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Truncation of Screwworm (Diptera: Calliphoridae) Development by Irradiation of Embryos

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ABSTRACT Gamma irradiation of screwworm, Cochliomyia hominivorax (Coquerel), embryos was used to truncate the life cycle at the prepupal stage. Egg hatch and larval survival were not reduced by dosages ≤8 kilorads (kr). Pupariation and adult emergence were inhibited by dosages ≥2.8 and 1.2 kr, respectively. Larvae, irradiated as embryos with dosages ≤8 kr, successfully infested wounded sheep, although larval weight was lower than normal. A dosage of 5.8 kr was sufficient to prevent pupariation, while permitting larval development to the third instar. Truncation of screwworm development permits the use of artificially wounded and infested animals for screwworm population surveillance and research without danger of accidentally releasing fertile screwworms.

KEY WORDS Insecta, development, radiation, surveillance

The population surveillance of screwworm, Cochliomyia hominivorax (Coquerel), normally consists of collecting egg masses from artificially wounded sentinel animals. Wounds, preinfested with screwworms, are more attractive to gravid females for oviposition than uninjured wounds (Hammack & Holt 1983, Knipping & Travis 1937). However, escape of fertile flies from infested wounds of sentinel animals is counter-productive to control efforts. A safe but sensitive screwworm surveillance technique is needed to monitor the progress of the eradication effort in zones under treatment with sterile flies, to detect reinfections of screwworm-free zones, and to monitor the area surrounding the mass production facility of the Comision Mexico-Americano para la Eradicacion del Gusano Barrenador del Ganado, at Tuxtla Gutierrez, Chiapas, Mexico for the escape of fertile insects. In addition, recent advances in the eradication program have left the USDA-ARS Screwworm Research Laboratory in Tuxtla Gutierrez, Chiapas, Mexico in a screwworm-free zone. Biological security regulations restrict the use of fertile screwworms outside of secure facilities, curtailing research programs on host immunity and the interaction of screwworm and exotic blowflies in wounds.

Studies on other insect species have indicated that radiation can truncate the life cycle. Irradiation of Musca domestica L. (Morgan et al. 1986) and Ephesia kuhniella Zeller (Kuzin et al. 1968) pupae with 20 kilorads (kr) inhibited metamorphosis. Irradiation of Aedes aegypti (L.) (Asman & Rai 1972) and Drosophila melanogaster Meigen (Hilliker 1985) embryos with 4 kr permitted embryos to develop to the prepupal stage, but inhibited adult emergence. The purpose of our study was to determine if gamma irradiation of embryos could be used to truncate screwworm development at the prepupal stage.

Materials and Methods

Screwworms of the VF-84 strain were used for this study. Eggs, ±15 min old, were maintained at room temperature (about 25°C) for 3.5 h while being divided into 100-mg-samples and placed in Petri dishes (4 cm diameter) on dampened paper toweling, after which they were incubated for 6 h at 37 ± 3°C. Following incubation, larval spines were visible through the egg chorion and embryos were approximately 1 h from eclosion. Embryos were irradiated with cesium 137 in a Husman irradiator (Hofmann 1985). Two samples were removed from the irradiator cylinder before irradiation and served as controls. Remaining samples were irradiated for 7-, 35- or 70-s intervals to produce the incremental dosages of 0.4, 2, and 4 kr, respectively. Two samples were removed after each exposure period and labeled. After irradiation, eggs were incubated at 37 ± 3°C for 18 h with 5 g of horse meat upon which the newly emerged larvae fed.

Our study consisted of four experiments. In the first experiment, embryos were exposed to radiation dosages of 0-60 kr in increments of 2 kr from 0 to 20 kr and then in increments of 4 kr from 20 to 60 kr. In the second experiment, radiation dosages ranged from 0 to 8 kr in 0.4-kr increments. Following irradiation and incubation, larvae for experiments one and two were transferred to rearing pans (19 by 9 by 9 cm) with 0.5 liter of Water-Lock G-400 (Grain Processing Corporation, Mus-
Artificially wounded sheep were used as hosts for the third and fourth experiments. Embryos were irradiated and incubated as previously described. Sheep were wounded on the shoulder and infested with 20-30 first-instar screwworm larvae. In the third experiment, 20 sheep were infested with larvae that had been exposed to 2, 4, 6, and 8 kr of radiation (five sheep per dosage). In the fourth experiment, 10 sheep were infested with screwworm larvae exposed to 5.8 kr. Three days after infestation, wounds were examined for the presence of screwworm larvae and scored as positive or negative. Larvae from positive wounds were removed and weighed.

Analysis of variance (ANOVA) (Proc GLM, SAS/STAT, pp. 183-260) was used to determine the effect of radiation on the development of screwworm embryos. Multiple regression with backward elimination (Proc GLM, SAS/STAT, pp. 183-260) was used to detect linear and curvilinear trends in developmental parameters.

Results

The first experiment examined the effects of radiation dosages between 0 and 60 kr. Egg hatch was significantly affected by radiation ($F = 3.32; \text{df} = 20, 21; P = 0.004$), decreasing as a curvilinear function of dosage in kr ($d$) where percent egg hatch = 91.5 - 0.009$d^2$ ($F = 31.87; \text{df} = 1, 40; P < 0.0001; r^2 = 0.44$) (Fig. 1). The adverse effect of radiation on hatch was most noticeable after the dosage exceeded 40 kr. The number of larvae developing to the third instar also was reduced significantly by radiation dosage ($F = 23.13; \text{df} = 20, 21; P < 0.0001$). No larvae developed to third instar if radiation exposure exceeded 14 kr. Within the range of 0-16 kr, the number of larvae surviving to the third instar decreased as a negative linear function of radiation dosage where larvae = 1,466.2 - 90.1$d$ ($F = 86.48; \text{df} = 1, 16; P < 0.0001; r^2 = 0.84$).

The second experiment examined the effects of radiation within the range of 0-8 kr. Percent eclosion of irradiated eggs ($F = 1.80; \text{df} = 20, 21; P = 0.094$) and number of larvae developing to third instar ($F = 1.34; \text{df} = 20, 21; P = 0.254$) were not significantly reduced by radiation dosages within this range (Fig. 2). Morphologically normal puparia were recovered from treatments of ≤2.4 kr. The number of puparia decreased as a linear function of dosage, where puparia = 759.1 - 337.3$d$ ($F = 145.97; \text{df} = 1, 12; P < 0.0001; r^2 = 0.92$). Adult screwworms were recovered from treatments of ≤0.8 kr. Adults from the 0.4-kr treatment had a normal rate of emergence (98% versus 96% in the controls) and appeared morphologically normal. Adults from the 0.8-kr treatment had a reduced rate of emergence (31%), were malformed,
and most died 1–2 d after emergence. The number of adult flies recovered decreased as a curvilinear function of dosage where adults = 710.19 - 550.5d² (F = 27.74; df = 1, 6; P = 0.001). Variation in fertility and fecundity was high among irradiated flies outcrossed to unirradiated flies, making statistical analysis difficult. Control females (unirradiated females mated to unirradiated males) oviposited eggs with a mean hatch of 66%. Eggs from unirradiated females mated to 0.4-kr males had a mean hatch rate of 60%, and eggs from 0.4-kr females mated to unirradiated males had 65% hatch. No 0.8-kr females survived long enough to oviposit, and only four unirradiated females crossed to 0.8-kr males were mated (sperm present in the spermathecae); however, 70% of the eggs from those females hatched.

In the third experiment, 95% of the sheep infested with larvae exposed to 2–8 kr as embryos had third-instar larvae 3 d after infestation. This infestation rate was within the 80–90% range observed for unirradiated larvae (R.L.M., unpublished data). Larval weight decreased to <50 mg at dosages of ≥4 kr (62.3, 49.7, 34.5, and 43.8 mg, respectively). Because of the danger of accidental escape, no unirradiated controls were possible, but larvae weighing about 80 mg would be expected at this age (R.L.M., unpublished data). In the fourth experiment, where embryos were irradiated with 5.8 kr, all 10 sheep had >5 third-instar larvae (mean weight 36.6 mg) 3 d after infestation.

Discussion

Exposure of screwworm embryos to ≤8 kr of gamma radiation did not reduce egg hatch or larval survival, whereas dosages of ≥1.2 kr prevented adult emergence. Adult sterilization was not obtained with sublethal dosages of embryonic irradiation. The dosage–response curves observed in our study were similar to those obtained by Asman & Rai (1972) for A. aegypti and Hilliker (1985) for D. melanogaster indicating that, among Diptera, the response to embryonic irradiation was relatively uniform.

A dosage of 5.8 kr was suitable for irradiating screwworm embryos. This dosage was used by the eradication program to sterilize pupae, thus eliminating the need to modify the irradiators for embryonic irradiation. The 5.8-kr dosage was >4.5 times higher than that needed to eliminate adult emergence and >2 times higher than that required to prevent normal pupariation, but it did not reduce larval survival. Larvae (exposed to this dosage as embryos) infested and developed in wounded sheep. However, low larval weights indicated that this technique was unsuitable for studies of screwworm development.

Sheep in the sentinel flock used to monitor escapes of fertile screwworms from the mass production facility currently are being infested with larvae irradiated as embryos (5.8 kr). Because of the small number of egg masses collected (fewer than three per month) and declining numbers of flies escaping (because of improved biological security measures), direct comparisons of the attractiveness of wounds infested with irradiated larvae and uninfested wounds are not possible. However, no problems with establishing infestations or with development of irradiated embryos beyond the prepupal stage have been observed.

As the screwworm eradication program proceeds into Central America, detecting escapes of fertile flies from the production facility and reinstatements of screwworm-free zones will be a high priority of the eradication program. The use of larvae (irradiated as embryos) to infest sentinel animal wounds provides field workers with a more effective and sensitive method of detecting screwworms without the risk of releasing fertile flies into the environment.

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