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The Effect of Gamma Radiation on Larval Resistance to *Bacillus thuringiensis* Infection[1]

During the past 25 years a great deal of evidence has accumulated indicating that ionizing radiation adversely affects the mammalian immune response. Except for a few scattered reports (R. H. Jafri: *J. Invertebr. Pathol.* **7,** 66–70, 1965; **10,** 355–360, 1968; W. A. Smirnoff: *J. Invertebr. Pathol.* **9,** 264–266, 1967), this area of research has not been exploited in insects.

In this study we have explored some relationships between radiation dose and susceptibility to *Bacillus thuringiensis* var. *thuringiensis* infection in *Hemerocampa leucostigma* larvae, reared and infected per os and irradiated as described (H. W. Rossmoore, L. Elder, and A. E. Hoffman: *J. Invertebr. Pathol.* **16,** 102–106, 1970; H. W. Rossmoore and A. E. Hoffman: *J. Invertebr. Pathol.* **17,** 277–281, 1971). Combinations of ^{137}Cs radiation doses and spore–crystal mixtures that alone produced significantly less than 100% mortality within a selected test-observation period were chosen from previous data (see above).

In addition, radiation infection studies were done on larvae injected intrahemocoelically with the largest dose of spore–crystals found to produce significant mortality. We used at least 100 larvae in each of the experiments listed in Table 1. None of the combinations used even hinted that ionizing radiation had affected immune responsiveness. Thus, varying both dose of radiation and spore–crystals, route of-administration, variety of pathogen (virulent and avirulent), and maintaining postradiation infecting time constant proved ineffective. As a last resort, we extended the postradiation time for infection to 10 days.

TABLE 1

LARVAL SUSCEPTIBILITY TO CRYSTALLIFEROUS STRAINS 4 DAYS POSTRADIATION[a]

	Larval state	Radation dose (kr)	Spore–crystal dose	Method of administration	% Survival 4 days postinfection
1	3rd	0	3×10^4[b]	Per os	96.6
2	3rd	40	3×10^4	Per os	89.4
3	3rd	60	3×10^4	Per os	94.1
4	3rd	0	3×10^4	Per os	56.5
5	3rd	30	3×10^4	Per os	66.7[c]
6	5th and 6th	0	16–18	Intrahemocoel.	74.6
7	5th and 6th	30	16–18	Intrahemocoel.	77.9
8	3rd	0	1.4×10^7[b]	Per os	87.5
9	3rd	30	1.4×10^7	Per os	81.8[c]

[a] In center well of ^{137}Cs Radcell; 10 kr/hr.
[b] Sprayed on leaf surface and calculated from plate counts/cm² of surface; checked for crystal ratios microscopically (1 spore: 1 crystal); area eaten was determined by planimeter, then divided by number of larvae/container; nos. 1–7 were infected with *Bacillus thuringiensis* var. *thuringiensis*; nos. 8 and 9 were infected with *Bacillus finitimus* var. *finitimus*.
[c] Not significant by chi-square test; $p > .05$ (compared to nonirradiated controls).

[1] Contribution No. 261, Department of Biology.

This period, plus the 4 days of infection, brings the third-instar test larvae practically into pupation, making 10 days maximal for these observations. We could not exceed 30 kr, since above this level there is extensive mortality within the 10 days. The results (Table 2) indicate that at 10 days postradiation, there is a depression of resistance to bacterial intoxication and/or infection.

It should be pointed out that in this latter group resistance seems less than in animals represented in Table 1 (56% vs 74% survival). We have no way to account for this difference, except that the egg masses were stored at 10°C an additional 4–6 months before hatching.

We are now attempting to evaluate the role of midgut epithelium integrity and hemocyte population shifts during the postradiation period. Preliminary evidence suggests that midgut cells become disorganized at radiation doses used as early as 1 day after irradiation, while discernible depression in total hemocyte count does not appear until 7 days. This latter effect also parallels a change in the electrophoretic pattern of whole hemolymph. These studies hopefully may locate the anatomical and biochemical lesions responsible for the reduction in resistance to *B. thuringiensis* var. *thuringiensis* infections.

TABLE 2

Larval Susceptibility to *Bacillus thuringiensis* var. *thuringiensis* Infection 10 Days Postradiation[a]

Treatment: Radiation dose	Treatment: Infecting dose[b] (spore–crystals)	No. of larvae treated	No. of larvae treated	% Survival (4 days postinfection)
30 kr	3.3×10^7 (autoclaved)	235	222	94.5
0 kr	3.3×10^5	228	169	74.1
30 kr	3.3×10^5	233	132	56.7[c]

[a] In center well of ^{137}Cs Radcel[d]—10 kr/hr.

[b] Sprayed on leaf surface and calculated from plate counts/cm^2 of surface; checked for crystal ratios microscopically (1 spore: 1 crystal); area eaten was determined by planimeter, then divided by number of larvae per container.

[c] Significant at $p < .05$ by chi-square test between 0 kr radiation-infected and 30 kr radiation-infected.

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