

Posttreatment Survival, Development, and Feeding of Irradiated Indianmeal Moth and Navel Orangeworm Larvae (Lepidoptera: Pyralidae)¹

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J. Econ. Entomol. 81(1): 376-380 (1988)

ABSTRACT Larvae of the Indianmeal moth, *Plodia interpunctella* (Hübner), and the navel orangeworm, *Amyelois transitella* (Walker), reared individually in small beakers containing artificial diet, were irradiated at doses ranging from 149 to 627 Gy. Adult emergence was prevented by all doses. Only 13% of the oldest Indianmeal moth larvae irradiated at the lowest dose were able to pupate. No pupation occurred at any other treatment level. Higher radiation doses decreased the time required for 100% larval mortality. Longevity of young larvae was reduced compared with that of controls, whereas longevity of older larvae was increased. Weight gain and food consumption of irradiated larvae were reduced, indicating that while radiation-induced larval mortality may be delayed, damage from feeding would be reduced.

KEY WORDS Insecta, Gamma radiation, *Plodia interpunctella*, *Amyelois transitella*

THE U.S. Food and Drug Administration has recently approved the use of low doses (≤ 1.0 kGy)² of ionizing radiation to inhibit the growth and maturation of fresh fruits and vegetables and to disinfest food of arthropod pests (Young & Bowen 1986). In anticipation of this approval, considerable work has been done on the effects of ionizing radiation on insects within postharvest food commodities (Tilton & Burditt 1983). Much of this work has centered on reduction of lepidopteran postharvest species, either through commodity disinfestation or sterile insect release. Because of emphasis on the latter, most studies have been on the effect of radiation on adult reproduction, while research on the effects of radiation on lepidopterous larvae is less complete.

Nearly all U.S. dried fruits and nuts are produced in California (U.S. Department of Agriculture 1985). A major problem in the processing and storage of these commodities is insect infestation, particularly Indianmeal moth (IMM), *Plodia interpunctella* (Hübner), and navel orangeworm (NOW), *Amyelois transitella* (Walker). Current control practices rely on chemical fumigants such as methyl bromide and hydrogen phosphide, but nonchemical alternatives such as ionizing radiation are being considered. The lack of chemical residues and relatively quick treatment times for radiation are attractive advantages.

To determine the potential of radiation treatments for controlling moth species in dried fruits and nuts, the effect on larval stages must be examined. While earlier studies (Brower & Tilton 1970, 1971, 1972, Tilton et al. 1978) have demonstrated the efficacy of radiation in controlling IMM populations in various commodities, less information is available on the longevity and post-treatment feeding of irradiated larvae. The objective of this study was to determine the effect of gamma radiation on survival, development, and feeding of IMM and NOW larvae.

Materials and Methods

All test larvae were irradiated in 2-ml polystyrene diSPo automatic analyzer beakers (American Scientific Products, Sunnyvale, Calif.) containing about 1 ml of proprietary codling moth diet (BioServ Inc., Frenchtown, N.J.) (Kellen & Hoffmann 1982). Polyethylene caps with small holes covered with stainless steel screen (150 mesh) were used to provide ventilation. Neonate larvae, <24 h old, were obtained from cultures maintained on bran diet (Finney & Brinkman 1967). A camel's-hair brush was used to place one larva in each beaker. Before and after treatment, larvae were kept at 27°C with a photoperiod of 12:12 (L:D). All tests consisted of 20 larvae replicated five times at each treatment level. Samples of larvae were frozen at -18°C at the time of treatment, and their head capsules were measured to estimate instar distribution.

Irradiation of test insects was conducted at the Sandia National Laboratories in Albuquerque, N. Mex. The day before irradiation, test beakers

¹ This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by the USDA.

² The Systeme Internationale (SI) unit for expressing absorbed radiation is the Gray (Gy), which replaces the older, more familiar kilorad (1 kilorad [krad] = 10 Gy).

were pressed into styrofoam blocks, one replicate of 20 beakers per block. The blocks were wrapped in cheese cloth secured with rubber bands and shipped overnight to Albuquerque in plastic ice chests. After treatment, the blocks were repacked and returned to Fresno, Calif., for evaluation.

To determine larval survival and longevity after irradiation, 6- and 11-d-old IMM and 7-, 12-, and 15-d-old NOW larvae were irradiated at four different doses. A cesium-137 chamber irradiator with a dose rate of ca. 12.5 Gy/min was used to irradiate IMM larvae. For the NOW, a cesium-137 prototype sludge irradiator with a dose rate of ca. 15.0 Gy/min. was used (Morris 1980). In both cases, actual doses were determined by dosimeters (TLD-400 [LiF]). Average applied doses were 149 ± 5.7, 294 ± 19.2, 428 ± 38.4, and 545 ± 33.7 Gy for IMM, and 162 ± 16.6, 285 ± 36.9, 451 ± 21.2, and 627 ± 54.5 Gy for NOW ($\bar{x} \pm SD$). Controls were also shipped, but left untreated. After treatment, larvae were checked daily for mortality. Dead larvae were stored frozen until head capsule measurements could be made.

The chamber irradiator was used to determine the effect of two dose levels on development and feeding of 6-d-old IMM and NOW larvae. Dosimeters showed that IMM larvae received average doses of 317 ± 22.6 and 505 ± 27.3 Gy. Average doses applied to NOW larvae were 295 ± 16.8 and 444 ± 41.7 Gy. Again, controls were shipped, but left untreated. Immediately after treatment and return to Fresno, larvae were individually weighed on an analytical balance and transferred to beakers containing fresh codling moth diet. Thereafter, individual larval weights were recorded daily for NOW and every other day for IMM. Larvae were transferred to beakers containing fresh diet on the 6th and 10th day posttreatment. Feeding activity was determined by visually rating the amount of diet consumed in each beaker. The categories used were no visible feeding, a trace to 10% of the diet consumed (slight feeding), 10% to 50% consumed (moderate feeding), and more than 50% consumed (extensive feeding). The data were subjected to analysis of variance with arcsine transformations. Significant differences in treatment means ($P \leq 0.05$) were separated by Duncan's (1955) multiple range test.

Results and Discussion

Complete mortality of irradiated IMM larvae did not occur until ≥2 wk posttreatment, depending on dose and larval age (Fig. 1). All doses prevented pupation of 6-d-old larvae. Only 13% of the 11-day-old larvae irradiated at 149 Gy were able to pupate, and none of these emerged as adults.

IMM larvae irradiated when 11 d old survived longer at all doses than did younger larvae. Mortality of irradiated 6-d-old IMM larvae began almost immediately after treatment. Complete mortality for 6-d-old larvae treated with 545, 428, 294,

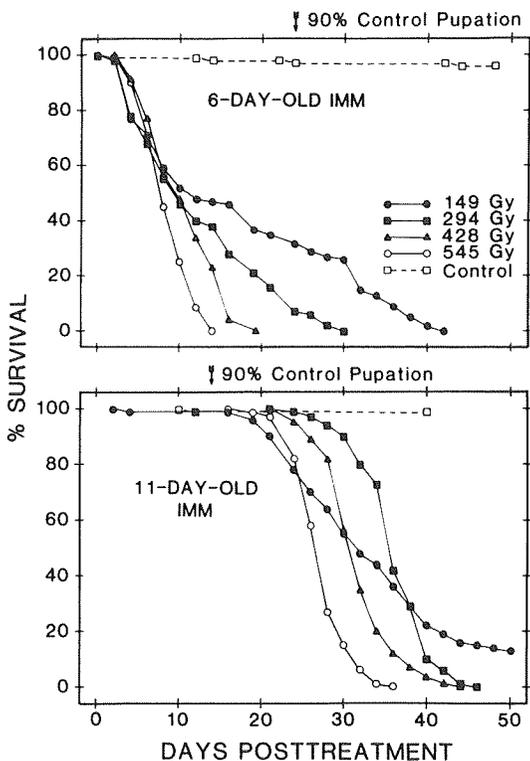


Fig. 1. Survival of 6- and 11-d-old IMM larvae following exposure to gamma radiation.

and 149 Gy was reached 14, 19, 30, and 42 d posttreatment, respectively. In contrast, mortality of 11-d-old larvae was delayed and did not begin until 19 d posttreatment. Maximal mortality for 11-d-old larvae treated with 545, 428, 294, and 149 Gy was reached at 36, 44, 46, and 50 d posttreatment, respectively. Mortality in control IMM was 4% for 6-d-old larvae and 1% for 11-d-old larvae.

While irradiation did not cause immediate mortality of IMM larvae, it did reduce longevity of younger larvae. Pupation of control larvae reached 90% 24 d after treatment. None of the 6-d-old larvae irradiated with 428 and 545 Gy survived for more than 20 d posttreatment. At 24 d posttreatment, survival of larvae irradiated with 294 and 149 Gy was 7 and 32%, respectively.

The longevity of 11-d-old larvae was increased after irradiation. Very little mortality of irradiated larvae had occurred by 14 d posttreatment, while 90% of the control larvae had successfully pupated. Twelve days later, survival of irradiated larvae was still >50% at all doses.

Brower (1980) irradiated nondiapausing and diapausing mature fifth instar IMM. Doses of ≤200 Gy had little effect on the pupation of nondiapausing larvae, although doses of ≥100 Gy completely prevented adult emergence. Diapausing larvae irradiated with 100 to 200 Gy often lived for weeks after treatment, but few (2%) were able to pupate. Pupation of nondiapausing larvae, both

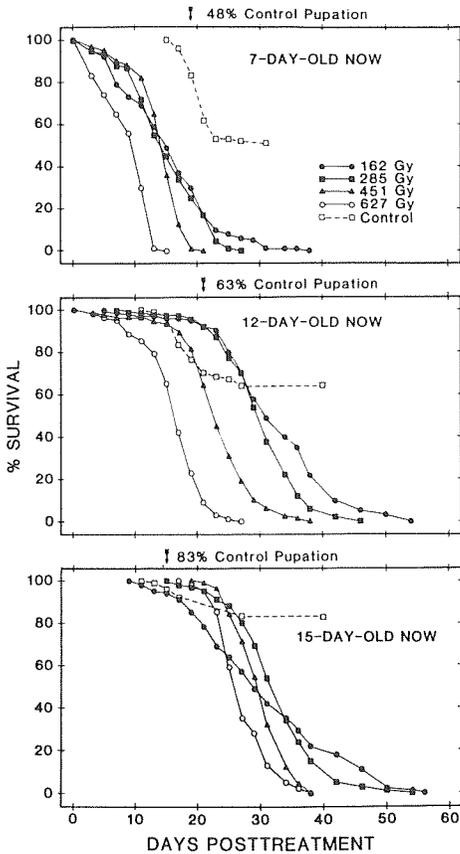


Fig. 2. Survival of 7-, 12-, and 15-d-old NOW larvae following exposure to gamma radiation.

irradiated and control, was mostly complete within 4 d posttreatment, indicating that a more developed stage was used than in the present study. Brower (1980) reported that pupation of diapausing control larvae neared 90% 8 d posttreatment. The similarity between our results from 11-d-old larvae and those from diapausing larvae may be due to a similarity in developmental stage at the time of irradiation.

Survival of irradiated NOW larvae (Fig. 2) forms a pattern similar to that of IMM. As larval age at irradiation increased, posttreatment longevity also increased. When 7-d-old NOW larvae were irradiated, the occurrence of complete mortality ranged from 15 d posttreatment for 627 Gy to 38 d posttreatment for 162 Gy. Complete mortality took from 27 to 54 d to occur for larvae irradiated when 12 d old, and ranged from 38 to 56 d for the oldest larvae, depending upon dose.

Control mortality for NOW larvae was higher than for IMM. The small amount of agar-based diet used in the test beakers sometimes dried out before the larger, slower-growing NOW larvae could complete their development. Even so, while no irradiated larvae successfully pupated, maximal pupation of control larvae was 48, 63, and 83% for 7-, 12-, and 15-d-old larvae, respectively.

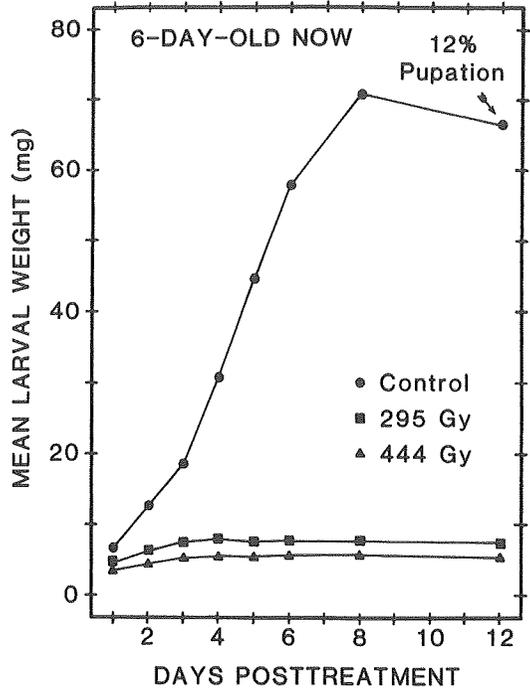


Fig. 3. Fresh body weight of 6-d-old NOW larvae following exposure to gamma radiation.

As with the IMM, radiation increased posttreatment longevity of older NOW larvae. While pupation of surviving 15-d-old control larvae was completed within 15 d posttreatment, very little mortality of irradiated larvae had occurred. At 25 d posttreatment, survival of irradiated larvae at all doses was still above 50%. In contrast, few larvae irradiated when 7 d old survived for more than 19 d posttreatment, when pupation of surviving control larvae had been completed.

Husseiny and Madsen (1964) irradiated mature NOW larvae with 30 to 400 Gy. Doses ≤ 240 Gy had little effect on pupation, although 120 and 240 Gy considerably reduced adult emergence. After exposure to 300 and 400 Gy, pupation was greatly reduced and no adults emerged. In all cases, pupation occurred within one week posttreatment, again indicating that a more advanced developmental stage was being treated. The higher doses reported by Husseiny and Madsen (1964) as necessary to prevent pupation, therefore, may be due to their use of an older, more radio-resistant stage.

Postmortem head capsule measurements of irradiated larvae were made to determine larval development. Accurate measurements were difficult to make due to the frequent occurrence of deformed head capsules. Frequency distributions of the measurements did not always yield discrete size classes, nor did measurements correspond with those of untreated larvae cultured on bran diet or with measurements reported by other workers (Curtis & Barnes 1977, Caltagirone et al. 1983). Tissue

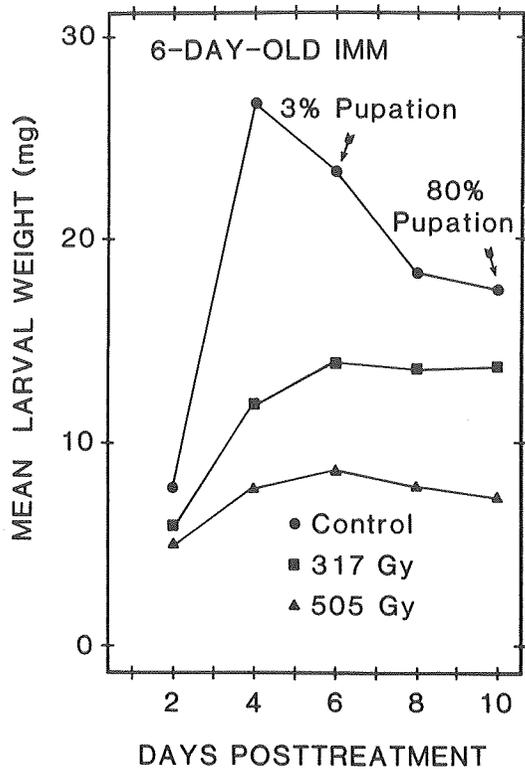


Fig. 4. Fresh body weight of 6-d-old IMM larvae following exposure to gamma radiation.

sensitivity to radiation is dependent on the degree of cellular reproductive activity and differentiation (Bergonie & Tribondeau 1959), and radiation damage to nondividing cells may be delayed until mitosis occurs (Baldwin & Salthouse 1959). Consequently, the gross effects of radiation on immature insects are often not expressed until molting, when the epidermis begins mitosis in the production of new cuticle. The occurrence of "doubled" head capsules, resulting when the old head capsule was not completely shed, indicated that death most often occurred during molts, but this and other abnormalities made accurate determinations of larval instars impossible. However, by comparing head capsule widths of irradiated larvae with those of untreated larvae sacrificed at the time of radiation, one trend could be seen—for both IMM and NOW larvae, the lowest dose allowed some of the younger larvae to develop successfully to the next instar, while higher doses killed larvae before or during their next molt.

Because immediate mortality of feeding larvae did not occur after irradiation, it was important to determine the extent of posttreatment development and feeding. Mean larval weights of irradiated NOW increased very little after treatment, while control larval weights increased 10-fold before pupation (Fig. 3). Maximal mean weights of larvae irradiated with 295 and 444 Gy were only 11.3 and 8.3% of control larval weight, respective-

Table 1. Extent of posttreatment feeding on artificial diet by irradiated NOW larvae

D post-treatment	Dose (Gy)	Categories of diet consumption ^a			
		0	Trace-10% (slight)	10-50% (moderate)	>50% (extensive)
1-6	0	0	6.2a	76.5c	17.3c
	295	0	73.4b	25.4b	1.2b
	444	0	94.6c	5.4a	0a
6-10	0 ^b	0a	6.0a	78.4b	15.6b
	295	23.4c	76.6b	0a	0a
	444	14.8b	85.2b	0a	0a

^a Values given are mean percent beakers within replicates (*n*, 100). Analysis of variance performed with arcsine transformation. Means within columns followed by the same letter are not significantly different (*P* > 0.05 level; Duncan's [1955] multiple range test).

^b 3.8% of control larvae (0 Gy) successfully pupated.

ly. Mean IMM larval weights formed a similar, though less obvious, pattern (Fig. 4). The maximal larval weights for IMM larvae irradiated with 317 and 505 Gy were, respectively, 52.1 and 32.2% that of control larvae. Thus, radiation inhibited larval growth.

The extent of posttreatment feeding on artificial diet by irradiated NOW larvae showed that radiation also inhibited feeding (Table 1). Most (94%) control larvae displayed moderate or extensive feeding activity. Between 1 and 6 d posttreatment, over 70 and 90% of larvae irradiated with 295 and 444 Gy, respectively, were capable of only slight feeding. The reduction in feeding of irradiated larvae continued, with all treated larvae showing no more than slight feeding between 6 and 10 d posttreatment.

Feeding activity of irradiated IMM was similar, with 83.8% of control larvae exhibiting moderate feeding between 1 and 6 d posttreatment, while few treated larvae were capable of more than slight feeding (Table 2). Between 6 and 10 d posttreatment, no moderate or extensive feeding was exhibited by any IMM larvae, including control larvae. However, the reduction in feeding activity in the irradiated larvae can be attributed to the treatment, while for control larvae it was a result of pupation of nearly 90% of the larvae.

In many insects, cells of the midgut epithelium are continually replaced by groups of undifferentiated regenerative cells. Thus, the midgut epithelium should be sensitive to radiation damage. Ashraf et al. (1971) examined the histopathological effects of radiation on the midgut of fifth-instar IMM and found histolysis of the epithelium and destruction of regenerative cells. A reduction in feeding activity was also noted and degeneration of the midgut was suggested as a primary cause of developmental failure in irradiated larvae. Thus, larval mortality may be due to a combination of radiation damage to the epidermis, expressed during molting, and to the midgut epithelium.

Table 2. Extent of posttreatment feeding on artificial diet by irradiated IMM larvae

D post-treatment	Dose (Gy)	Categories of diet consumption ^a			
		0	Trace-10% (slight)	10-50% (moderate)	>50% (extensive)
1-6	0 ^b	1a	15.2a	83.8c	0
	317	7.2b	87.2b	5.6b	0
	505	7.1b	92.9b	0a	0
6-10	0 ^c	71.8b	28.2a	0	0
	317	50.0a	50.0b	0	0
	505	69.0b	31.0a	0	0

^a Values given are mean percent beakers within replicates (n , 100). Analysis of variance performed with arcsine transformation. Means within columns followed by the same letter are not significantly different ($P > 0.05$ level; Duncan's [1955] multiple range test).

^b 3.0% of control larvae (0 Gy) successfully pupated.

^c 89.9% of control larvae (0 Gy) successfully pupated.

Our results show that control of lepidopterous larvae infesting postharvest dried fruits and nuts is possible with relatively low doses of radiation. Previous work with pupal and adult stages has suggested doses of 200 to 500 Gy for practical control (Brower & Tilton 1970, 1971, 1972, Tilton et al. 1978, Johnson & Vail 1987). Similar doses applied to larvae would be adequate to prevent pupation and emergence of fertile adults. The delayed mortality exhibited by irradiated larvae presents a distinct disadvantage, even though damage from feeding is reduced. If suitable time does not elapse between treatment and marketing, it is possible that some living larvae might be found in the commodity by consumers.

Acknowledgment

We wish to thank Steve Glass and Neil Simmons of Sandia National Laboratories for their cooperation and patience, and to Christina Nunez for her assistance. Special thanks to Rodney Fries for supplying test insects, Darlene Hoffmann for technical advice, and John Brower, Art Burditt, and Wayne Tilton for helpful review of this manuscript. Our thanks also to the Raisin, Walnut and Almond Advisory Boards for their coverage of transportation costs to and from Albuquerque. This study was supported in part by a contract (DE-A104-83AL24327) from the U.S. Department of Energy.

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Received for publication 23 March 1987; accepted 16 September 1987.