *Cryptorhynchus mangiferae* (F.), has prevented the export of mangoes from Hawaii to the U.S. mainland for over 50 years because there were no approved quarantine treatments to control this pest. Irradiation was explored as a method to prevent adult emergence in, or to sterilize, mango seed weevil. Mixed-age mango seed weevils in mangoes were irradiated with target doses of 50, 100, or 300 Gy and held for adult emergence. The 300 Gy treatment (dose range 180–310 Gy) did not prevent adult emergence. Emerging adults from the 100 and 300 Gy treatments were lethargic and short-lived, and laid no eggs indicating sterility. An irradiation quarantine treatment (100 Gy) to sterilize mango seed weevil in mangoes has been proposed. Approval of this treatment will open U.S. mainland markets to mango exports from Hawaii.

Key words: Cryptorhynchus mangiferae, Sternochetus mangiferae, disinfestation, postharvest treatment

## Introduction

With the demise of the sugarcane industry in Hawaii in recent years, vast acreages have become available for diversified agriculture. A tropical exotic fruit industry is rapidly expanding to help fill the void and is expected to be an important part of Hawaii's crop diversification. An important step in this expansion is the development of quarantine treatments to permit exports. Currently, irradiation is an accepted quarantine treatment for export of papaya, carambola, lychee, rambutan, longan, atemoya, sapodilla, and abiu from Hawaii to the U. S. mainland. Irradiation quarantine treatments up to this time have been developed exclusively for fruit flies. In addition to fruit flies, many other insect and mite pests are classified as regulatory pests and their appearance has equal status with fruit flies in interrupting export shipments.

The mango seed weevil, *Cryptorhynchus* (*=Sternochetus*) mangiferae (F.), first reported in Hawaii in 1905 (Kotinsky 1905), is a federally quarantined pest that has prevented the shipment of mangos from Hawaii into the continental United States for over 50 years (Armstrong 1993). Pest control research for mango weevil over the years has looked at a number of options, including field sanitation, chemical sprays (trunk and foliar), natural enemies (parasitoids, the fungus *Beauvaria bassiana*), host plant resistance, and x-ray fruit culling technology with little success (Hansen and Armstrong 1990, Hansen 1991, CAB and EPPO 1997, Pena et al. 1998). Therefore, the industry must depend on a postharvest disinfestation treatment. Postharvest researchers have attempted to kill mango seed weevil in mangoes, while maintaining fruit quality, using heat, cold, and fumigation treatments without success (Balock and Kozuma 1964). Irradiation is a viable alternative to disinfest mangoes of mango seed weevil.

A typical quarantine treatment for fruit flies is developed by testing hundreds of thousands of lab-reared insects, which are artificially inoculated into the fruit, so-called probit 9 (99.9968% mortality) level testing. Development of an irradiation quarantine treatment for mango seed weevil has been difficult because there is only one generation of weevils per year, and no artificial diet exists to culture the insect in the laboratory. Therefore, probit 9 level testing is impractical. Instead, we pursued an alternate approach: to generate data for an irradiation treatment with the numbers of insects available, and to show that the weevil would have no economic impact on fruit production and negligible impact on mango germplasm if it were introduced into the U.S. mainland (Follett 2000, Follett and Gabbard 2000). If the risk of mango seed weevil could be put in perspective, regulators could be convinced that a safe quarantine treatment could be developed by testing only a few thousand insects. In this paper, I present the evidence for an effective irradiation quarantine treatment to disinfest mangoes of mango seed weevil.

### Background

Studies on irradiation against mango seed weevil in Hawaii date back 40 years. Upadhya and Brewbaker (1966) irradiated approximately 100 infested fruits at each of four doses (300, 600, 900 and 1,200 Gy) and measured survival of adult weevils after 3 and 5 weeks. About 70% of mangoes treated with an irradiation dose of 300 Gy produced live but moribund adults, and no adults survived doses of  $\geq 600$  Gy after 5 weeks. The authors state that the adult stage is the most tolerant to irradiation but provide no data.

In the early 1970s, Seo et al. (1974) conducted a series of irradiation experiments with mango seed weevil in Hawaii, examining effects on mango seed weevil mortality and sterility. Irradiation treatment with a dose of 329 Gy killed all 1,143 weevils of mixed age infesting packaged mangoes. When the fruit density (number of fruit per fiberboard container) was varied at an irradiation dose of 206 Gy, 4 out of 1,324 adults survived at the highest density (3.92 g/cm<sup>2</sup>), 1 out of 1,206 at the middle density and 0 out of 1,206 at the lowest density (2.56 g/cm<sup>2</sup>). Adults from untreated control fruit remained alive after 6 months. To examine sterility, 30 mating pairs in individual glass vials were treated with a dose of 50, 75, 100 or 200 Gy, or left untreated as controls. Twenty-nine females survived treatment with 50 or 75 Gy (21 and 8 respectively) and laid a total of 18 sterile eggs. All sixty of the females irradiated with 100 or 200 Gy died without producing eggs. Seventeen untreated females produced 1,175 eggs of which 639 hatched.

In Australia in the late 1980s, 1,956 'Kensington' mangoes, with a natural mango seed weevil infestation estimated as 1,065 (54.6%) larvae, 725 (37.2%) pupae, and 161 (8.3%) adults, were irradiated at 300 Gy (Heather & Corcoran 1992) The 300 Gy treatment was selected based on the results of Seo et al. (1974) (N. Heather personal communication). The 300 Gy treatment prevented adult weevil emergence from the mangoes and resulted in 100% mortality after 8 months. By comparison, 522 control fruit with a natural infestation consisting of an estimated 32 larvae (6.5%), 354 pupae (71.7%), and 108 adults (21.9%) produced 110 surviving adults after 8 months.

In South Africa, Thomas (1975) reported that 500 Gy reduced adult emergence almost to zero but 750 Gy was required to completely inactivate the weevil and prevent emergence. Milne et al. (1977) measured direct mortality to all life stages and prevention of emergence of the adult weevils with early- and late-maturing varieties of mangoes irradiated at doses from 300–850 Gy. After irradiation, seeds containing weevils were checked weekly for 6 weeks. In the early maturing variety, 60% of the population was in the adult stage; in the late-maturing variety 80% of the population was in the adult stage. According to the authors, preliminary studies indicated that adult weevils were, by far, the most tolerant stage. Irradiation doses of 300 and 375 Gy were inadequate in preventing adult emergence from fruit, particularly in the late-maturing cultivar where a larger proportion of the population

		Irradiation dose (Gy)		
Study	Country	Prevent adult emergence	Cause sterility	
Upadhya & Brewbaker 1966	USA/Hawaii	300	_	
Seo et al. 1974	USA/Hawaii	329	50 <sup>z</sup> 100 <sup>y</sup>	
Heather & Corcoran 1992	Australia	300	_	
Thomas 1975	South Africa	750	_	
Milne et al. 1977	South Africa	>500		
Shukla and Tandon 1985	India	>500	_	

	ı doses to disinfes	

<sup>2</sup>Irradiated adults laid eggs but none hatched. <sup>y</sup>Irradiated adults died without laying eggs.

was in the adult stage. At 500 Gy, one adult emerged from the seed but died soon afterward. In seeds treated with 500 Gy or more, adults inside the seed that were alive appeared moribund and incapable of emerging. No tests were conducted to determine sterility of adults at various irradiation doses. More recent work suggests a dose of 600 Gy is required to prevent mango seed weevil emergence from fruit under South African conditions (L. Milne, personal communication).

In India, Shulka and Tandon (1985) irradiated mango seed weevils in harvest-mature 'Alphonso' mangoes at 10, 100, 200, 300, 400, and 500 Gy. Fruit were cut open after an unspecified period to examine the presence of dead or live insects. The 500 Gy dosage killed immature stages but not adults.

Different treatment dose intervals, dosimetry, methods, and mango cultivars used in each of the irradiation studies discussed above make it difficult to draw general conclusions about an effective irradiation dose for mango seed weevil (Table 1). Based on the studies of Seo et al. (1974) and Heather and Corcoran (1992), an irradiation dose of 100 Gy is adequate to sterilize mango seed weevil and 300 Gy will usually prevent adult emergence. Studies by Thomas (1975) and Milne et al. (1977) suggest a higher dose is required to prevent adult emergence in South Africa. Research in South Africa has focused on obtaining a quicker kill because, in late season, mango seed weevils occasionally emerge from the ripening fruit on the tree (Heather 1992). This event is unlikely under conditions in Hawaii and Australia with early and mid-season mango varieties, where weevils typically emerge from fruit only after the flesh has decayed on the ground and the seed is exposed. Consequently, it is more difficult to prevent some emergence of weevils with irradiation in South Africa.

Dose (Gy)	Fruits treated	Adults emerged	Adults dead in seed	Eggs layed	Hatched
0	255	160	9	190	37
50	330	95	23	57	15
100	331	61	15	0	0
300	335	53	49	0	0

Table 2. Effect of irradiation on adult emergence and sterility in mango seed weevil.

Age structure at treatment: 39% early larvae, 19% late larvae, 18% pupae, and 24% adults.

We conducted studies from 1997 to 1999 to confirm the results of irradiation treatment tests reported by other researchers.

#### **Materials and Methods**

Harvest-mature 'Haden' mangoes were collected from the Yamada orchard in Kalapana, HI in 1998, and infestation was determined based on oviposition scars. Infested mangoes were transported to the Hawaii Research Irradiator (HRI) at the University of Hawaii at Manoa and irradiated at target doses of 50, 100, or 300 Gy, or left untreated as controls. The Hawaii Research Irradiator uses a <sup>60</sup>Co source of gamma radiation and the dose rate at the time of the tests was 5.3–5.8 Gy min<sup>-1</sup>. Details on dose mapping, dosimetry, and dose variation are given in Follett and Lower (2000). A sub-sample of fruit was taken beforehand and opened to determine the age structure of weevils in the infested fruit. After irradiation, the fruit flesh was cut away and the seeds in their husks were held over an 8-month period and inspected weekly for adult emergence. Emerging adults were placed in screen cages with immature mango fruits to examine reproduction. The experiment was replicated 4 times and all emerging adult weevils for a given irradiation treatment (control, 50, 100, 300 Gy) were held in one cage.

### Results

Although adult emergence occurred at all irradiation doses, emergence was reduced in irradiated fruits (Table 2). The 300 Gy treatment did not prevent adult emergence, unlike previous studies in Hawaii and Australia. This is probably explained by dose variation in the treatment chamber: the dose uniformity ratio when using the full volume of the chamber at HRI is 1.6 to 1.8, with the dose distribution skewed toward lower doses (see Follett and Lower 2000). Therefore, the dose range of the 300 Gy treatment was estimated to be 180–315 Gy, and many individuals received a radiation dose less than 300 Gy.

Emerging adults from the 100 and 300 Gy treatments were lethargic and short-lived, and no eggs were laid indicating sterility. Egg laying by untreated control weevils was consistent but at a relatively low rate compared with weevils in the study by Seo et al. (1974). This suggests that cage conditions in our studies were not ideal for mating and/or oviposition. Nonetheless, sterility was achieved in this study with an irradiation dose of 100 Gy (estimated dose range, 60–105 Gy), as it was in the study by Seo et al. (1974) (Table 2).

## Discussion

Mango seed weevil is monophagous and requires mango to complete development. Eggs are laid on the surface of young fruit and neonates burrow down to the developing seed; larvae feed within the seed, which becomes encased in a tough fibrous husk, and development to the adult stage occurs entirely in the seed (Balock and Kozuma 1964, Follett and Gabbard 2000). Therefore, mango seed weevil attack usually goes unnoticed and does not reduce fruit marketability.

The mango industry on the U. S. mainland is small and concentrated in southern Florida. Florida's mango industry focuses on growing mango for seed production in nurseries and orchards. The high-risk quarantine pest status given to mango weevil is mainly in response to concerns from the mango industry in Florida that *C. mangiferae* infestation would reduce seed germination and therefore limit seed production in nurseries and orchards (Balock and Kozuma 1964). Recent studies suggest that infestation of, and damage to, mango seeds by mango seed weevil does not adversely affect seed germination as previously thought (Follett 2000, Follett and Gabbard 2000). Therefore, mango industry. Nevertheless, mango seed weevil is a pest and may cause some crop loss due to premature fruit drop and damage to fruit pulp, albeit rarely (Follett and Gabbard 2000).

Our irradiation data were submitted to USDA-APHIS for a quarantine treatment along with data from field studies showing that mango seed weevil is a less serious pest of mangoes than previously thought (Follett and Gabbard 2000). A proposed rule was published in the Federal Register on May 26, 2000 recommending an irradiation dose of 100 Gy to control mango seed weevil in exported mangoes. Publication of the final rule for the irradiation quarantine treatment will be the first time that irradiation has been approved for an insect other than a fruit fly. This will open U.S. mainland markets to mango exports from Hawaii.

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