Ionizing Irradiation Quarantine Treatment Against Plum Curculio (Coleoptera: Curculionidae)

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ABSTRACT Plum curculio, *Conotrachelus nenuphar* (Herbst), is a quarantine pest of many temperate fruits, such as pomes, *Malus* spp.; stone fruits, *Prunus* spp.; and blueberries, *Vaccinium* spp.; in North America east of the Rocky Mountains and a small area in Utah. There are two strains, a northern univoltine one that undergoes obligate diapause as an adult and a southern multivoltine strain that usually has facultative diapause. Current quarantine treatments for shipment to areas that do not have the pest include methyl bromide fumigation and cold storage for several weeks. The cold storage treatment may not be effective against northern strain adults in diapause. The objective of this research was to develop an irradiation quarantine treatment against plum curculio. The estimated dose to kill southern strain plum curculio adults in one day is ≈4 kGy. Diapausing northern strain plum curculios were prevented from reproducing with 40 Gy. Reproduction of southern strain plum curculios was prevented with a target dose of 80 Gy, and the dose recommended as a quarantine treatment, that would prevent adults from reproducing, is 92 Gy, the maximum absorbed dose measured when a target dose of 80 Gy was sought. At that dose, oviposition may still occur for up to 1 wk and some of the eggs may hatch, but there is no development beyond the first instar. Hosts of the plum curculio would tolerate that dose well. Immature plum curculios were prevented from reproducing with lower doses.

KEY WORDS *Conotrachelus nenuphar*, gamma radiation, phytosanitary treatment, disinfestation

THE PLUM CURCULIO, *Conotrachelus nenuphar* (Herbst), occurs in temperate North America east of the Rocky Mountains and north of latitude 28 degrees North with one isolated introduced population in Box Elder County, UT. There are two strains: the northern strain is univoltine and undergoes obligate diapause, and the southern strain is multivoltine and usually undergoes facultative diapause. The two strains are somewhat reproductively incompatible (Padula and Smith 1971). Plum curculios infest a wide variety of fruits, including pomes, *Malus* spp.; stone fruits, *Prunus* spp.; and blueberries, *Vaccinium* spp. Much of the world, including the west coast of the United States, has quarantine barriers against fruits that might be infested with this pest. Hosts of plum curculio shipped out of the infested range are subjected to methyl bromide fumigation if there is a risk of infestation. Continued use of methyl bromide worldwide is in jeopardy because the fumigant is considered to play a significant role in stratospheric ozone depletion. Although postharvest quarantine uses of methyl bromide have been exempted from phasing out, there is no certainty that the fumigant will not be lost as a quarantine treatment or at least become more scarce and expensive in the future. The price of methyl bromide has more than tripled since 1999. Alternative quarantine treatments are needed. Cold treatments of 40 d at 0°C or 45 d at 2.2°C are tolerated by apples, but other fruits, such as stone fruits, cannot tolerate them.

Ionizing radiation has been studied as a quarantine treatment for many insects, especially tephritid fruit flies (Hallman 1999). Commodities are exposed to gamma or x-rays, which are able to penetrate loads in palletized or similar arrangements. The mode of action of ionizing irradiation as a quarantine treatment involves breaking of chemical bonds. When this happens to DNA, normal development or reproduction of the organism may be prevented. There is no residual activity, and irradiation does not make food harmful to human health. Currently several fruits that are hosts of tephritids are irradiated in Hawaii and shipped to the rest of the United States. Guavas, *Psidium guajava* L., and sweetpotatoes, *Ipomea batatas* (L.) Lam., are irradiated for quarantine control of the Caribbean fruit fly, *Anastrepha suspensa* (Loew), and the sweetpotato weevil, *Cylas formicarius elegantulus* (Summers), respectively, in Florida for shipment to Texas and California. The United States has accepted irradiation quarantine treatments against 11 fruit fly species and mango seed weevil, *Cryptorhynchus mangiferae* (F.) and has proposed it for green scale, *Coccus viridus* (Green) (USDA–APHIS 2002a, 2000b). Efficacy, host tolerance, consumer acceptance, and economic returns with irradiation have been favorable. The expanded use of irradiation as an effective quarantine

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treatment with no health or environmental detriments looks positive.

A quarantine treatment must be virtually 100% effective. Irradiation is unique among quarantine treatments in that the objective is not acute mortality but prevention of development or reproduction. Achieving 100% acute mortality requires doses of irradiation not tolerated by fresh commodities. Adult plum curculios exposed to 1 kGy still lived up to 4 d (Lippold et al. 1968). Hosts of the plum curculio tolerate 0.4–0.9 kGy (Drake and Neven 1997, 1998; Drake et al. 1999; Miller and McDonald 1996). The objective for irradiation of tephritids is prevention of adult emergence; only eggs and larvae are present in fruit. Because adult plum curculios may be present in packed fruits, an irradiation treatment would have to ensure their sterility. Small numbers of plum curculio adults irradiated with 80 Gy produced few eggs and none of them hatched (Jacklin et al. 1970).

The objective of this research was to establish irradiation quarantine treatment doses for plum curculio based on sufficient numbers of insects to achieve a high level of security. A quarantine treatment must be effective against the most tolerant stage of the quarantine pest that may be on the commodity when it is shipped. The most tolerant stage to irradiation invariably seems to be that which is most developed (Hallman 2001). Although the egg and larvae are the most common stages that could be present on harvested fruit, adult plum curculios could also be present. Therefore, the research reported here was focused on determining the dose necessary to stop reproduction in actively reproducing adults. Another objective was to confirm that immatures were more susceptible to radiation than adults.

Materials and Methods

Plum Curculio. Southern strain plum curculios were obtained from a colony at the United States Department of Agriculture, Agricultural Research Service facility in Byron, GA. They had originally been collected in the field near Gainesville, FL. The insects were reared at ≈25°C, 70% RH, on immature apples that were picked when ≈3 cm in diameter. Larvae emerging from the apples were placed on sterilized potting soil until adult emergence.

Northern strain plum curculios were collected as larvae in apples from the field in Massachusetts and sent to Weslaco as adults.

Irradiator. Radiation was applied with two ^{137}Cs sources (Husman model 521A, Isomedix, Inc., Whippany, NJ) that were delivering calculated centerline gamma ray dose rates of ≈20 and 40 Gy·min^{-1} during the time of this research. While transiting the machine for exposure to the ^{137}Cs source, the product being irradiated rotates a total of ≈245° on a horizontal axis in two roughly equal steps (one before and one after exposure), moving at a speed of ≈7 cm·sec^{-1}. Reference standard dosimetry was done on one irradiator in 1985 and the other in 1996 using the Fricke system. Routine dosimetry was done with radiochromic film (Gafchromic MD-55, ISP Technologies, Inc., Wayne, NJ) and read with a spectrophotometer at 510 nm (Milton Roy Spectronic 401, Ivyland, PA). Two routine dosimeters were used in every fifth cylinder, one placed in the center of the load and one on the outside. These locations were shown by reference standard dosimetry to receive the minimum and maximum, respectively, absorbed doses.

Irradiation of Immature Stages. Immature apples ('Red Delicious', mean diameter 3 cm) that were picked in Washington State in June were infested with southern strain plum curculio by allowing 90–111 adults to oviposit on them in cages for 2 d (eggs) or 5 d (larvae). At 26°C, the first late fourth instars began emerging from the apples to pupate in the soil at ≈2 wk. Several apples containing 2-d-old eggs or approximately third-instar larvae (1 wk after removal from infestation cages) were placed inside cylindrical paper containers (0.5 liter) that in turn fit into stainless steel perforated cylinders (11.4 cm i.d., 50 cm long) and were irradiated with target doses of 0, 20, 40, 60, 80, 100, 150, and 300 Gy. The unirradiated cohort of infested apples was used as the control and to estimate the number of insects present in the apples. There were two and four replicates of 13–17 apples per dose for eggs and larvae, respectively, with an estimated mean of 69 and 243 insects per dose for eggs and larvae, respectively. After irradiation, the apples containing eggs and larvae were placed in plastic containers at ≈26°C to allow for continued development of the larvae. Emerging larvae were counted and placed on moist, loose, loamy potting soil for pupation. Apples were opened and examined for additional larvae. Pupation, adult emergence, and reproduction were recorded. Data were subjected to analysis using PROC PROBIT with normal and Gompertz probability density functions (SAS Institute 1988).

Fifty early (≈7 d after pupation) and 50 late (≈2 d before first adult emergence was expected) pupae in soil were irradiated with 0, 20, 40, 60, or 80 Gy. Adult emergence and reproduction on small apples were recorded (two replicates).

Irradiation of Adults. Actively reproducing (2–3-wk-old) southern strain adults were irradiated to determine both the dose required for adult mortality and that required to prevent reproduction. In both cases, adults were irradiated with small apples in the same paper and stainless steel cylinders as with larvae. Doses were 0, 80, 150, 300, 500, 1000, and 2000 Gy for mortality determination and 0, 20, 40, 60, 80, 100, 150, and 300 Gy for reproductive determination. After irradiation, adults were maintained on small apples at ≈25°C, and mortality was determined every 3–4 d by placing apparently dead adults on a hot plate at ≈60°C to check for movement. The apples were replaced every 3–4 d and maintained at 25°C for development of any immatures inside. Larvae emerging from apples were collected every 2–3 d and placed on potting soil for pupation and adult emergence. After larvae were no longer emerging, the apples were opened and any remaining insects collected. Data were subjected to analysis using PROC PROBIT with normal and Gomp-

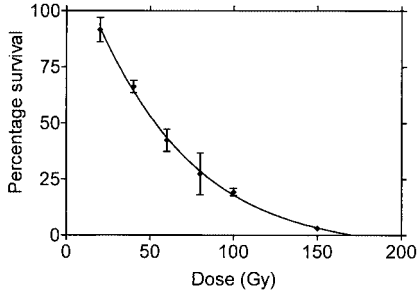

Fig. 1. Mean survival (SE) to fully formed fourth instars after irradiation of approximate third-instar southern strain plum curculio in apple. At 300 Gy, no larvae completed development.

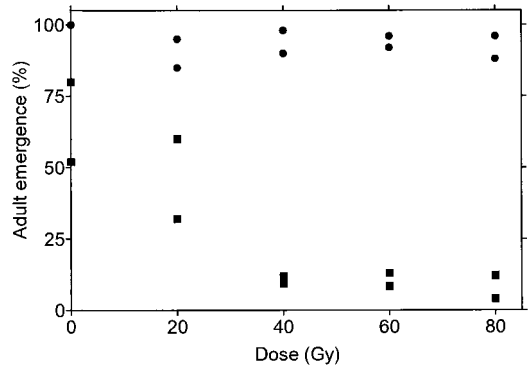

Fig. 2. Adult emergence from early (≈7 d after pupation and represented by squares) and late (≈2 d before first expected adult emergence and represented by circles) plum curculio pupae at various irradiation doses.

ertz probability density functions (SAS Institute 1988).

Northern strain adult plum curculios (100, 100, 200, 400, and 970, respectively) with small apples in the same containers used for southern strain adults were irradiated with 0, 20, 40, 60, or 80 Gy in 1997 and 0, 20, or 80 Gy (100, 100, and 395, respectively) in 1998. Two weeks after irradiation, the plum curculios were subjected to a temperature regime to break diapause: 1 mo at 10°C, 1 mo at 5°C, 3 wk at 1°C, 2.5 wk at 5°C, 2.5 wk at 10°C, and finally 1 wk at 21°C, with a photoperiod of 16:8 (L:D) h. Afterwards, dead weevils were counted, and survivors were offered immature apples and allowed to reproduce.

Because quarantine treatments demand such a high level of control (near 100%), they should be confirmed by treating a large number of the most tolerant stage with no survivors. Twenty five thousand 2-wk-old southern strain adults were irradiated with a target dose of 80 Gy (a dose indicated by this research to possibly provide quarantine security) and held with small apples in the same manner as before until they died to observe reproduction compared with nonirradiated adults.

Results

Immatures. No eggs developed beyond early instars at any of the irradiation doses as no mature larvae emerged from these treatments, whereas a mean of 69 fully developed fourth instars was produced in the control. Therefore, tests with eggs were halted after two replicates, and it was concluded that the egg stage was quite susceptible to irradiation and would be controlled by doses required to prevent reproduction by adults.

The number of approximately third-instar plum curculios that failed to complete larval development after irradiation increased with dose until 97% failed at 150 Gy and 100% failed at 300 Gy (Fig. 1). The data fit probit analysis, normal probability density function (PDF) using the normal logarithm of dose (F value = 9.7; intercept = -6.5 ± 0.43; slope = 3.7 ± 0.24; n = 243 larvae/dose), but did not fit the Gompertz PDF or normal PDF without log transformation of dose. The estimated doses for 50 and 99% control were 54 Gy (95% fiducial limits: 49–59 Gy) and 225 Gy (95% fiducial limits: 185–293 Gy), respectively. There was no correlation between weights of surviving larvae (mean = 17.4 mg, range = 12.4–24.4 mg) and dose; the closest fit ($y = a + bx^{0.5}\ln x$) to the 21 simplest equations in TableCurve (version 1.0, Jandel Scientific, San Rafael, CA) had an r^2 of only 0.004. Forty seven percent of unirradiated larvae pupated and 34% formed adults, whereas 48% of larvae irradiated with 20 Gy pupated and 25% formed adults; no larvae exposed to ≥40 Gy pupated. Adults that were exposed as larvae to 20 Gy did not successfully reproduce, whereas control adults did.

Adult emergence from pupae is given in Fig. 2. Although all of the nonirradiated late pupae emerged as adults, only 66% of the early pupae did. The lower emergence of early pupae was probably a result of handling, which would be expected to have a greater detrimental effect to pupae early in development compared with those close to adult emergence. Doses ≥40 Gy lowered adult emergence of early pupae considerably, but evenly. The highest dose, 80 Gy, did not reduce adult emergence of late pupae markedly. None of the adults irradiated as pupae reproduced.

Adults. Two-week-old southern strain plum curculio adults lived up to a mean of 5.8 and 24.8 d when irradiated with 2000 and 80 Gy, respectively (Fig. 3). The first death from those irradiated with 2000 Gy was at 20 h and the last at 7 d. The data fit the equation: $t = 50.5 + 6.0 \cdot \ln(d)$, where t is days to 100% mortality and d is dose in Gy (F statistic = 200; r^2 = 0.98). This indicates that the dose required to provide near 100% mortality in 1 day would be ≈4 kGy, upon extrapolation. After 25 d, only 13.3 ± 2.3% of unirradiated weevils had died. None of the irradiated (≥80 Gy) adults reproduced. Adults irradiated with 2000 Gy did not feed. Those irradiated with 0.5 or 1000 Gy fed very little, whereas the rest fed more, although less than control weevils.

A mean of 34.4 fourth-instar offspring per southern strain female was achieved in the unirradiated control

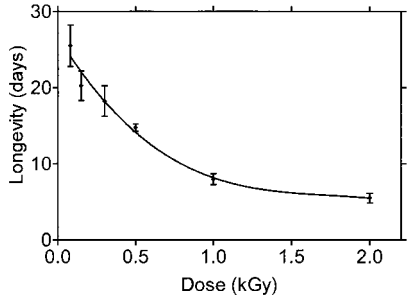

Fig. 3. Mean longevity (SE) of 2-wk-old southern strain plum curculio adults irradiated with apples at 80–2000 Gy. When the last of the irradiated (80 Gy) adults died (after a mean of 25.5 d), a mean of 86.8% of control (nonirradiated) adults were still alive.

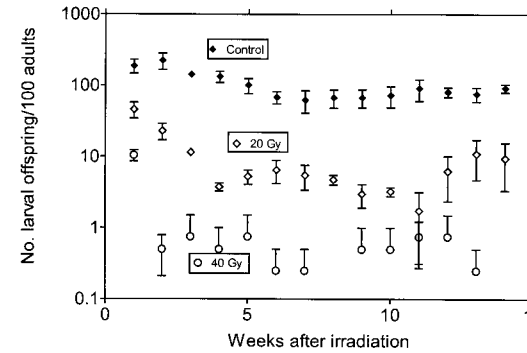

Fig. 5. Mean numbers (log scale, SE) of fourth instars by week produced by 2-wk-old southern strain plum curculio adults irradiated with apples at 0, 20, or 40 Gy.

with progressively less being produced by irradiated plum curculio until one resulted from a total of 400 adults irradiated at 60 Gy; at ≥80 Gy, no fourth-instar progeny were produced (Fig. 4) and larval tunneling was not observed in the apples. Quantity of reproduction was reduced slightly from the first few weeks for the control and adults irradiated at 20 Gy (Fig. 5). As in the tests done at higher doses (Fig. 3), all irradiated weevils, including those subjected to the lowest dose (20 Gy) had shorter lives than unirradiated weevils. This agrees with the literature for the great majority of arthropods (Hallman 2000).

Although no larvae were observed developing from southern strain adults irradiated with 80 Gy, they laid eggs for up to 1 wk after irradiation, with the number falling off exponentially from the first day to the eighth day (Fig. 6).

In 1997, 1,770 northern strain plum curculio adults did not reproduce until after being exposed to the temperature regime to end diapause. In 1998, 35 fourth instars resulting in 28 adults were produced by 600 adults in the 4 wk before irradiation. In the 2 wk between irradiation and the initiation of cold treatment, five more fourth instars were produced by 100 control adults and none by 100 or 395 adults irradiated with 20 or 80 Gy, respectively.

Upon termination of the temperature regime to end diapause in 1997 and 1998, the survival of adults in the control was 59 and 79%, respectively, and in those irradiated with 80 Gy, survival was 3.7 and 10.1%, respectively (Fig. 7).

One week after terminating the diapause-breaking temperature regime, control plum curculio collected in 1997 began to lay eggs for 3 wk (Fig. 8). One hundred eleven fourth instars were produced by the 59 remaining weevils. After that, no more reproduction occurred in the control although they lived up to 9 more months. Three weeks after terminating diapause, the 33 surviving adults irradiated with 20 Gy produced four fourth instars and did not produce any more, although the last adult died 10 d after the last control adult. Adults irradiated with 40–80 Gy did not reproduce. The 74 (of 100) surviving weevils irradiated with 20 Gy in 1998 produced 11 fourth instars over a 7-wk period beginning 2 wk after termination of diapause. In 1998, the surviving (79 of 100) control weevils produced 381 fourth instars over 5.5 months beginning 2 wk after removal from the diapause-breaking temperature regime.

No late-instar larvae were found from apples infested by the 25,000 southern strain adults irradiated

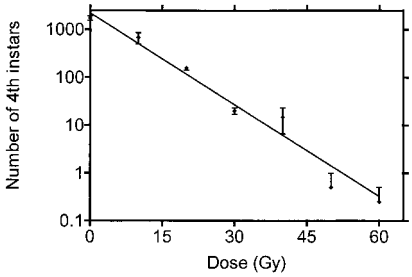

Fig. 4. Mean numbers (log scale, SE) of late fourth instars resulting from irradiation of 50 female and 50 male 2-wk-old southern strain plum curculio adults.

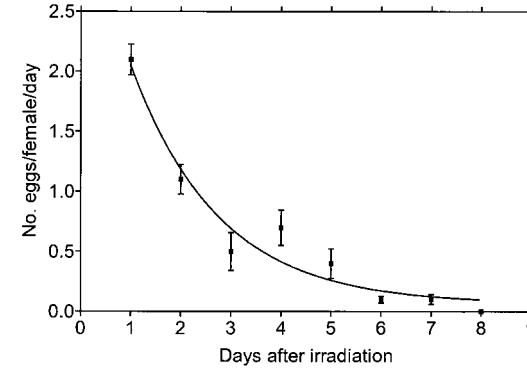

Fig. 6. Mean number (SE) of eggs per female southern strain plum curculio per day after irradiation with 80 Gy. $R^2 = 0.83$. No oviposition beyond the seventh day occurred, although 83% of weevils were still alive. No development beyond the first instar occurred.

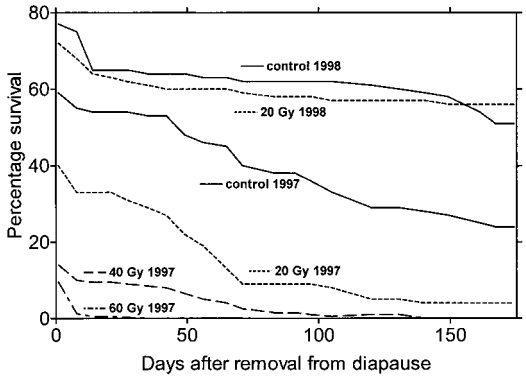

Fig. 7. Survival of northern strain plum curculio after irradiation and termination of diapause. Data shown are up to 175 d after removal from temperature regime to break diapause. In 1997, last control and 20 Gy adult died after 312 and 322 d, respectively. In 1998, mortality was recorded for only 225 d after termination of diapause, at which survival was 42 and 53%, respectively, for the control and 20 Gy. Treatments at 80 Gy not shown; in 1997, survival at day 1 was 3.7%, and all had died by day 14. In 1998, survival at day 1 (80 Gy) was 10.1% and all had died by day 15.

with a target dose of 80 Gy, whereas the nonirradiated adults produced a mean of 36.2 fourth instars per female over the 3 wk that the irradiated weevils lived. Some eggs were laid soon after adults were irradiated and some appeared to hatch, but with no development beyond the first instar observed. The maximum absorbed dose during this part of the research was 92 Gy.

Discussion

Twenty Gy prevented development of plum curculio eggs beyond the early instars, 40 Gy applied to three fourth instars in apples prevented pupation, irradiation of pupae with 20 Gy prevented reproduction, and 80 Gy was needed to prevent reproduction of adults. These results agree with an abundance of literature relating increased level of development to increased radiotolerance (Hallman 2001). Research into irradiation quarantine treatments would probably be justified in selecting the most developed stage that may be shipped with a commodity as the most radiotolerant stage, thus negating the need for preliminary studies to establish the most tolerant stage before further tests are conducted.

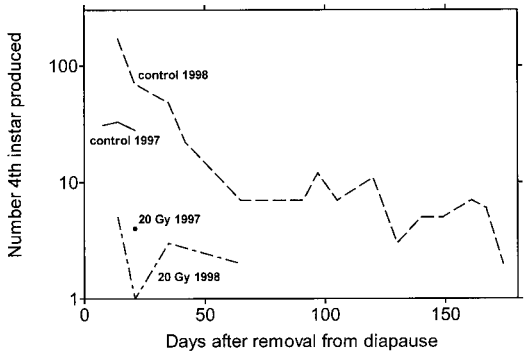

Fig. 8. Numbers (log scale) of F1 fourth instars produced from northern strain plum curculio irradiated with 20 Gy and nonirradiated control. Reproduction occurred on only one day for 20 Gy 1997. Each group started with 100 adults (sex ratio ≈50: 50); survival curves are given in Fig. 7.

The fact that a dose of 2 kGy did not kill all plum curculio adults until ≈6 d after irradiation reaffirms the contention that irradiation cannot be used as a quarantine treatment for which efficacy is based on acute mortality, as are all other commercially used treatments (Hallman 2001). In any case, a high dose, such as the estimated 4 kGy needed for all adults within 24 h, would damage fruit hosts of the insect (Drake and Neven 1998, Drake et al. 1999).

When adults are present, acute mortality is not a viable goal for irradiation and lack of reproduction would be the objective. Twenty five thousand actively reproducing plum curculio adults were irradiated with a target dose of 80 Gy with no successful reproduction observed. Because the maximum absorbed dose during this part of the research was 92 Gy, that dose should be recommended as the minimum absorbed dose for a commercial quarantine treatment.

Northern strain plum curculio was more radiosusceptible than the southern strain based on reproduction. At 20 Gy, the former produced 0.07 fourth instar per female, whereas the southern strain produced over one level of magnitude as much (0.8). Furthermore, northern strain plum curculio, undergoing obligate diapause, must surmount additional hurdles not faced by southern strain, such as the proper conditions and sufficient time to break diapause, before any beetles surviving a quarantine treatment would pose a threat. Therefore, an irradiation quarantine treatment effective against southern strain would serve against northern strain with a much lower level of risk.

Although a partial second generation has been observed from northern plum curculios in the Midwest (Sarai 1969), I am unaware of it occurring in plum curculios from the Massachusetts area, as was observed during this study.

Over a 6-yr period in the field in New York, mean survival of overwintering plum curculios was 57% (Smith and Flessel 1968). That is lower than the survival observed for northern strain plum curculios during this study indicating that the level of overwintering mortality found during this study was normal.

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