Irradiation as a Methyl Bromide Alternative for Postharvest Control of *Omphisa anastomosalis* (Lepidoptera: Pyralidae) and *Euscepes postfasciatus* and *Cylas formicarius elegantulus* (Coleoptera: Curculionidae) in Sweet Potatoes

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ABSTRACT Irradiation studies were conducted with three sweet potato, *Ipomoea batatas* (L.) Lam., pests to determine an effective dose for quarantine control. Dose–response tests indicated that the most radiotolerant stage occurring in roots was the pupa of sweetpotato vine borer, *Omphisa anastomosalis* (Guenee), and the adult of West Indian sweetpotato weevil, *Euscepes postfasciatus* (Fairmaire), and sweetpotato weevil, *Cylas formicarius elegantulus* (Summers). In large-scale confirmatory tests, irradiation of 60,000 *C. formicarius elegantulus* adults, 62,323 *E. postfasciatus* adults, and 30,282 *O. anastomosalis* pupae at a dose of 150 Gy resulted in no production of F_1 adults, demonstrating that this dose is sufficient to provide quarantine security.

KEY WORDS phytosanitary treatment, quarantine pest, x-ray irradiation

HAWAII'S VEGETABLE GROWERS produce several unique varieties of sweet potatoes, *Ipomoea batatas* (L.) Lam., including a purple-fleshed type. Sweet potato growers are unable to ship sweet potatoes to the U.S. mainland without a quarantine treatment because of three quarantine pests. West Indian sweetpotato weevil, *Euscepes postfasciatus* (Fairmaire) (Coleoptera: Curculionidae), and the sweetpotato vine borer, *Omphisa anastomosalis* (Guenee) (Lepidoptera: Pyralidae), are federal quarantine pests (USDA–APHIS–PPQ 2002), and the sweetpotato weevil, *Cylas formicarius elegantulus* (Summers) (Coleoptera: Curculionidae), is an actionable pest for California. Until recently, growers have been exporting sweet potatoes to the U.S. mainland by using methyl bromide fumigation to control these pests. Methyl bromide was identified as an ozone-depleting substance, and many uses are being phased out (UNEP 1992). Methyl bromide fumigation adversely affects sweet potato root quality and has become costly because of reduced availability. Irradiation treatment is a viable alternative to chemical fumigation for sweet potato growers. The main advantage of irradiation as a phytosanitary treatment is that it is broadly effective against arthropod pests (Follett and Griffin 2006) at dose levels that do not cause injury to most types of fresh fruits and vegetables, including sweet potatoes (Wall 2004, 2005).

Unlike other quarantine treatments, irradiation does not cause high acute mortality. The goal of a quarantine treatment is to prevent reproduction in the target pest. This goal can be achieved by killing the pest outright, preventing development to the reproductive stage, or sterilizing adult insects. The sweet potato pests considered in this study are all internal feeders. For species, eggs are laid on the surface of the root, and hatching neonates bore into the root to feed and develop. *O. anastomosalis* pupates near the surface of the root and adults break through the root epidermis to emerge. *E. postfasciatus* and *C. formicarius elegantulus* pupate in the root and emerging adults typically feed then bore out, but some adults can remain in the root for an extended period. Therefore, the pupa is the most advanced stage of *O. anastomosalis* occurring in the root, and the adult is the most advanced stage of *E. postfasciatus* and *C. formicarius elegantulus* found in the root. The goal of irradiation treatment is to prevent successful reproduction, which, for sweet potato pests, is measured as no successful emergence of adults from roots in the subsequent generation (F_1).

Irradiation research has been carried out on *E. postfasciatus* and *C. formicarius elegantulus* in Okinawa as part of a sterile insect release program to eradicate the weevils from the island of Kume (Shimoji and Miyatake 2002), and unpublished reports indicate that an irradiation dose of 150 Gy sterilizes adult females of the two species (M. Yamagishi, personal communication). Dawes et al. (1987) and Hallman (2001) studied radiotolerance in *C. formicarius elegantulus*, and Hallman (2001) recommended treatment at an irradiation dose of 165 Gy to prevent reproduction in adults. No irradiation research has been performed with *O. anastomosalis.* USDA-Animal and Plant Health Inspection

Service (APHIS) published a rule allowing export of irradiated sweet potato from Hawaii to the U.S. mainland based on preliminary research and information on effective doses for related species (Federal Register 2003, 2004; Follett and Neven 2006). A conservative irradiation dose of 400 Gy was set until research demonstrates that a lower dose is effective. Irradiation studies were conducted to determine the most tolerant life stage and an effective irradiation dose to control the three sweet potato quarantine pests.

Materials and Methods

O. anastomosalis. An experiment was conducted on sweet potatoes collected from the field to determine the approximate radiotolerance of *O. anastomosalis.* Naturally infested sweet potato roots were collected from an abandoned field in Pepekeeo, HI, and irradiated the next day. Roots infested with *O. anastomosalis* were identified by inspection for small "windows" (the thin, papery epidermis where emerging adults exit) at the root surface, indicating the presence of late instars or pupae (Talekar and Pollard 1991). Other immature life stages were probably also present. Equal numbers of roots were randomly assigned to four nominal treatment doses (0, 100, 200, and 400 Gy). The experiment was repeated on four dates, which served as replicates. Collectively, all roots treated at each dose weighed ≈2.8 kg in the first replicate and 3.4 kg in subsequent replicates. Irradiation treatment was conducted at a nearby commercial x-ray facility (Hawaii Pride LLC, Keaau, HI) by using an electron linear accelerator (5 MeV, model TB-5/15, SureBeam Corp. [now L-3 Communications Titan Corp.], San Diego, CA) at ambient temperature. Dosimeters (Opti-chromic detectors, FWT-70-83M, Far West Technology, Goleta, CA) were placed inside two representative roots at each dose in each replicate to measure dose variation. The dosimeters were read with a FWT-200 reader (Far West Technology) at 600-nm absorbance to verify the minimum absorbed dose and dose variation in each replicate. To minimize the dose uniformity ratio (the ratio of the maximum/minimum dose), infested sweet potatoes were placed upright in plastic tubs in a single row perpendicular to the beam. Dose mapping demonstrated that doses were sometimes lower near the sides and floor of the metal carrier, so the tubs with sweet potatoes were elevated by placement on a cardboard box and positioned in the exact center of the carrier. Each carrier passed in front of the beam in a forward then reverse orientation. The dose uniformity ratio during the sweet potato research was consistently <1.2. After irradiation treatment, roots were held in ventilated plastic tubs for adult emergence. (These irradiation methods also were used in all subsequent experiments.)

Laboratory tests were conducted with *O. anastomosalis* to determine the most tolerant stage occurring in the root and to identify a dose to control the most tolerant stage. A laboratory colony of *O. anastomosalis* was maintained on whole sweet potato roots in 3.8-liter plastic tubs (Rubbermaid, Wooster, OH) at 25 ± 2°C and a photoperiod of 14:10 (L:D) h. Fresh roots were irradiated at a high dose (350–450 Gy) to eliminate any natural insect infestation and then placed in a cage for 24 h with mated moths for oviposition. Roots with eggs were held in plastic containers for hatch and larval and pupal development. At 2, 16, 23, 30, 37, and 44 d, a subset of infested roots was irradiated at 100 Gy and then held for adult emergence. These intervals corresponded to increasingly older test subjects, beginning with early larvae and progressing to late pupae. Talekar and Pollard (1991) reported average developmental times for eggs, larvae, and pupae as 7, 32, and 14 d, respectively, at unspecified temperatures. Under our laboratory rearing conditions, adult moths begin to emerge at 42–44 d and continued emerging for ≈4 wk. Roots selected for each age treatment started with approximately the same total number of eggs. Adult emergence was recorded. For each replicate, an equal number of infested roots were left unirradiated as a control.

Adult moths also were tested. Adults newly emerged to 3 d old were placed in plastic tubs and irradiated at 50, 100, 150, 200, and 250 Gy to evaluate the effects of irradiation on sterility. Tests were replicated four times with 20 female and 20 male moths in each replicate. For each replicate, an equal number of adults were left unirradiated as a control. After irradiation, moths were placed in 25 by 25 by 25-cm screen cages with fresh sweetpotatoes to allow oviposition. After all adults had died, sweet potatoes from the cages were transferred to plastic tubs. Frass jettisoned from the root by developing larvae within was weighed weekly to estimate relative larval survival and development among treatments. F_1 adult emergence was recorded.

Small-scale confirmatory tests were conducted with adult moths. In each replicate, 50 male and 50 female adult moths were transferred to plastic containers containing sweet potato roots and treated 2 h later with an irradiation dose of 250 or 150 Gy. Twenty-five male and 25 female moths were held under identical conditions but not irradiated as controls. After treatment, irradiated adults and controls were placed in separate plastic containers with fresh uninfested roots to examine reproduction and development of any progeny to adulthood.

Large-scale confirmatory tests were conducted with pupae. Female moths were allowed to oviposit for 24 h on sweet potato roots. Roots were held for 7 wk to allow time for development to the pupal stage. Infested roots were irradiated at 150 Gy and held in plastic tubs for adult emergence. Each test included 500-1200 pupae (number determined by emergence of moths), and 37 tests in total were conducted on different dates. Emerging adults were held with fresh uninfested roots to examine reproduction and development of any progeny to adulthood. A subset of uninfested roots was held similarly but not irradiated as a control.

E. postfasciatus. Laboratory tests were conducted with *E. postfasciatus* to determine the most tolerant stage occurring in the root and to identify a dose to control the most tolerant stage. A laboratory colony of *E. postfasciatus* was maintained in plastic tubs on whole sweet potato roots at 25 ± 2°C and a photoperiod of 14:10 (L:D) h. Fresh roots were irradiated at a high dose (350–450 Gy) to eliminate any natural infestation then placed in a plastic container for 24 h with 100 reproductive adults for oviposition. Roots with eggs were transferred to new plastic containers for hatch and larval and pupal development. At 14, 21, 28, 35, 42, and 49 d a subset of infested roots was irradiated at 75 Gy and then held for adult emergence. Raman and Alleyne (1991) reported average development times for eggs, larvae, and pupae as 8, 27, and 8 d, respectively, at 24°C. Under our laboratory rearing conditions, adult weevils begin emerging at 40–42 d and are at ≈30% emergence at 49 d and continue to emerge for 3 wk. Roots selected for each age treatment started with approximately the same total number of eggs. Adult emergence was recorded.

E. postfasciatus pupates within the root and emerging adults may remain in the root and feed before leaving. To evaluate the effects of irradiation on sterility, adult weevils newly emerged to 1wk old were placed in sweet potato roots with the centers bored out and irradiated at 50, 100, 150, and 200 Gy. Tests were replicated four times with 100 adults in each replicate. A 1:1 sex ratio was assumed (Raman and Alleyne 1991). At each irradiation dose, additional roots were included containing dosimeters placed in bore holes at the center of the root to estimate dose variation. For each replicate, an equal number of adults were left unirradiated as a control. After irradiation, weevils were placed in plastic tubs with fresh sweet potatoes to allow oviposition. Adults were removed after 3 wk, and roots were held for F_1 adult emergence.

In large-scale confirmatory tests, mated adult weevils were transferred into sweet potato roots with the centers bored out and treated 24 h later with an irradiation dose of 150 Gy. Each test included 3,000–10,000 weevils divided evenly among 10–30 sweetpotato roots, and 15 tests in total were conducted on different dates. Treated adults were placed on uninfested roots to examine reproduction. Fresh roots were provided every 2 wk until all weevils had died, and roots were held for F_1 adult emergence. Unirradiated adult weevils were held similarly on uninfested roots as controls.

C. formicarius elegantulus. Large-scale confirmatory tests were conducted with an irradiation dose of 150 Gy to determine whether *C. formicarius elegantulus* could be controlled at the same irradiation dose as the other two pests. The adult was assumed to be the most radiotolerant stage (Hallman 2001). Mated adult weevils were transferred into sweet potato roots with the centers bored out and treated 24 h later with an irradiation dose of 150 Gy. Each test included 10,000–15,000 weevils divided evenly among 30–40 sweetpotato roots, and five tests in total were conducted on different dates. Treated adults were placed on uninfested roots to examine reproduction. Fresh roots were provided every 2 wk until all weevils had died, and roots were held for F_1 adult emergence. Unirradiated adult weevils were held on uninfested roots as controls.

Statistical Analysis. Adult emergence and frass data were subjected to regression analysis and analysis of variance (ANOVA), and mean separations were done with a Tukey's test (SAS Institute 2000). Data were log $(x + 1)$ transformed before ANOVA.

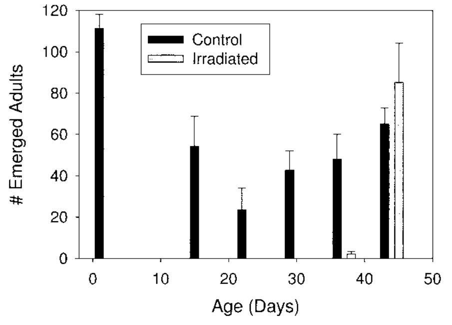

Fig. 1. Mean emergence of adults after treating immature *O. anastomosalis* of various ages with an irradiation dose of 100 Gy.

Results

O. anastomosalis. Emergence of adult *O. anastomosalis* after irradiation of sweet potatoes naturally infested with mixed age larvae and pupae was significantly reduced at all doses compared with the control ($P \leq 0.05$, Tukey's test). Mean adult emergence in the 0- (control), 100-, 200-, and 400-Gy treatments was 49.0, 2.0, 1.8, and 0.0, respectively. Moth emergence began 5 d after treatment and continued for 30 d in both the control and irradiation treatments. Fertility of irradiated moths was not evaluated in this study. Measured doses were 94 (range 85–97), 186 (range 176–197), and 365 (range 356–383) for the 100-, 200-, and 400-Gy treatments, respectively.

Irradiation of immature *O. anastomosalis* in roots with a dose of 100 Gy resulted in no adult emergence in the 2-, 16-, 23-, and 30-d-old treatments (Fig. 1). In total, eight and 341 moths emerged from the 37- and 44-d-old treatments, which represented 2.3 and 97.7% of the total number of irradiated individuals emerging, respectively. Developmental rate studies showed that most 37–44-d-old *O. anastomosalis* were in the pupal stage. Therefore, the pupal stage is the most radiotolerant stage occurring in the sweetpotato root. For the untreated controls, 1,380 moths in total emerged from all the age treatments (Fig. 1). Differences among treatment dates in the number of adults emerging from control roots represent random variation. Adult emergence from the 2-d-old control treatment was significantly different from the other age treatments ($P \leq 0.05$).

When reproductive adult moths were irradiated in roots at various irradiation doses, F_1 adult emergence decreased with increasing dose [$\log(F_1 \text{ moths}) = 4.14 - 0.06(\text{irradiation dose})$, $r^2 = 0.96$] (Table 1).

Table 1. Effect of irradiation dose applied to adults of *E. postfasciatus* and *O. anastomosalis* in sweet potatoes on development and production of F_1 adults

Dose (Gy)	*n*	*E. postfasciatus*				*O. anastomosalis*	
		No. F_1 adults[a]		Frass (g)[b]		No. F_1 adults[a]	
		Mean	SEM	Mean	SEM	Mean	SEM
0 (control)	4	1910.8a	157.8	906.3a	186.6	64.0a	15.8
50	4	105.5b	24.6	15.8b	18.1	3.0b	2.4
100	4	1.5c	1.0	0.1c	0.2	0.0c	0.0
150	4	0.0c	0.0	0.0c	0.0	0.0c	0.0
200	4	0.0c	0.0	0.0c	0.0	0.0c	0.0
250	4	0.0c	0.0	0.0c	0.0	0.0c	0.0

Means followed by different letters are significantly different ($P < 0.05$) by Tukey's test.
[a] Progeny of irradiated adults that emerge after completing development in the root.
[b] Frass weight is an indicator of the number and age of larvae developing in the root.

Frass production by progeny also decreased with increasing dose [log(frass weight) = 6.54–0.08(irradiation dose), $r^2 = 0.82$]. Frass production and F_1 adult emergence were greatly reduced in the 50-Gy treatment compared with the untreated control. A small amount of frass was produced in the 100-Gy irradiation treatment, but no adult emergence was observed. No frass and no F_1 adult emergence were observed in the 150-, 200-, and 250-Gy irradiation treatments.

In small-scale confirmatory tests at a single dose with gravid moths, 900 females irradiated at 250 Gy laid many eggs, but no progeny survived to the adult stage. Also, no frass was observed on the outside of the sweet potato roots, indicating that any larvae hatching from eggs did not successfully develop. Unirradiated moths produced 585 F_1 adults, and 5,482 g of frass was weighed from the outside of roots. Gravid moths irradiated at 150 Gy also laid many eggs but produced no F_1 adults, and no frass was observed on the outside of the roots. Unirradiated moths produced 709 F_1 adults.

Large-scale confirmatory tests were conducted with pupae, the most tolerant stage associated with sweet potato roots. Dose–response tests suggested a dose of 150 Gy would be sufficient to prevent reproduction in adults. In confirmatory tests with pupae, 30,282 adults emerged after irradiation treatment at 150 Gy and laid many eggs, but they produced no F_1 adults, whereas 6,300 unirradiated pupae (controls) produced 10,331 F_1 adults (Table 2). All measured irradiation doses were <150 Gy.

E. postfasciatus. No adult weevils emerged in the 7-, 14-, 21-, and 28-d-old treatments when roots were irradiated at 75 Gy (Fig. 2). One adult emerged from the 35-d-old treatment, 78 adults emerged from the 42-d-old treatment, and 248 adults emerged from the 49-d-old treatment, which represented 0.003, 23.9, and 75.8% of the total number of irradiated individuals emerging, respectively. Developmental rate studies showed that most 35–49-d-old weevils were in the pupal stage. In the 49-d-old treatment, a few adults had emerged at the time of treatment, confirming that many individuals were likely to be in the pupal stage. In total, 1,026 adults emerged from the unirradiated controls (Fig. 2). Differences among treatment dates in the number of adults emerging from control roots represents random variation, and none of the differences were significant ($F = 0.9$; df = 5, 18; $P = 0.5$).

When adults were irradiated in roots at various irradiation doses, F_1 adult emergence decreased with increasing dose [log(F_1 weevils) = 7.62–0.06(irradiation dose), $r^2 = 0.97$] (Table 1). In the 50-Gy treatment, mean adult emergence was 105.5, which was significantly less than the mean adult emergence from control roots ($t = -11.3$; df = 1, 6; $P = 0.001$). A small number of adults emerged in the 100-Gy treatment, and no F_1 adult emergence was observed in the 150- and 200-Gy irradiation treatments. In unirradiated control roots, 7,643 adults emerged.

Radiotolerance of the adult and pupal stages of *E. postfasciatus* were not specifically compared. The adult stage was selected for large-scale testing because

Table 2. Large-scale confirmatory tests irradiating three sweet potato quarantine pests

Species	Stage treated[a]	Target dose (Gy)[b]	Measured doses (Gy)	No. tested	F_1 adults[c]	Control	
						No. tested	F_1 adults
O. anastomosalis	Pupa	150	135–148	30,282	0	6300	10,331
E. postfasciatus	Adult	150	130–145	60,000	0	1550	10,543
C. formicarius elegantulus	Adult	150	125–140	62,623	0	600	6398

[a] Sweet potatoes were infested by natural oviposition in a cage (*O. anastomosalis*) or artificial inoculation (*E. postfasciatus* and *C. f. elegantulus*).
[b] Maximum absorbed dose.
[c] F_1 generation adults emerging after irradiation of parental generation as pupae (*O. anastomolsalis*) or adults (*E. postfasciatus* and *C. formicarius elegantulus*).

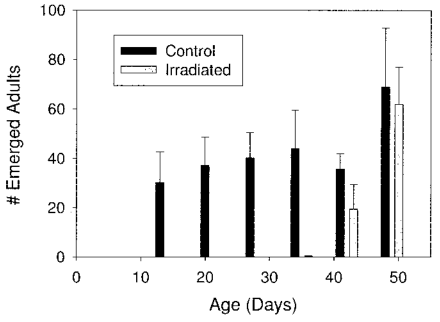

Fig. 2. Mean emergence of adults after treating immature *E. postfasciatus* of various ages with an irradiation dose of 75 Gy.

it is the most advanced stage occurring in the root and the trend from our data suggested that weevils become more radiotolerant as they mature (Fig. 2). Dose–response tests suggested a dose >100 Gy would be necessary to prevent reproduction in adults (Table 1). In large-scale confirmatory tests, 60,000 adult weevils were treated at an irradiation dose of 150 Gy with no successful reproduction and development to the next generation (Table 2). All measured irradiation doses were below 150 Gy. In the unirradiated control treatments, 10,543 F_1 adults emerged from roots exposed to 1,550 reproductive adults.

C. formicarius elegantulus. In large-scale confirmatory tests, 62,623 adult weevils were treated at an irradiation dose of 150 Gy with no successful reproduction (Table 2). All measured irradiation doses were >150 Gy. In the unirradiated control treatments, 6,398 F_1 adults emerged from roots exposed to 600 reproductive adults.

Discussion

Based in part on results from the early stages of this research, USDA–APHIS published an interim rule (Federal Register 2003), and then a Final Rule (Federal Register 2004), allowing interstate movement of sweet potatoes from Hawaii with an irradiation treatment of 400 Gy. This was the first time USDA–APHIS considered the high-dose approach for controlling a pest complex until research is completed to confirm a lower dose. Immediately after the interim rule was published, 18,000–28,000 kg of sweet potatoes per week was being exported from Hawaii to the U.S. mainland with irradiation treatment. Irradiated sweet potato exports from Hawaii are now averaging 34,000–45,000 kg/wk or 1.8–2.3 million kg/yr.

In our study, large-scale confirmatory tests demonstrated that irradiation treatment of the three sweet potato pests at a dose of 150 Gy results in no F_1 progeny, indicating that 150 Gy is sufficient to prevent reproduction and provide quarantine security. Lowering the irradiation dose from 400 to 150 Gy will lower treatment costs. No sweet potatoes exported from Hawaii have been treated with methyl bromide fumigation since the irradiation treatment became available. A vapor heat treatment was recently proposed that would give sweet potato growers and exporters a quarantine treatment option for organic food markets (Shimabukuru et al. 1997, Federal Register 2005).

Unlike other quarantine control techniques, irradiation does not need to kill the pest immediately to provide quarantine security, and therefore live (but nonviable or sterile) insects may occur with the exported commodity (Follett and Griffin 2006). Many *O. anastomosalis* and *E. postfasciatus* in various tests were alive and continued to develop after irradiation treatment. For example, in the age tolerance tests, a significant number of *O. anastomosalis* pupae treated at 100 Gy and *E. postfasciatus* pupae irradiated at 75 Gy emerged as adults. When *O. anastomosalis* pupae were irradiated at 150 Gy, many eclosed and laid eggs as adults, but eggs did not hatch. Lowering the quarantine irradiation treatment dose from 400 to 150 Gy might result in a greater number of sterile adult pests emerging from sweet potato roots after export. In our study with naturally infested roots, more sweetpotato vine borers emerged from roots irradiated at 100 and 200 Gy than from those irradiated at 400 Gy. In commercial practice, product is culled to eliminate any roots with obvious insect damage before export, and this procedure coupled with irradiation treatment should result in few live or emerging pests.

The dose–uniformity ratio for commercially irradiated sweet potatoes in Hawaii is ≈2.0. The current requirement for a minimum absorbed dose of 400 Gy results in some roots receiving a dose of 800–900 Gy. Research indicates that roots irradiated at 600 Gy suffer minimal loss in quality (Wall 2004); higher doses have not been tested. Adoption of the proposed 150-Gy dose will result in commercial doses up to 300–400 Gy, which will minimize any deleterious effects on quality (Wall 2005).

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