Gamma Radiation Effects on Reproductive Biology of the Tick *Amblyomma americanum* (Acari: Ixodidae)[1]

Mary Ann Stanley and James H. Oliver, Jr.

Institute of Arthropodology and Parasitology, Georgia Southern University
Statesboro, GA 30460-8056, USA

J. Entomol. Sci. 28(3):267-277 (July 1993)

ABSTRACT Male *Amblyomma americanum* were irradiated at 0.5, 1, 2, 3, 4, 8, and 16 krad in ambient air and were mated with untreated females. Mating was impaired at 16 krad. The number of females engorging and ovipositing decreased as the radiation dosage of their mates increased. Attachment period of females increased as male radiation dosage increased. No relationship was seen between radiation dosage of males and preoviposition period of females mated to them, number of eggs laid per female, or weights of eggs.

KEY WORDS Ticks, Acari, Ixodidae, *Amblyomma americanum,* radiation, reproduction.

Amblyomma americanum is a vector of Rocky Mountain spotted fever, American Q fever, tularemia, and possibly Lyme disease (Schulze et al. 1984, Magnarelli et al. 1986). It is an important pest of livestock in the southern United States and can cause tick paralysis in mammals. Release of sterile males into a pest population is one method of control that has been successful in several insect species (Knipling 1982). Thus, this study was designed to gather basic information on the feeding, host attachment, oviposition, and egg characteristics of untreated females mated to irradiated *A. americanum* males. A previous study (Oliver and Stanley 1987) found decreased hatch of eggs from such crosses and described radiation-induced changes in gross testicular morphology and spermatogenesis.

Male ixodid ticks exposed to 16 krad or less of gamma radiation develop chromosomal aberrations such as bridges and rings. The testes often are underdeveloped, abnormal cells are prominent, and production of spermatids may be slowed or halted (Oliver et al. 1972, Pappas and Oliver 1972). In our previous study (Oliver and Stanley 1987), we noted no recognizable alteration in timing of spermatogenesis in *A. americanum* treated with 0.5 to 16 krad, but severe breakdown and depletion of germinal cells was seen at 4, 8, and 16 krad.

Ixodid ticks generally are sterilized by a minimum of 2 to 4 krad of gamma or X-irradiation. When either parent is exposed to these dosages, oviposition and embryonic development are impaired. Hatch of eggs from a tick treated with 2 krad or above is rare (Sidorov and Grokhovskaya 1964, Drummond et al. 1966, Kitaoka and Morii 1967, Gregson 1969, Oliver et al. 1972, Purnell et al. 1972, Beuthner 1975, Oliver and Stanley 1987). These minimum sterilization dosages appear most promising for biological control of ixodid ticks; higher dosages may result in loss of competitive ability, feeding difficulty, and mating impairment.

[1] Accepted for publication 26 April 1993.

Materials and Methods

Amblyomma americanum engorged nymphs were obtained from the USDA Livestock Insects Laboratory, Kerrville, Texas, approximately 2 mo before the experiment. The ticks were kept at 85% RH and 25°C.

Unfed adult males were separated into 8 groups and 7 groups were treated at the USDA Stored Products Insect Laboratory, Savannah, Georgia, with a Cobalt-60 source (strength = 1,235 Ci; rate = 1.1 krad/min) in ambient air at levels of 0.5, 1, 2, 3, 4, 8, and 16 krad of gamma radiation. Control ticks were maintained identically to experimentals except for the radiation exposure. After treatment the ticks were returned to 85% RH and 25°C for 28 h before they were placed on hosts.

Ten males representing each radiation level were combined with an equal number of untreated females and were placed on the left ears of New Zealand white rabbits. Attachment was checked daily. Engorged females from each group were collected as they detached and were placed in individually labeled vials to await oviposition. Those that did not detach of their own volition were removed from the host after 21 to 30 days. Date of oviposition was recorded for each female. Egg masses were maintained in desiccators at 80% RH and 25°C. Each egg mass was weighed on day 22 after the start of oviposition. Eggs were later counted individually with a dissecting microscope. The entire experiment was then repeated.

Mating studies were evaluated statistically using correlation, regression, and ANOVA with unequal sample sizes. In cases where ANOVA showed a significant difference, multiple comparisons were made using Scheffé's F test. Confidence intervals were computed (Sokal and Rohlf 1969). Experiments One and Two are presented separately because a t-test for paired comparison of means indicated that some data for numbers of females engorging, attachment and preoviposition periods of females, and egg weights differed significantly ($P < 0.05$) between experiments.

Results

Ticks at all radiation levels in both experiments usually attached in mixed clusters of males and females. Males from all groups were seen in the venter-to-venter position of mating, but many of the males that had been exposed to 16 krad detached from their host and wandered about until they died without mating. Females confined with the 16-krad-treated ticks remained at partial engorgement, indicating that the males either had not mated or had not successfully transferred spermatophores to the females.

Number of females engorging. In Experiment One, the number of females engorging and dropping decreased as radiation exposure level of their mates increased ($P < 0.05$, regression analysis). High percentages of females engorged in the control, 0.5-, 1-, and 2-krad groups (Table 1). Half of the females mated to 3-krad-treated males engorged. The 4-krad group was lost when its host scratched off an earbag, enabling all the ticks to escape. Few females engorged when confined with males treated at 8 or 16 krad; most were eventually removed after a 30-d attachment period. Similar results were recorded in Experiment Two (Table 2). Because many control females died or escaped in Experiment Two, the regression is not as distinct as in Experiment One.

Attachment period of females. In Experiment One, average attachment period of females increased as dosage level of their mates increased ($P < 0.05$). Average time spent on the host for a control female was 9.6 d, while females confined with males exposed to 16 krad remained attached an average of 17.0 d (Table 1). Ticks mated to males that received intermediate krad dosages showed intermediate average attachment periods.

Table 1. Results of matings between irradiated males and untreated females of *A. americanum* (Experiment One).

Male radiation dosage (krad)	No. females engorging (n = 10)	Attachment period of engorged females (d)		Number ovipositing	Preoviposition period (d)	
		Mean	Range		Mean	Range
0	10	9.6 a	9-11	10	9.3	8-11
0.5	10	10.5 a	9-15	8	8.6	6-12
1	8	10.6 a	9-12	5	8.0	8
2	9*	12.0 a, b	9-18	2	10.0	9-11
3	5	13.6 a, b	11-16	4	8.2	7-9
4	ND†	ND	ND	ND	ND	ND
8	5‡	15.8 b,c	13-18	2§	13.0	13
16	3‡	17.0 b, c	11-20	1	9.0	9

Means within a column followed by the same letter are not significantly different ($P > 0.05$, Scheffé's F test). Means within a column not followed by any letter showed no significant differences in a one-way ANOVA.
ND, No data available.
* 5 of the 9 engorged females subsequently died of causes other than experimental.
† Entire group lost from host animal.
‡ The majority of females in these groups did not fully engorge and drop. They were removed from the host animal after a 30-day attachment period.
§ These egg masses contained few eggs, which appeared normal.

ANOVA indicated a significant difference in mean length of attachment period among dosage levels ($P < 0.05$) for both Experiment One and Experiment Two. Results of Scheffé's F test for multiple comparisons are given in Table 1 for Experiment One and in Table 2 for Experiment Two. In Experiment Two, there were some technical problems with the control group of females and also the 16-krad group exhibited a drop in mean length of attachment that did not occur with the females in Experiment One. The multiple comparison test showed some differences among the groups, but the results are not as clear as in Experiment One.

Table 2. Results of matings between irradiated males and untreated females of *A. americanum* (Experiment Two).

Male radiation dosage (krad)	No. females engorging (n = 10)	Number removed after 21 days	Attachment period of engorged females (d)		Number ovipositing	Preoviposition period (d)	
			Mean	Range		Mean	Range
0	4*	0	10.5 a	10-12	3†	8.3	8-9
0.5	8	0	11.7 a, b	10-16	8	9.8	9-10
1	10	0	12.9 a, b, c	11-16	10	9.2	8-10
2	6‡	0	11.6 a, b, c, d	10-12	4	10.3	10-11
3	2	6	12.0 a, b, c, d	12	1†	9.0	9
4	2	6	17.5 b, c, d	16-19	§	–	–
8	1	6	21.0 c	21	1	–	–
16	2	5	14.0 a, b, c, d	10-18	§	–	–

Means within a column followed by the same letter are not significantly different ($P > 0.05$, Scheffé's F test).
Means within a column not followed by any letter are not significantly different by a one-way ANOVA.
* The remaining females were lost early in the experiment.
† One female from each of these groups died before ovipositing.
‡ Two of these females were dead when they dropped; several not listed here became stuck in rabbit ear exudate.
§ Females mated to males treated with 4 and 16 krad died without ovipositing.

Number of females ovipositing. The number of females ovipositing was drastically reduced by irradiation of males in both experiments. However, a statistically significant regression relating these factors was not obtained. A strong correlation existed between the number of females engorging and the number of females ovipositing at each radiation dosage ($P < 0.05$); this indicated that most of the ticks that fed and dropped went on to lay eggs.

Preoviposition period of females. ANOVA revealed no differences in average preoviposition times among the various groups in either experiment. Most ticks laid their eggs within 8 to 11 d after they dropped from their hosts. No relationship was seen between attachment period and preoviposition period of individual females in either experiment.

Number of eggs laid per mass. For Experiment One, ANOVA and regression analysis showed no significant differences in the number of eggs laid per mass that could be attributed to the radiation dosage of the male parent. Total eggs laid by individual females ranged from 166 to 6,450 (Table 3). A similar result was obtained in the second experiment (Table 4). Ranges of egg numbers contained in the individual masses varied widely. Thus, although differences in average numbers of eggs laid did exist from group to group, they were not in inverse linear relationship to increasing radiation dosage.

Weight of egg mass on day 22 after initiation of oviposition. Each egg mass was allowed to develop for 22 d and was then weighed. ANOVA showed no significant differences among the weights of the masses at that time for the control, 0.5-krad, and 1-krad groups in Experiment One. ANOVA could not be applied to other groups because of insufficient numbers of egg masses for analysis. Both large, heavy masses and small, light masses were scattered among all radiation levels.

In Experiment Two, ANOVA again demonstrated no significant differences in average weight of egg masses among the groups ($P > 0.05$). These data indicate no effects on average egg-mass weight that could be attributed to males' radiation dosage. There was no correlation between length of attachment periods of individual females and weights of their egg masses in either experiment.

Weight per egg. In both experiments, ANOVA indicated no significant differences among the weights of individual eggs at the various radiation levels. Average weights of eggs fell between 63 and 79 μg (Tables 3, 4). There was no significant correlation between weight per egg and dosage level of male parent. Also, weight per egg was not influenced by length of attachment period of the female parent or by the total number of eggs in the mass.

Discussion

Attachment and feeding of the treated *Amblyomma americanum* males in our study were unaffected at all but the highest radiation levels (8 and 16 krad). Ticks exposed to 16 krad were the most affected, often detaching from the host and wandering about for days. Most died during the second week after irradiation. These findings agree with Oliver et al. (1972), who stated that feeding of *Rhipicephalus sanguineus* was normal at all but 16 krad (at which level the ticks attached loosely and dislodged easily). These data also agree generally with those on *Rhipicephalus appendiculatus* (Purnell et al. 1972) which show

Table 3. Egg numbers and weights from matings of irradiated males of *A. americanum* with untreated females (Experiment One).

Male radiation dosage (krad)	No. eggs laid per mass		Egg mass wt. day 22 (mg)		Weight per egg (µg)	
	Mean	Range	Mean	Range	Mean	Range
0	2,984.5	687-4,586	186	41.5 - 311	66.2	60.4 - 77.9
0.5	3,041.7	326-6,450	208.9	25.7 - 458.8	72.5	60.9 - 78.8
1	3,438.4	454-5,667	209.5	188.5 - 230.5	70.5	65.3 - 75.7
2	693	361-1,025	*	–	–	–
3	3,068	2,002-4,133	296.8	296.8	71.8	71.8
4	ND	ND	ND	ND	ND	ND
8	1,011	166-1,856	*	–	–	–
16	5,147	5,147	321.9	321.9	62.5	62.5

Means within a column not followed by any letter are not significantly different by a one-way ANOVA.
ND, No data available.
* No weights were available.

Table 4. Egg numbers and weights from matings of irradiated males of *A. americanum* with untreated females (Experiment Two).

Male radiation dosage (krad)	No. eggs laid per mass		Egg mass wt. day 22 (mg)		Weight per egg (µg)	
	Mean	Range	Mean	Range	Mean	Range
0	1,845.6	526-3,371	133.6	51.5-235	79.1	69.6-97.9
0.5	3,867.5	367-5,929	329.7	277.4-375.5	67.5	61.6-73.8
1	4,495	3,061-5,665	300.8	177.3-403.5	65.1	57.9-76.8
2	2,055	485-3,139	175.1	120.3-264.3	65.8	52.1-84.1
3	3,294	3,294	252.6	252.6	76.6	76.6
4	ND*	ND	ND	ND	ND	ND
8	1,133	1,133	†	-	-	-
16	ND*	ND	ND	ND	ND	ND

Means within a column not followed by any letter are not significantly different by a one-way ANOVA.
ND, No data available.
* Females mated to males treated with 4 and 16 krad died before ovipositing.
† No weights were available.

that dosages up to 16 krad had no obvious effect on attachment of males two weeks after irradiation. Also, male *Dermacentor occidentalis* exposed to 7.5 krad mated, but those that received 15 krad did not mate (Oliver and Al-Ahmadi, unpublished information). It seems unanimous that doses of approximately 15-16 krad greatly affect feeding of irradiated males; and because feeding is necessary for sperm production in almost all metastriate species (Oliver 1974, 1986a, b), 15-16 krad prevents mating of the above mentioned species. Dosages much lower than 15-16 krad affect "successful" mating, although not feeding, of irradiated males. Our data indicate fewer *A. americanum* females fed and detached of their own volition after mating with males exposed to radiation levels of 3 krad and above. Moreover, there was a trend toward long average attachment periods in these ticks. The underlying explanation of why these females did not complete feeding is that female metastriate ticks hold at partial engorgement on the host until they are mated, after which they rapidly and fully engorge and drop off. The specific stimulus during mating that causes complete engorgement is still not known (Pappas and Oliver 1971, 1972, Oliver 1974, 1986a, b). Nevertheless, ability of male ticks to supply females with this stimulus is apparently impaired or destroyed by irradiation.

Our results generally agree with those reported by Drummond et al. (1966) on *A. americanum.* They found that females engorged normally when mated to males receiving 2.5 krad, but that only one of 20 females engorged after mating with males treated with 5 krad; no females engorged when paired to 7.5-krad treated males. Also, another study (Darrow et al. 1976) showed that male *A. americanum* treated with 2.5 krad did not compete successfully with normal males when provided normal females. Additional confirmation that dosages greater than 2 krad affect successful mating of metastriate male ticks is provided by Beuthner (1975), who reported a significant increase in engorgement periods of females paired with male *Amblyomma variegatum, Hyalomma anatolicum excavatum,* and *Rhipicephalus appendiculatus* treated at such levels. An exception is the report that unfed female *Dermacentor andersoni* confined with males exposed to 4 krad mated normally within the usual time period (Gregson 1969).

Fewer female *A. americanum* oviposited after mating with males exposed to the higher radiation levels (Tables 1, 2). This is a reflection of fewer females engorging and dropping of their own volition in these groups, and probably also indicates lack of successful sperm transfer in some cases. Szlendak et al. (1987) noted decreased spermatophore transfer by irradiated male *Acarus siro* mites. Results similar to ours were reported in *A. americanum* (Drummond et al. 1966). Shanbaky et al. (1979) reported no significant difference in percentages of females ovipositing between control *Argas arboreus* and those mated to 1-, 2- and 3-krad-treated males, but fewer females oviposited when mated with males treated with 4-25 krad. When irradiated male *Argas persicus* were mated with untreated females, the number of females ovipositing decreased with increasing dosage (Sternberg et al. 1973).

No clear-cut relationship was found between dosage level of males and average preoviposition period of females paired with them during our study with *A. americanum* (Tables 1, 2). This result is supported by Oliver et al. (1972), who detected no significant differences in preoviposition periods of *R. sanguineus* females mated to irradiated males. Drummond et al. (1971) and Sweatman

(1967) thought that length of preoviposition period was influenced by environmental factors such as temperature and relative humidity.

Irradiation of male *Amblyomma americanum* did not significantly influence number of eggs per mass laid by their mates if the females fed sufficiently to oviposit (Tables 3, 4). Females with mates from any treatment level deposited a range of a few hundred to several thousand eggs. Shanbaky et al. (1979) found that mean egg numbers per oviposition of *Argas arboreus* females mated with 1-krad-treated males did not differ significantly from control results, but fewer eggs were laid by females whose mates had been exposed to 2-25 krad.

There was no significant difference among the weights of the egg masses on day 22 after oviposition that could be attributed to the males' radiation exposure (Tables 3, 4). The same appears true regarding weights of individual eggs. Egg weight averaged between 63 and 79 μg in our study. These figures are close to those of Drummond et al. (1971), who reported an average weight of 59-60 μg for freshly-laid eggs and 52 μg for eggs on the verge of hatching in *Amblyomma americanum*. Sactor et al. (1948) recorded a weight of 54 μg for the same species.

Data from our study and from Oliver and Stanley (1987) suggest that if the conventional sterile-male technique is considered for *A. americanum,* 2 krad is probably the most effective exposure, as this dosage provides complete sterility in males without significantly altering their longevity or mating ability, at least initially. Reproductive impairment becomes severe at higher dosages, coinciding with cellular damage in the distal half of the testes.

Negation of sterile-male matings through subsequent inseminations by normal males (Gregson 1969) and unsuccessful competition by treated males (Sternberg et al. 1973, Darrow et al. 1976) remain problematic in control efforts. Use of sterile females also has disadvantages. Introduction of sterile females increased mating activity in untreated males of the soft tick *Argas persicus* (Galun et al. 1972).

A strategy not fully investigated in ticks is that of using substerilizing dosages of radiation to cause detrimental mutations. Khalil and Abdu (1979) showed that substerilizing dosages caused induction of delayed lethal genes in germinal cells of male *Argas arboreus,* and this subsequently caused embryo death. Detrimental genes also were expressed in the F_1 generation of treated ticks.

Acknowledgments

Thanks to C. Claiborne and M. Joiner (Georgia Southern University) for editorial assistance, S. Thomas (Georgia Southern University) for typing and graphics generation, and to Professors F. Clark (Memphis State University) and J. Schlager (University of Kansas) for statistical advice. Dr. J. George supplied the ticks and Dr. J. Brower kindly irradiated them.

Supported in part by Grants AI 09556 and AI 24899 from the National Institute of Allergy and Infectious Diseases and Contract # N00014-80-C-0546 from the Office of Naval Research.

References Cited

Beuthner, U. 1975. The sterile-male-release-technique: sterilization through gamma-radiation (60 Co) with *Amblyomma variegatum, Hyalomma anatolicum excavatum* and *Rhipicephalus appendiculatus.* Ph.D. Dissertation. Freien Univ., Berlin.

Darrow, D. I., W. J. Gladney and C. C. Dawkins. 1976. Lone star tick: mating behavior of gamma-irradiated males. Ann. Entomol. Soc. Am. 69: 106-108.

Drummond, R. O., J. G. Medley and O. H. Graham. 1966. Engorgement and reproduction of lone star ticks (*Amblyomma americanum* (L.)) treated with gamma-radiation. Int. J. Radiation Biol. 10: 183-188.

Drummond, R. O., T. M. Whetstone and W. J. Gladney. 1971. Oviposition of the lone star tick. Ann. Entomol. Soc. Am. 64: 191-194.

Galun, R., S. Sternberg and C. Mango. 1972. The use of sterile females for the control of the tick. *Argas persicus* (Oken). Israel J. Entomol. 7: 109-115.

Gregson, J. D. 1969. Evidence of multiple mating in *Dermacentor andersoni.* J. Parasitol. 55: 260.

Khalil, G. M. and R. M. Abdu. 1979. The subgenus *Persicargas* (Ixodoidea: Argasidae: *Argas*). 32. Effect of substerilizing doses of gamma radiation on male fertility and progeny of *A.* (*P.*) *arboreus.* J. Med. Entomol. 16: 339-342.

Kitaoka, S. and T. Morii. 1967. Effects of gamma radiation and chemosterilants on the cattle tick, *Boophilus microplus.* Japanese J. Sanitary Zool. 18: 126.

Knipling, E. F. 1982. Present status and future trends of the SIT approach to the control of arthropod pests, pp. 3-23. *In* Sterile insect technique and radiation in insect control. International Atomic Energy Agency, Vienna.

Magnarelli, L. A., J. F. Anderson, C. S. Apperson, D. Fish, R. C. Johnson and W. A. Chappell. 1986. Spirochetes in ticks and antibodies to *Borrelia burgdorferi* in white-tailed deer from Connecticut, New York state, and North Carolina. J. Wildl. Dis. 22: 178-188.

Oliver, J. H., Jr. 1974. Symposium on reproduction of arthropods of medical and veterinary importance. IV. Reproduction in ticks (Ixodoidea). J. Med. Entomol. 11: 26-34.

1986a. Relationship among feeding, gametogenesis, mating and syngamy in ticks, pp. 93-99. *In* D. Borovsky and A. Spielman [eds.], Host regulated developmental mechanisms in vector arthropods. Univ. of Florida Press IFAS, Vero Beach, Florida.

1986b. Induction of oogenesis and oviposition in ticks, pp. 233-247. *In* J. Sauer and J. Hair (eds.), Recent advances in morphology, physiology, and behavioral biology of ticks. Ellis Harwood, Chichester, England.

Oliver, J. H., Jr., R. L. Osburn and J. R. Roberts, Jr. 1972. Cytogenetics of ticks (Acari: Ixodoidea). 9. Chromosomes of *Rhipicephalus sanguineus* (Latreille) and effects of gamma radiation on spermatogenesis. J. Parasitol. 58: 824-827.

Oliver, J. H., Jr. and M. A. Stanley. 1987. Effects of gamma radiation on spermatogenesis and fertility of male *Amblyomma americanum* (Acari: Ixodidae). J. Parasitol. 73: 309-313.

Pappas, P. J. and J. H. Oliver, Jr. 1971. Mating necessary for complete feeding of female *Dermacentor variabilis* (Acari: Ixodidae). J. Ga. Entomol. Soc. 6: 122-124.

1972. Reproduction in ticks (Acari: Ixodoidea) 2. Analysis of the stimulus for rapid and complete feeding of female *Dermacentor variabilis* (Say). J. Med. Entomol. 9: 47-50.

Purnell, R. E., J. D. Dargie, B. Gilliver, A. D. Irvin and M. A. Ledger. 1972. Some effects of irradiation on the tick *Rhipicephalus appendiculatus.* Parasitology 64: 429-440.

Sactor, B., M. Hutchinson and P. Granett. 1948. Biology of the lone star tick in the laboratory. J. Econ. Entomol. 41: 296-301.

Schulze, T. L., G. S. Bowen, E. M. Bosler, M. F. Lakat, W. E. Parkin, R. Altman, B. G. Ormiston and J. K. Shisler. 1984. *Amblyomma americanum:* A potential vector of Lyme disease in New Jersey. Science 224: 601-603.

Shanbaky, N. M., G. M. Khalil and R. M. Abdu. 1979. The subgenus *Persicargas* (Ixodoidea: Argasidae: *Argas*). 30. The effect of gamma radiation on fertility and longevity of adult *Argas (P.) arboreus.* J. Med. Entomol. 16: 116-120.

Sidorov, V. E. and I. M. Grokhovskaya. 1964. The effect of x-rays on adult *Hyalomma asiaticum.* Communication I. Meditsinskaya Parazitologiya 33: 560-563.

Sokal, R. and F. J. Rohlf. 1969. Biometry. W. H. Freeman and Co., San Francisco, California.

Sternberg, S., B. A. Peleg and R. Galun. 1973. Effect of irradiation on mating competitiveness of the male tick *Argas persicus* (Oken). J. Med. Entomol. 10: 137-142.

Sweatman, G. K. 1967. Physical and biological factors affecting the longevity and oviposition of engorged *Rhipicephalus sanguineus* female ticks. J. Parasitol. 53: 432-445.

Szlendak, E., J. Boczek, W. Bruce and R. Davis. 1987. Effects of gamma radiation on spermatophore production and reception and subsequent fecundity and egg viability in *Acarus siro* L. (Acari: Acaridae). Exp. Appl. Acarol. 3: 33-44.