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Irradiation quarantine treatment for control of *Sitophilus oryzae* (Coleoptera: Curculionidae) in rice

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ABSTRACT

Irradiation is a quarantine treatment option for stored products pests. Dose–response tests were conducted to identify a post-harvest radiation treatment that would control rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) in rice. Rice infested with adult or immature weevils was treated at radiation doses of 30, 60, 90, or 120 Gy, or left untreated as a control. Live and dead beetles were counted weekly for 15–24 weeks. Treatment of adult weevils at a radiation dose of 120 Gy resulted in no live adults after two weeks, indicating that this radiation dose caused adult mortality and sterility, whereas a total of 1261 adult beetles emerged during 24 weeks in the untreated controls. Treatment of immature life stages (a mixture of eggs, larvae and pupae) with a radiation dose of 90 or 120 Gy resulted in no adults emerging after five or two weeks, respectively, indicating that these doses prevented reproduction, whereas a total of 4275 adults emerged throughout 15 weeks in the untreated controls. Weight loss of rice infested with immature or adult weevils was significantly reduced by irradiation treatment at 60 Gy and 120 Gy. In a large-scale confirmatory test, a radiation dose of 120 Gy applied to 38,025 adult weevils in rice resulted in no reproduction. Irradiation at 120 Gy will provide quarantine security for rice weevil, and prevent post-irradiation weight loss caused by insect feeding in the commodity. Irradiation may be particularly helpful in controlling phosphine-resistant populations, and could help manage resistance by preventing the spread of resistant weevils in exported grains.

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1. Introduction

An estimated 5–10% of cereals is lost during storage as a result of insect infestation (Ahmed, 2001). Most domestic and export markets have zero tolerance for grain contamination by insects. Fumigation treatment with phosphine ensures that grain can be stored and exported in a clean and uninfested condition. The primary source of storage loss in rice in terms of weight and sensory quality is the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). The adult female rice weevil bores an oblong shallow cavity in the kernel and deposits an egg, then seals the egg cavity with a clear and gluey secretion (Metcalf et al., 1962; Plarre, 2010). Each kernel only hosts one rice weevil egg. The entire larval and pupal period of the beetle is spent hidden inside the kernel, and during the larval period the kernel is completely hollowed out. Once the adult beetle emerges from the pupa, it remains for a short time inside the kernel until it has bored its way to the outside. Adult weevils may crawl back into hollow kernels where they are difficult to detect. Adult weevils can live for 6–8 months or more, and females may lay 300–400 eggs in their life time (Metcalf et al., 1962; Longstaff, 1981).

Resistance to phosphine poses a major challenge to the control of rice weevil and other stored product pests, and alternative control methods are needed (Donahaye, 2000; Chaudhry, 2000; Nayak et al., 2007). Also, it may become advantageous to slow the spread of traits conferring phosphine resistance in rice weevil and other stored product pests by using quarantines for areas showing high levels of resistance, so alternative quarantine treatments or approaches would be useful to safely move the commodity out of the quarantine area.

Irradiation has potential as a method to control rice weevil. Hoedaya et al. (1973) irradiated stored rice infested with rice weevil and 99% were killed at 200 Gy after three weeks; Tilton et al. (1966) found 100% mortality of rice weevils treated at 175 Gy after three

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weeks; and Tuncbilek (1995) irradiated adult rice weevils at 90 Gy and mortality was 100% after three weeks. Ignatowicz (2004) irradiated immature rice weevils (eggs, larvae and pupae) at 80 and 100 Gy and observed no development to the adult stage. Radiation treatment of adults of the closely related weevil, *Sitophilus granarius* (L.), required 100 Gy to achieve sterility (Aldryhim and Adam, 1999). Immediate kill of rice weevil and other stored product insects may require much higher doses, in the range of 1–3 kGy (Ignatowicz, 2004). Data on the effectiveness of irradiation using criteria acceptable to regulatory authorities needs to be developed before a quarantine treatment can be recommended.

Research was conducted to identify a radiation dose that will prevent development of immature rice weevils or sterilize adults. Large-scale tests were performed to confirm the efficacy of the dose as a quarantine irradiation treatment (Follett, 2009).

2. Methods

2.1. Dose–response tests

A rice weevil colony was started from a heavily infested bag of whole grain rice (Shirakiku Sukoyaka Genmai, Nishimoto Trading Co., Santa Fe Springs, California, USA) obtained from a residence in Hilo, Hawaii. The stock colony was maintained in multiple 3.8-L plastic containers with approximately 1 kg rice at 24 °C (±2 °C) and a photoperiod of 12:12 (L:D) h. Adult weevils were periodically moved to new containers with fresh rice. Dose–response tests were conducted with mixed-age immature (eggs, larvae, pupae) or adult rice weevils. For adult dose–response tests, 15 adults were collected randomly from the stock colony, and placed on 500 g fresh rice (Pearl Blossom Natural Brown Calrose Rice, American Rice Co., Houston, Texas, USA) in each of 20 plastic containers. A 1:1 sex ratio was assumed (Howe, 1952). Containers with weevils and rice were treated with radiation doses of 30, 60, 90, or 120 Gy or left untreated as a control. The experiment used a completely randomized design with four replicates per dose. The number of live and dead adults in each container was counted weekly for five months (sufficient time to complete 2–3 generations), and dead adults were removed.

The dose–response tests with immature weevils used infested stock colony rice with adult weevils removed. The rice was mixed thoroughly and divided by 400 g lots into 20 containers (four replicates for control, 30, 60, 90, and 120 Gy treatments). Containers with rice and a variable number of immature insects were irradiated using a completely randomized design with four replicates per dose. The number of live and dead adults in each container was counted weekly for five months, and dead adults were removed. The time of adult emergence in untreated controls from weekly counts was used to estimate the number of eggs, larvae and pupae in containers at the time of irradiation using development times reported in Sharifi and Mills (1971). In all experiments, containers with weevils and rice were held at 24 °C (±2 °C) and a photoperiod of 12:12 (L:D) h.

2.2. Weight loss tests

Whole grain rice (Shirakiku Sukoyaka Genmai, Nishimoto Trading Co., Santa Fe Springs, California, USA) purchased at a grocery store was stored at −18 °C for six days to ensure that rice weevils or other insects were killed. Adult and immature weevil responses to irradiation were evaluated using a completely randomized design with three treatments: irradiation of rice infested with weevils (at 60 or 120 Gy), rice infested with weevils left untreated (0 Gy), and uninfested and untreated rice. In the adult tests, fifteen adults were collected from the stock colony, irradiated in plastic cups at 60 or 120 Gy (8 replicates each), and placed on 500 g rice in 1-L plastic containers with screen lids. Four replicate containers of rice with adults were left untreated as controls, and four containers of rice without weevils were held as another control to determine natural weight loss due to drying. In the immature test, fifteen adult weevils were added to 250 g rice for one month before removal of adults and subsequent irradiation of the infested rice at 60 Gy (8 replicates). Four replicate containers of rice with immature weevils were left untreated as controls, and four containers of rice without weevils were held as another control to determine natural weight loss due to drying. In all experiments, the number of live and dead adults in each container was counted weekly for eight weeks, and dead adults were removed. The rice in containers was weighed each time weevils were counted and removed. For the immature test, adult emergence was counted daily in a separate 100 g sample of rice infested with immature rice weevils to estimate the number of eggs, larvae and pupae in containers at the time of irradiation using reported development times (Treiman, 1937; Sharifi and Mills, 1971). Insect density at the start of the adult tests was 30 adult weevils per kg, and the density at the start of the immature test (as estimated by adult emergence during the test) was approximately 550 immature insects per kg.

2.3. Large-scale confirmatory test

Confirmatory tests were conducted by treating large numbers of adult rice weevils with a radiation dose of 120 Gy. Approximately 1500 adults from the stock colony were counted and placed on 1 kg rice in 1.5-L ventilated plastic containers 1–4 days before irradiation. As controls, approximately 145 adults were placed on 1 kg rice in 1.5-L ventilated plastic containers and held similarly. Irradiated containers were replicated 25 times and unirradiated containers were replicated five times in a completely randomized design. Adults were removed from containers after 30 days, and the rice was held for an additional 30 days for any emergence of F_1 adults. No emergence of F_1 adults from buckets containing rice and irradiated rice weevils would indicate that adults were sterilized, whereas emergence of F_1 adults would indicate that the radiation treatment was not completely effective. Large-scale confirmatory tests were also conducted at 150 Gy using similar methods.

2.4. Irradiation and dosimetry

Irradiation treatment was conducted at a nearby commercial X-ray irradiation facility (CW Hawaii Pride, Keaau, HI) using an electron linear accelerator (5 MeV, model TB-5/15, Titan Corp., San Diego, CA). ROW dosimeters (Opti-chromic detectors, FWT-70-83/40M, Far West Technology, Goleta, California) were placed in several containers with rice in each treatment to measure dose variation. Dosimeters were read with an FWT-200 reader (Far West Technology, Goleta, CA) at 600-nm absorbance.

2.5. Statistical analysis

Repeated measures analysis of variance (MANOVA) (SAS Institute, 2010) was used to separate the effect of irradiation dose on population size in the dose–response tests. A univariate test with a Huynh–Feldt Epsilon correction factor was used to test the effect of time after treatment, and the time by dose interaction on population numbers. When the irradiation dose by time interaction was significant, paired contrasts were used to make comparisons between dose treatments. Data on mean % weight loss were subjected to ANOVA, and when treatment effects were significant, means separations were done by using a Tukey's HSD test.