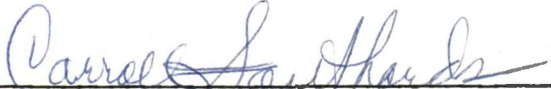
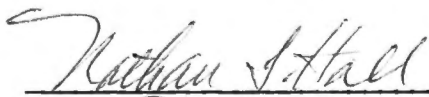
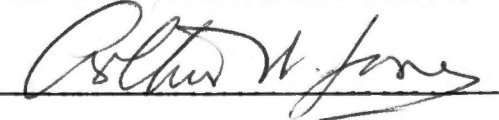


To the Graduate Council:

I am submitting herewith a thesis written by Robert J. Cox entitled "The Effects of Neutron-Irradiation on Meloidogyne incognita (Kofoid and White, 1919) Chitwood, 1949." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Agricultural Biology.


Carroll J. Southards, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council:


Linton A. Smith
Vice Chancellor
Graduate Studies and Research

Ag-VetMed

Thesis

76

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SOME EFFECTS OF FAST NEUTRON IRRADIATION UPON MELOIDOGYNE

INCOGNITA (KOFOID AND WHITE, 1919) CHITWOOD, 1949

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Robert J. Cox

December 1976

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DEDICATION

To My Grandparents

JOHN and GOLDIE COX

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ABSTRACT

Two groups of Meloidogyne incognita (Kofoid and White, 1919) Chitwood, 1949 eggs (Stage I = 50% of total number of eggs at a stage of embryological development exceeding the four-cell stage; Stage II = 50% of total number of eggs at a stage of embryological development equal to or less than the four-cell stage) were irradiated at 7, 22, and 32°C with 0, 1, 2, 3, or 4 Kilorads (Krad) of fast neutrons.

Radiation treatment had an effect on the hatch of both Stage I and Stage II eggs. The hatch of Stage I eggs was inversely proportional to the radiation levels and was independent of irradiation temperature. However, the hatch of Stage II eggs was not proportional and was associated with irradiation temperature. More Stage II eggs hatched at 2 Krad, 7°C treatment than at any of the other treatments at the same temperature. Significantly fewer eggs hatched at the 1 Krad treatment. The 0, 3, and 4 Krad, 7°C hatches were intermediate between and differed from the hatches at 1 and 2 Krad. At 22°C, all radiation treatments at Stage II showed significant increases over the hatch at 0 Krad with peaks at 1 and 3 Krad. The Stage I eggs followed a pattern similar to that noted at 7°C. At 32°C, the greatest number of eggs hatched at Stage I.

Host differential studies showed that eggs produced by females developing from Stage II eggs receiving 1, 2, and 3 Krad at 7°C and by females developing from both Stage I and Stage II eggs receiving 1, 2, 3, and 4 Krad at 32°C developed into larvae capable of parasitizing

and reproducing on a resistant variety of cowpea, Vigna sinensis 'Breeding Line M57-13N'. A change in the ability of Stage I, 7°C and Stage I, 32°C 3 Krad larvae to parasitize a normally susceptible watermelon, Citrulus vulgaris 'Charleston Grey', was noted. It was also observed that larvae developing from eggs laid by females developing from Stage I eggs exposed to 2 Krads at 7°C and from Stage II eggs exposed to 1 and 3 Krads at the same temperature were able to parasitize and reproduce on Nicotiana tabacum 'NC-95', a normally resistant variety of tobacco. Similar changes were noted in larvae developing from eggs laid by females developing from Stage II eggs exposed to 1 Krad at 32°C.

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CHAPTER I

INTRODUCTION

Meloidogyne incognita (Kofoed and White, 1919) Chitwood, 1949 is an important parasite of a large number of economically important plants. In the United States M. incognita causes several millions of dollars worth of damage annually. While chemical treatments and cultural control methods reduce this loss, the greatest degree of control is achieved by cultivating plant varieties resistant to this nematode. However, the utility of this practice is limited by the existence of a large number of "pathotypes" or "physiological races" of M. incognita. These pathotypes are morphologically identical to but physiologically different from other members of this species. Because of physiological differences one pathotype may have the ability to parasitize a plant or a variety of plant that all other pathotypes of that species are unable to parasitize. Pathotypes may exist in a natural population, expressing themselves only when environmental stress (e.g., resistant plants, chemical treatments, etc.) is exerted on the total population or may arise de novo from a natural population when it is placed under similar stress (Sturhan, 1971).

Presently there is little knowledge relative to the origin and biology of pathotypes. This report is part of a regional research project (S-76) studying these and related problems. As part of this project, the University of Tennessee concentrated on developing new

pathotypes of M. incognita by subjecting previously characterized populations of the nematode to mutagenic agents such as radiation or chemicals. The use of such agents might shorten the time required for the development of new pathotypes and perhaps allow for the collection of new information regarding the biology and origin of pathotypes. Such information could possibly be used to predict when and where new pathotypes might arise.

Since neutrons are approximately ten times more efficient than X-rays or gamma radiation in producing biological changes (Dertinger and Jung, 1970), neutron irradiation was used in this study. This was made possible by the University of Tennessee's close proximity to the Oak Ridge National Laboratory in Oak Ridge, Tennessee.

While the questions pertaining to pathotypes constituted a major portion of this research, they were not the sole objectives of the project. Three other phenomena were also investigated: (i) the hatching of larvae from irradiated eggs, (ii) the development of these larvae into males, and (iii) the production of eggs by females developing from these larvae. Information obtained from these studies might indicate the amount and type of change induced by the radiation. Also, an investigation of the effect of neutron irradiation on the hatching of larvae from irradiated eggs might increase our understanding of the processes involved in the normal hatching mechanism of Meloidogyne incognita.

CHAPTER II

LITERATURE REVIEW

Most radiation studies involving nematodes have been concerned with a few economically important forms such as Trichinella spiralis, the causative agent of trichinosis. The majority of these studies have centered around the possible use of ionizing radiations to kill eggs, larvae, and/or adults encysted in meat products (See Fassuliotis (1958) for a bibliography of such studies) or the development of radiation-attenuated vaccines (Yarinsky, 1962).

Relatively little work has been done on the effects of radiation on free-living or phytoparasitic nematodes. The first radiation studies involving phytoparasitic nematodes were reported by N. A. Cobb (1920). In these studies a species of Meloidogyne, the root-knot nematode, was exposed to various levels of X-rays. Cobb reported no detrimental morphological or genetic changes. In 1941, Evans, et al., reported that the larvae of Rhabditis pellio, a semisaprophytic nematode, died after exposure to 5 kiloroentgens of X-rays. They also noted that X-rays at 7 and 8 kiloroentgens killed embryos and larvae but not adult worms. Thomas and Quastler (1950) irradiated adult Rhabditis strongyloides with 40 kiloroentgens of X-rays and observed that the progeny of these nematodes were bisexual and sterile.

Fassuliotis and Sparrow (1955) used X-rays in an attempt to disinfect potatoes infested with Heterodera rostochiensis, the golden

nematode. They reported that wet cysts exposed to a dose of 20 or more kiloroentgens gave rise to sterile females. Fassuliotis (1958) expanded this work to include both X-rays and gamma radiation at levels between 0 and 1,280 kiloroentgens. He discovered that (i) at or above 160 kiloroentgens a delay in larval emergence (hatching) occurred, (ii) embryonated eggs differed among themselves with regard to radio-sensitivity, (iii) larvae emerging from cysts exposed to levels greater than 80 kiloroentgens suffered from so-called "motility pattern disturbances," (iv) no larvae emerged from cysts exposed to levels equal to or greater than 640 kiloroentgens, (v) no differences in the number of eggs laid by females developing from irradiated and non-irradiated cysts were observed until radiation levels exceeded 20 kiloroentgens, and (vi) infectivity was decreased in individuals emerging from cysts exposed to levels greater than 20 kiloroentgens. He also observed that while nematodes emerging from cysts exposed to levels greater than 20 kiloroentgens could still infect plants, they could not produce viable eggs. Eggs laid by females developing from cysts exposed to 20 or less kiloroentgens were viable but often contained abnormal chromosomes.

Weischer (1957) reported on the effects of X-rays on Heterodera rostochiensis and H. schachtii. He found that levels up to and including 29 kiloroentgens had no effect on the emergence of larvae from the cysts. At levels exceeding 39 kiloroentgens hatching was reduced in H. schachtii but stimulated in H. rostochiensis.

Wood and Goodey (1957) reported that between 48 and 96 kilo-roentgens of X-rays were needed to kill nematodes infesting cultivated mushroom beds.

Myers and Dropkin (1959), after studying the effects of gamma radiation on eleven species of phytoparasitic nematodes, concluded that the control of phytoparasitic nematodes in the soil and the sterilization of nematodes infesting plant materials by irradiation was not feasible. Myers (1960) repeated this work and expanded it to include several species of free-living nematodes. His results agreed with those obtained earlier and reinforced the conclusions drawn in the 1959 paper.

Ishibashi (1965) exposed Meloidogyne incognita larvae in intact roots in soil to 0, 10, and 20 krads of gamma radiation. He observed a difference in response due to the worm's stage of sexual differentiation. Larvae irradiated before sexual differentiation developed into significantly high numbers of small males with one testis. The number of these males increased with increasing dosage. He observed that the growth rate of these larvae was retarded in proportion to the radiation dosage. If larvae were irradiated during sexual differentiation, an increase in the number of large males with two testes was observed. While the number of such males increased with increasing dosage, little or no growth retardation was observed in the population as a whole.

Ishibashi and Taguchi (1965A) separated M. incognita egg masses into two groups on the basis of color and age (white egg masses = young eggs; yellow egg masses = old eggs), suspended them in water, and

exposed them to between 0 and 350 Krads of gamma radiation. While the number of larvae emerging from the white egg masses decreased with increasing dosage, the number emerging from yellow egg masses receiving 150 or more kilorads increased. Also, larvae emerging from white egg masses receiving 35 or more kilorads were unable to mature or reproduce on host plants. The authors suggest that the eggs at the more advanced developmental stages (those in the yellow egg masses) are less radiosensitive than those at the less advanced developmental stages (those in the white egg masses). The same authors (1965B) exposed various developmental stages of the same nematode on soil-free intact roots to 0, 10, and 20 Krads of gamma radiation. At both 10 and 20 Krads larvae in the early stages of development (those having penetrated the plant root three days prior to irradiation) were extremely radiosensitive and failed to mature and reproduce. Radiosensitivity was observed to decrease with increasing developmental stage. This supported their earlier conclusion. No changes in the number of eggs produced was observed in irradiated females. However, development of these eggs was retarded. Also, an abnormally high number of adult male nematodes developed from the larvae irradiated during the earlier developmental stages.

Townshend (1967) exposed Heterodera schachtii cyst and mature females in both air and soil to between 0 and 512 Krads of gamma radiation. Larval emergence from cysts receiving 32 Krads increased while emergence from cysts exposed to 512 Krads was totally suppressed. Larval emergence from cysts developing from irradiated mature females decreased

at all radiation levels. Most larvae emerging from cysts receiving 128 or more kilorads failed to develop into adults; those that did lacked gonads. While germinal damage was observed at 32 Krads, somatic damage did not appear until the 128 Krad level was reached.

Evans (1970) exposed various developmental stages of Heterodera rostochiensis to between 0 and 64 Krads of gamma radiation. He noted that soaking the cysts in potato root diffusate three days prior to irradiation--a process inducing larval emergence--made them much more radiosensitive than dry cysts. Embryos in soaked cysts were killed by dosages as low as 2 Krads. Second stage larvae were less radiosensitive than embryos while adults were apparently unharmed by radiation. Evans also noted that doses above 8 Krads reduced the fertility of adult females and the ability of the second stage larvae to penetrate and parasitize host plants.

Siddiqui and Viglierchio (1970) exposed fourth stage larvae of Ditylenchus dipsaci to gamma radiation at levels ranging from 0 to 400 Krads. They observed that the motility of the nematodes exposed to between 48 and 192 Krads was initially reduced. However, this reduction usually disappeared by the fourth day after irradiation. The ability of the nematode to successfully parasitize alfalfa seedlings decreased with increasing dosage. Reproduction was decreased at dosages greater than or equal to 48 Krads. It was noted that morphological abnormalities were found only in the progeny of nematodes receiving 32 or more kilorads. In all cases the amount of damage was less in individuals irradiated in a nitrogen atmosphere as opposed to an oxygen atmosphere.

Lapp (1970) exposed Meloidogyne arenaria eggs and adult females to between 0 and 320 Krads of gamma radiation. He observed that (i) small radiation doses--5 and 10 Krads--had no effect on hatching, (ii) six days after irradiation an increase in the number of larvae emerging was noted in the 20 Krad treatment, (iii) doses higher than 20 Krads decreased larval emergence, and (iv) nematodes developing from eggs exposed to more than 80 Krads exhibited a loss in their ability to parasitize normally susceptible plants. No increase in the number of males developing from irradiated eggs was observed. Lapp concluded that eggs at relatively early developmental stages are more radiosensitive than those in relatively late stages.

In summary, several general stagements regarding the effects of radiation upon free-living and phytoparasitic nematodes can be drawn from the preceding discussion.

- i. Radiosensitivity as a function of developmental stage
(Evans, et al., 1941; Fassuliotis, 1958; Ishibashi, 1965; Ishibashi and Taguchi, 1965A and B; Evans, 1970; Lapp, 1970), with radiosensitivity decreasing with increasing developmental stage.
- ii. Irradiation may cause a depression (Fassuliotis, 1958; Weischer, 1957; Ishibashi and Taguchi, 1965A; Townshend, 1967; Lapp, 1970) or a stimulation (Weischer, 1957; Ishibashi, 1965; Ishibashi and Taguchi, 1965A; Townshend, 1967; Lapp, 1970) of the emergence of larvae from eggs.
- iii. Rather extreme morphological changes have been observed

in individuals emerging from irradiated eggs (Fassuliotis, 1958; Ishibashi, 1965; Townshend, 1967; Siddiqui and Viglierchio, 1970). Apparent physiological changes have also been noted (Fassuliotis, 1958; Ishibashi and Taguchi, 1965A; Evans, 1970; Siddiqui and Viglierchio, 1970; Lapp, 1970).

- iv. While irradiation can kill or sterilize phytoparasitic and free-living nematodes (Fassuliotis and Sparrow, 1955; Fassuliotis, 1958; Wood and Goodey, 1957; Evans, 1970), the many technical and biological problems involved make the use of radiation in nematode control unfeasible (Myers and Dropkin, 1959; Myers, 1960).

CHAPTER III

MATERIALS AND METHODS

Eggs of Meloidogyne incognita were obtained from cultures originally established by inoculating the roots of Lycopersicon esculentum Mill. var. 'Rutgers' plants with single egg masses (Southards and Priest, 1973). Stage I eggs (50% or more of the total number of eggs at a stage of embryological development exceeding the four-cell stage) were obtained by immersing galled roots in a 12% aqueous solution of commercial strength Chlorox[®] and agitating them for 1.5 minutes. The resulting slurry was differentially screened through a series of sieves (e.g., 45, 100, and 200 mesh) to remove plant debris. The eggs were collected on a No. 500 mesh sieve and suspended in 50 ml. tap water and the number of eggs determined. The suspension was equally dispensed into five 1.5 x 15 cm. Pyrex[®] test tubes. These tubes were stoppered and stored overnight at 10°C. Stage II eggs (50% or more of the total number of eggs at a stage of embryological development equal to or less than the four-cell stage) were obtained by immersing the same roots used above in a 12% aqueous solution of commercial strength Chlorox[®] and agitating them for another 1.5 minutes. The eggs obtained were treated as previously described.

Fast neutron irradiation was conducted with the unshielded, unmodified fast burst Health Physics Research Reactor (HPRR) at the Oak Ridge National Laboratory, Oak Ridge, Tennessee (Auxier, 1965). Eggs

were exposed to five different radiation levels (1, 1, 2, 3, and 4 Kilorads) at three different temperatures (approximately 7, 22, and 32°C). Within 30 minutes after irradiation, the water in which the eggs were suspended was decanted and replaced with an equal volume of fresh water. This was done to minimize the effects of any peroxides or free radicals that might have formed in the water during irradiation.

Eggs were irradiated at three different temperatures because the building housing the reactor lacked a temperature control unit. Thus, the ambient air temperature was the irradiation temperature. The first group of eggs was irradiated in early January when the mean ambient air temperature was about 7°C. The second group of eggs was irradiated in early July when the mean ambient air temperature was approximately 32°C. At this time, a temperature effect was noted and the decision was made to irradiate a third group of eggs at a temperature somewhere between these two extremes. This was done in early October when the mean ambient air temperature was 22°C.

To determine the effects of radiation treatment level and irradiation temperature on the emergence of larvae from the eggs, a one milliliter aliquant of each treatment was removed and diluted to a volume of 10 ml. These suspensions were incubated at 22-26°C for 12 days. Counts of the number of larvae present in each treatment were made every two days during this period. The remaining eggs from each treatment were used to inoculate the roots of 6-8 week-old L. esculentum var. 'Rutgers' plants (approximately 10,000 eggs per replicate per respective irradiation treatment). Each plant was transferred to a

Host differential studies were carried out using ten different host plants: tomato, Lycopersicon esculentum Mill. var. 'Rutgers' (Susceptible) and 'Wonder Boy' (Resistant); pepper, Capsicum frutescens L. var. 'California Wonder' (Susceptible); peanut, Arachis hypogaea L. var. 'Floor Runner' (Resistant); cowpea, Vigna sinensis (Torner) Savi 'Breeding Line M57-13N' (Resistant); cotton, Gossypium hirsutum L. var. 'DPL-16' (Intermediate Response); corn, Zea mays L. var. 'Minnesota A-401' (Intermediate Response); sweet potato, Ipomea batatas L. var. 'Puerto Rico' (Resistant); watermelon, Citrulus vulgaris Schrad. var. 'Charleston Grey' (Susceptible); and tobacco, Nicotiana tabacum L. 'North Carolina-95' (Resistant). Each plant received approximately 5,000 eggs.

Fifty days after inoculation, all plants were processed and indexed as previously described. After indexing, the roots were treated in the manner outlined above in order to collect the eggs. These eggs were used to establish subcultures on 'Rutgers' tomato plants.

CHAPTER IV

RESULTS

I. EMERGENCE OF LARVAE FROM IRRADIATED EGGS

Twelve days after irradiation, the number of larvae emerging from the control (0 Krads) treatments for both Stage I and Stage II eggs at 7°C was not different (Figure 1). However, the shape of the two response curves was quite different. The Stage I eggs at 1, 2, 3, and 4 Krads exhibited a classical radiation response--decreasing biological activity (hatching) was increasing dose level. The Stage II eggs exhibited an erratic response to radiation treatment. A highly significant ($P = 0.01\%$) drop below the control hatch number was observed at the 1 Krad treatment. At the 2 Krad treatment, a highly significant ($P = 0.01\%$) increase over the control hatch number was noted. This was followed by a gradual drop in numbers hatching so that the 3 and 4 Krad treatments were no different from the 0 Krad treatment. At this temperature no interactions between developmental stage, irradiation temperature, and/or treatment level were observed.

At 22°C the Stage I eggs exhibited a behavior pattern similar to that observed for Stage I at 7°C. The only observable difference was the occurrence of a slight, insignificant stimulation at 1 Krad (Figure 2). The numbers emerging from Stage II eggs irradiated with 1, 3, and 4 Krads were all significantly higher ($P = 0.01\%$) than those emerging at 0 and 2 Krads. A highly significant ($P = 0.01\%$) interaction between

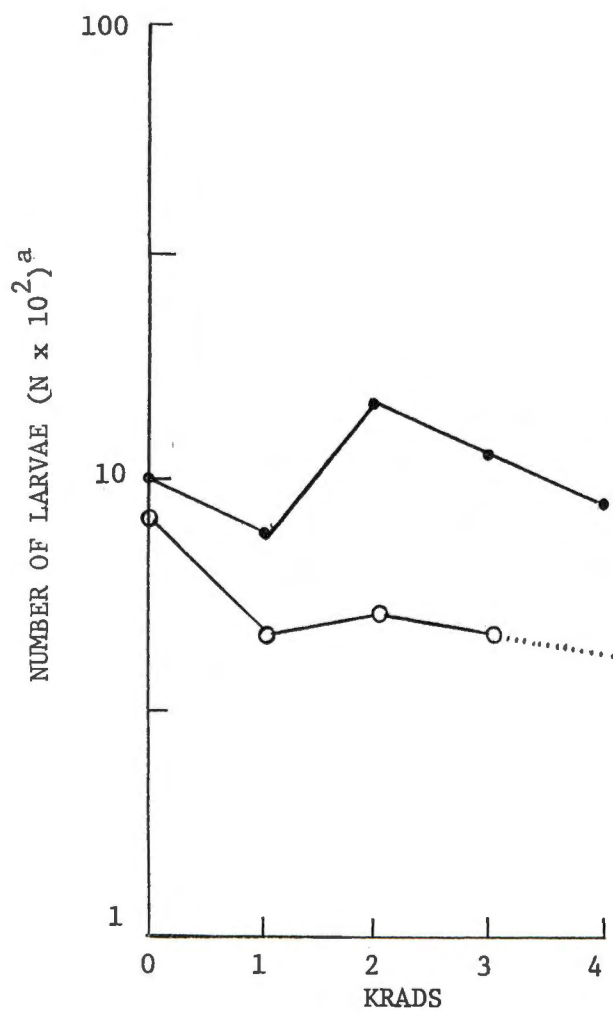


Figure 1. Number of larvae emerging from Stage I eggs (O) and Stage II eggs (•) irradiated at 7°C.

^aAverage of five replications.

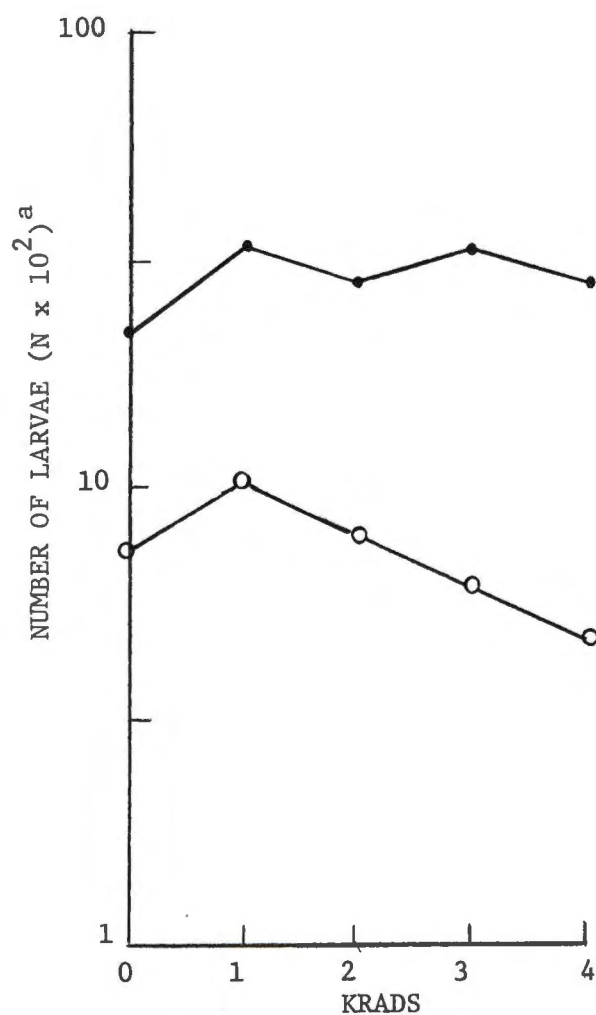


Figure 2. Number of larvae emerging from Stage I eggs (O) and Stage II eggs (●) irradiated at 22°C.

^aAverage of five replications

developmental stage and irradiation temperature was observed at this temperature.

At 7 and 22°C more larvae hatched from the Stage II eggs than from the Stage I eggs. At 32°C this trend was reversed (Figure 3). At 32°C the number of larvae emerging from the Stage I eggs at 4 Krads was significantly lower ($P = 0.05\%$) than the number emerging at 1 Krad. The numbers hatching at all other treatment levels were intermediate between these extremes. For Stage II eggs at 32°C, the greatest hatch was noted at 0 Krads, the lowest at 1 and 2 Krads ($P = 0.05\%$). A highly significant ($P = 0.01\%$) interaction between developmental stage and irradiation temperature was observed at this temperature.

When all treatment level and developmental stage hatch totals are pooled according to irradiation temperature, the number of larvae emerging is observed to increase with increasing temperature (Figure 4). The differences between all three temperatures are highly significant ($P = 0.01\%$).

II. NUMBER OF MALES DEVELOPING FROM IRRADIATED EGGS

25 DAYS AFTER INOCULATION ONTO 'RUTGERS'

TOMATO PLANTS

No significant increase in the number of males developing from irradiated eggs was observed at any temperature (Figure 5). At 7°C the ratio of males to non-differentiated larvae was about 1:2 for all treatment levels at both developmental stages. At Stage II 32°C no males were observed in the 0, 1, and 3 Krad treatments. While an

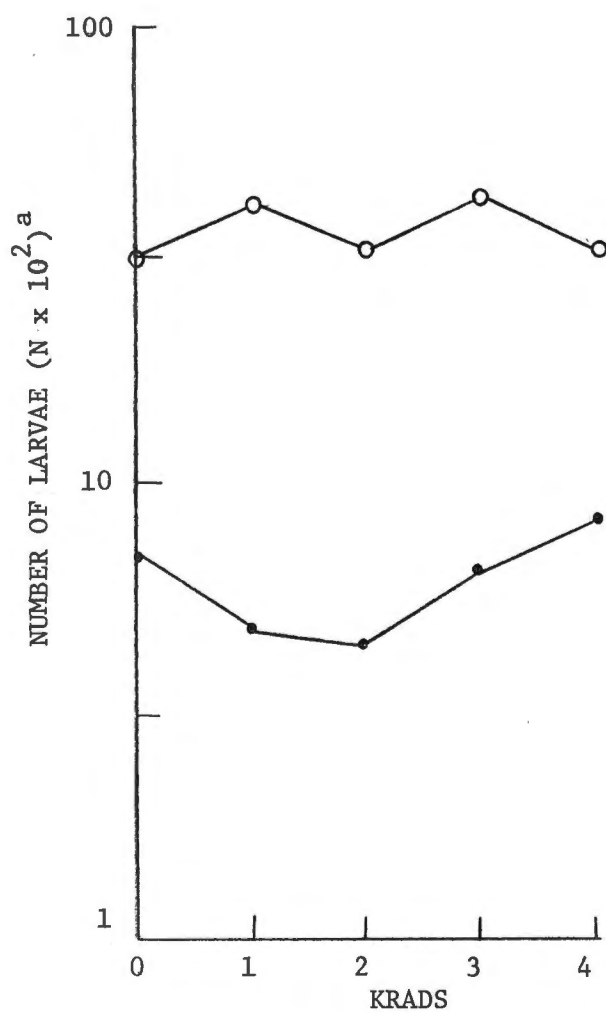


Figure 3. Number of larvae emerging from Stage I eggs (○) and Stage II eggs (●) irradiated at 32°C.

^aAverage of five replications.

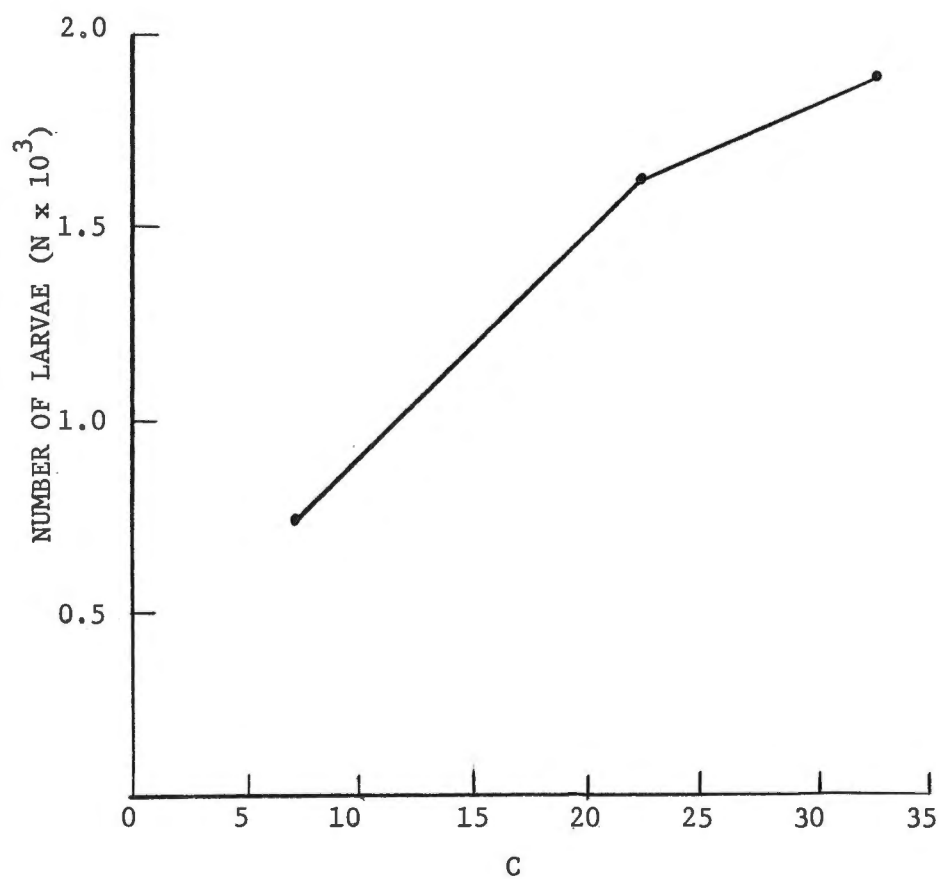


Figure 4. Number of larvae emerging from irradiated eggs with regard to irradiation temperature (pooled data).

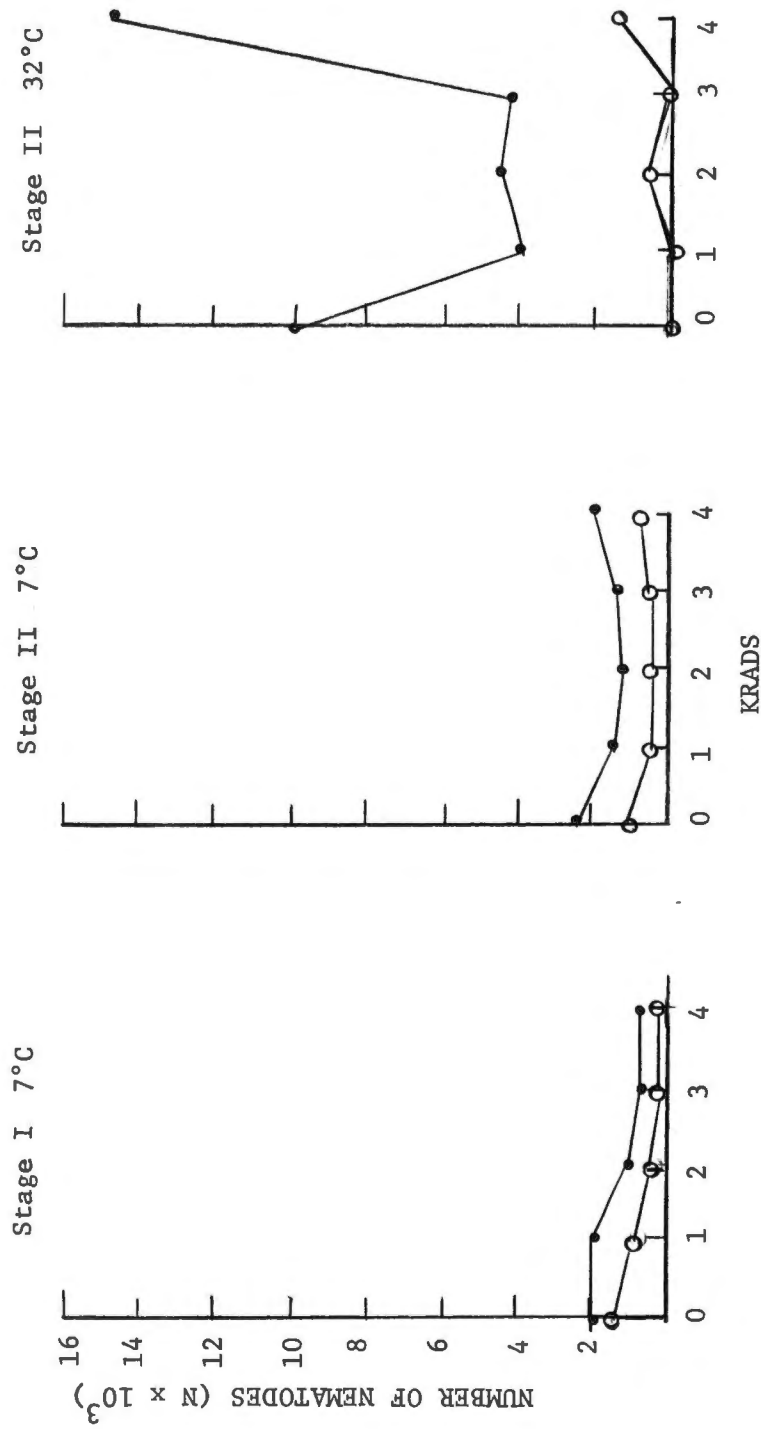


Figure 5. Number of adult males (O) and undifferentiated larvae (●) present 25 days after inoculation onto Lycopersicon esculentum var. 'Rutgers' plants.

increase over these numbers was observed at 2 and 4 Krads at 32°C, it was insignificant when compared to the numbers of non-differentiated larvae present at these treatment levels. The Stage I 32°C sample was lost during processing.

III. RESPONSE OF 'RUTGERS' TOMATO PLANTS TO LARVAE EMERGING FROM IRRADIATED EGGS

Fifty days after inoculation the highest gall size score for Stage I 7°C was noted at the 4 Krad level (Figure 6). While the scores at 0 and 3 Krads were no different from that at 4 Krads, the 2 Krad score was significantly less ($P = 0.01\%$) than the 4 Krad score. The 1 Krad score was intermediate between these extremes. The highest gall number score for Stage I 7°C was observed at 3 Krads. The 4 Krad score was no different from the 3 Krad score but the 1 Krad score was significantly lower ($P = 0.01\%$). The remaining scores were intermediate between these extremes. The highest egg mass size score for this stage and temperature was noted at 3 and 4 Krads. All other scores were significantly lower ($P = 0.05\%$). The highest egg mass number score for Stage I 7°C was observed at 3 and 4 Krads. While no differences were noted between this score and those observed at 0 and 2 Krads, the 1 Krad score was significantly lower ($P = 0.05\%$) than that at 3 and 4 Krads.

For Stage II 7°C the highest gall size score occurred at 3 Krads (Figure 6). The 0 Krad score did not differ from the 3 Krad score. However, the 4 Krad score was significantly less ($P = 0.01\%$) than the

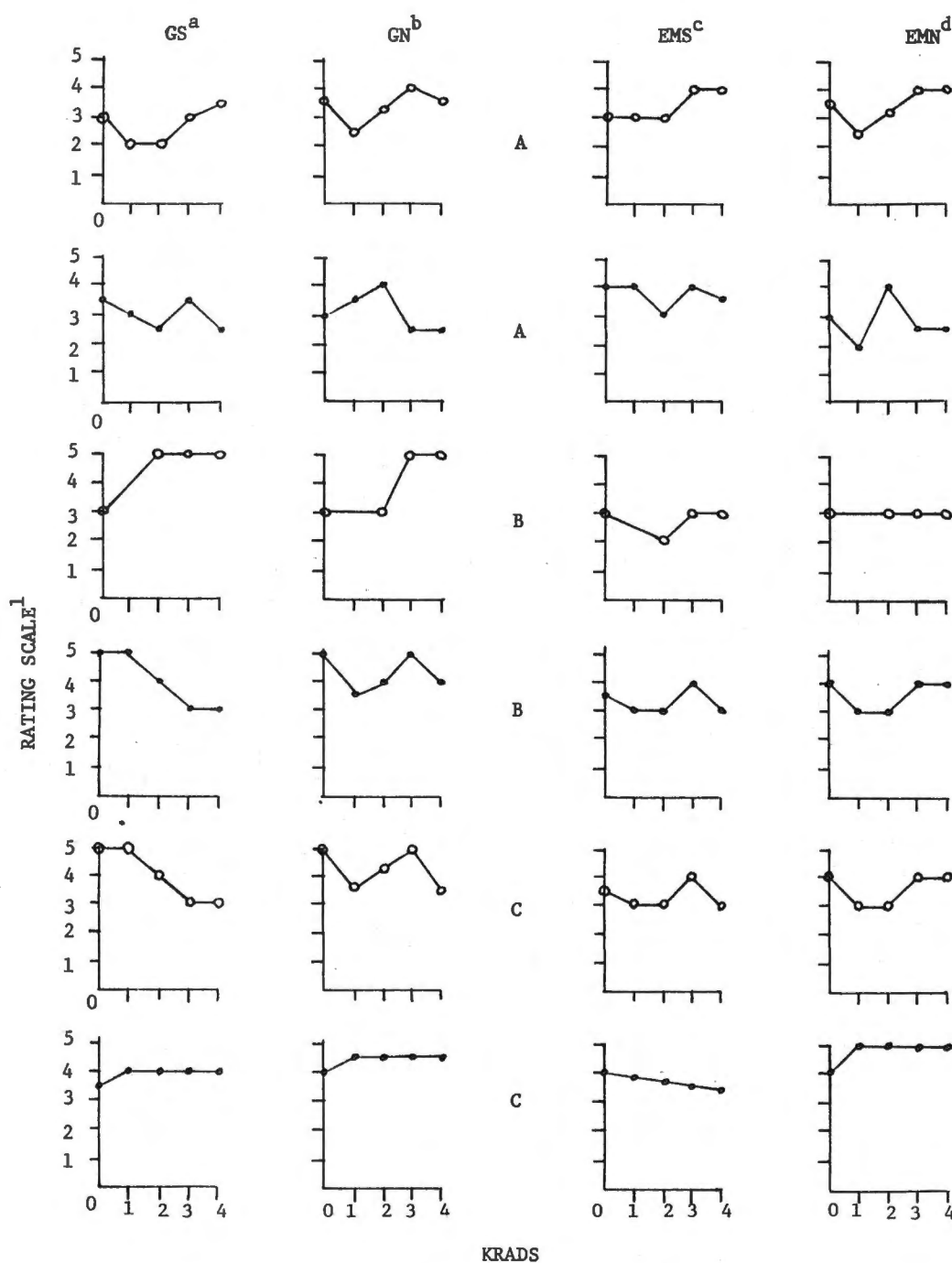


Figure 6. Response of *Lycopersicon esculentum* var. 'Rutgers' to larvae emerging from Stage I eggs (○) and Stage II eggs (●) irradiated at 7°C (A), 22°C, (B), and 32°C (C).

^aGall Size

^bGall Number

^cEgg Mass Size

^dEgg Mass Number

¹Average of five replications.

3 Krad score. The scores at 1 and 2 Krads were intermediate between these extremes. The highest gall number score for this stage and temperature was noted at 2 Krads. The lowest (significant difference, $P = 0.01\%$) occurred at 4 Krads. All remaining scores were intermediate between these two extremes. The highest egg mass size score for Stage II 7°C was observed at 0, 1, and 3 Krads. All remaining scores were significantly lower ($P = 0.05\%$). The highest egg mass number score occurred at 2 Krads and was significantly greater ($P = 0.01\%$) than the scores at 1, 3, and 4 Krads. The score at 0 Krads was intermediate between these extremes.

For Stage I 22°C the highest gall size score was noted at 2, 3, and 4 Krads (Figure 6). The lowest (high significant difference, $P = 0.01\%$) score was observed at 0 Krads. The highest gall number score for this stage and temperature was observed at 3 and 4 Krads. The gall number scores at 0 and 2 Krads were significantly lower ($P = 0.01\%$) than the 3 and 4 Krad scores. The highest egg mass size score for Stage I 7°C was noted at 0, 3, and 4 Krads. The 2 Krad score was significantly lower ($P = 0.01\%$). All egg mass number scores for this stage and temperature were identical. The Stage I 7°C 1 Krad treatment was lost.

For Stage II 22°C the highest gall size score was observed at 0 and 1 Krads. While the 2 Krad score did not differ from this score, the 3 and 4 Krad score was significantly lower ($P = 0.01\%$) than the 0 and 1 Krad score (Figure 6). The highest gall number score for this stage and temperature was noted at 0 and 3 Krads. The lowest score was observed at 1 Krad and differed significantly ($P = 0.05\%$) from the score

at 0 and 3 Krads. The scores at 2 and 4 Krads were intermediate between these extremes. The highest egg mass size score for Stage II 22°C was observed at 3 Krads. The scores at 1, 2, and 4 Krads differed significantly ($P = 0.05\%$) from the 3 Krad score. The 0 Krad score was intermediate between these extremes. The highest egg mass number score was noted at 0, 3, and 4 Krads; the scores at 1 and 2 Krads were significantly lower ($P = 0.05\%$).

At Stage I 32°C no significant differences were observed in gall size, gall number, egg mass size, or egg mass number at any treatment level (Figure 6). No significant differences in gall size, gall number, or egg mass number were observed in any treatment in Stage II at the same temperature. The highest egg mass size score for Stage II 32°C was noted at 0 Krads. The score at 2 and 4 Krads was not significantly different from this score. The scores at 1 and 3 Krads were significantly lower ($P = 0.05\%$) than the other scores.

When treatment level scores are pooled and only developmental stage and irradiation temperature is considered, no significant differences are noted between either stage at any temperature. However, when treatment level scores and developmental stage scores are pooled and only irradiation temperature is considered, highly significant differences ($P = 0.01\%$) are noted in gall and egg mass production at all temperatures.

IV. EGG PRODUCTION ON 'RUTGERS' TOMATO PLANTS BY FEMALES DEVELOPING FROM LARVAE EMERGING FROM IRRADIATED EGGS

Fifty days after inoculation, the greatest egg production for Stage I 7°C was observed at 2 Krads (Figure 7). Egg production at 0 Krads did not differ from that observed at 2 Krads. Highly significant differences ($P = 0.01\%$) were noted between these scores and the 1 Krad score. Production at 3 and 4 Krads was significantly lower ($P = 0.01\%$) than that noted at the other treatment levels. For Stage II 7°C the greatest egg production was observed at 2 Krads. Egg production at 0 Krads was significantly different ($P = 0.01\%$) from that at 2 Krads. Production at 4 Krads was significantly less ($P = 0.01\%$) than that observed at 2 and 0 Krads. Production at 1 and 2 Krads was intermediate between that noted for 0 and 4 Krads. Highly significant ($P = 0.01\%$) interactions between treatment level and irradiation temperature, developmental stage and irradiation temperature, and treatment level, developmental stage and irradiation temperature were observed in both stages at this temperature. These same interactions were observed in both stages at 22 and 32°C as well.

For Stage I 22°C, the greatest egg production was observed at 4 Krads (Figure 8). Egg production at 3 Krads was no different from that at 4 Krads. Production at 0 and 2 Krads was significantly lower ($P = 0.01\%$) than that at 3 and 4 Krads. The 1 Krad treatment for this stage and temperature was lost. For Stage II 22°C the greatest egg production was observed at 2 Krads. Production at 3 and 4 Krads was

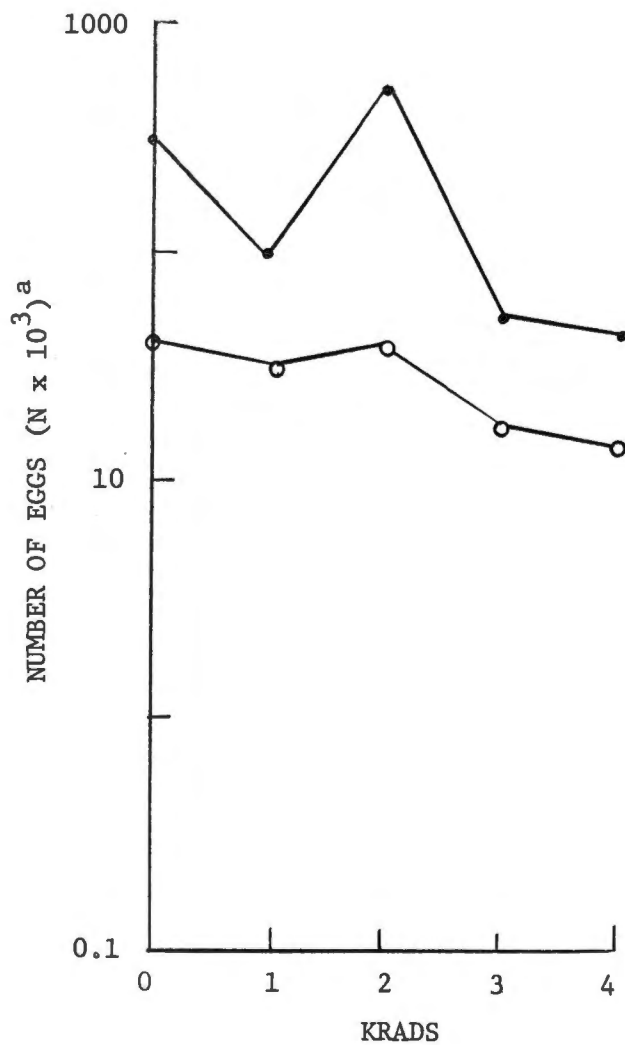


Figure 7. Egg production by females developing from larvae emerging from Stage I eggs (O) and Stage II eggs (●) irradiated at 7°C.

^a Average of four replications.

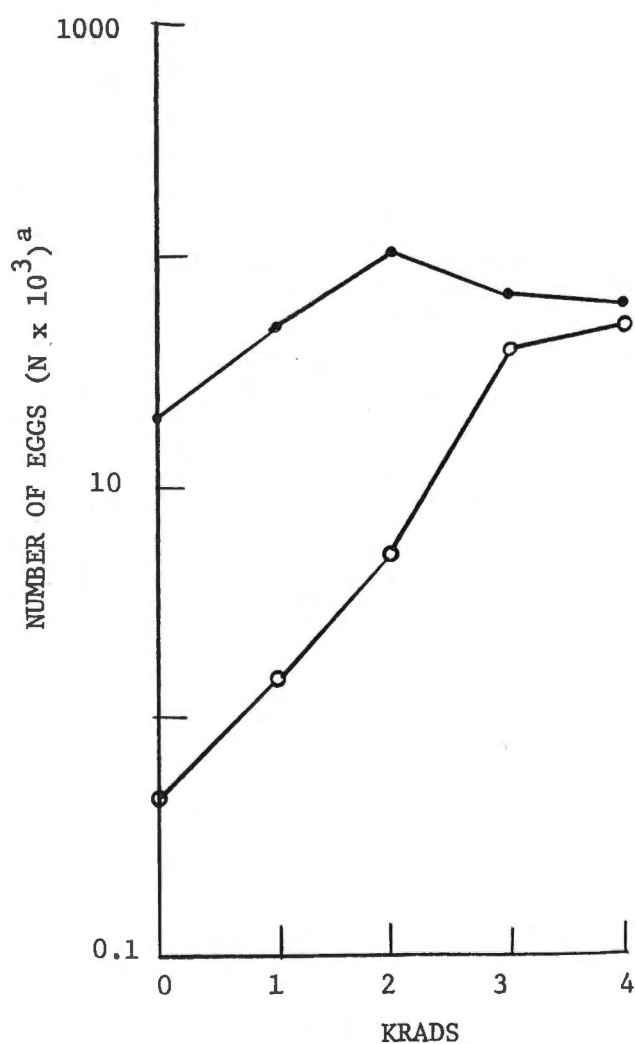


Figure 8. Egg production by females developing from larvae emerging from Stage I eggs (O) and Stage II eggs (●) irradiated at 22°C.

^a Average of four replications.

approximately equal and was significantly lower ($P = 0.01\%$) than that at 2 Krads. Egg production at 0 Krads was significantly lower ($P = 0.01\%$) than that observed at all other treatments. Production at 1 Krad was intermediate between that noted at 0 Krads and 3 and 4 Krads.

At Stage I 32°C the 4 Krad treatment exhibited the greatest egg production level (Figure 9). Production at 3 Krads did not differ significantly from that observed at 4 Krads. Egg production at 2 Krads was significantly lower ($P = 0.01\%$) than that noted at 3 and 4 Krads. Production at 0 Krads was significantly lower ($P = 0.01\%$) than that observed at 2, 3, and 4 Krads. Egg production at 1 Krad was intermediate between that noted at 0 and 2 Krads. For Stage II 32°C maximum egg production was observed at 4 Krads. Production at 3 Krads was significantly lower ($P = 0.01\%$) than that observed at 4 Krads. Egg production at 0 Krads was significantly lower ($P = 0.01\%$) than that at 3 and 4 Krads. Production at 1 and 2 Krads was intermediate between that for 3 and 4 Krads.

When treatment level totals are pooled according to developmental stage and irradiation temperature, egg production in Stage II is observed to be much greater ($P = 0.01\%$) than egg production in Stage I (Figure 10). This pattern holds for all three irradiation temperatures.

When developmental stage and treatment level totals are pooled according to irradiation temperature, a V-shaped curve is obtained (Figure 11). Maximum egg production is observed at 7 and 32°C , minimum at 22°C . Production at all three irradiation temperatures is significantly different ($P = 0.01\%$).

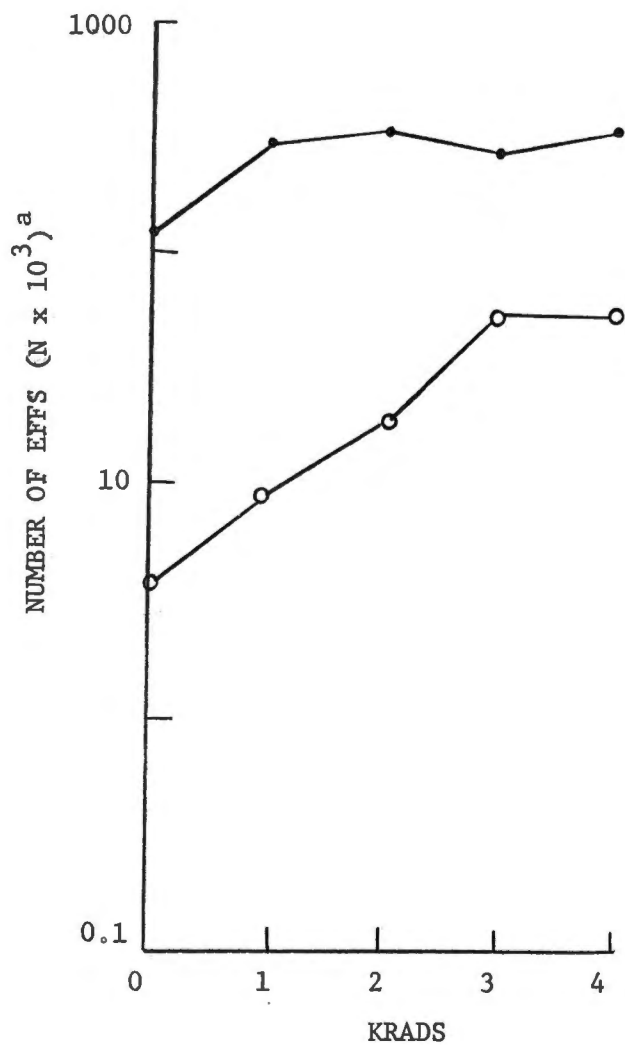


Figure 9. Egg production by females developing from larvae emerging from Stage I eggs (O) and Stage II eggs (●) irradiated at 32°C.

^a Average of four replications.

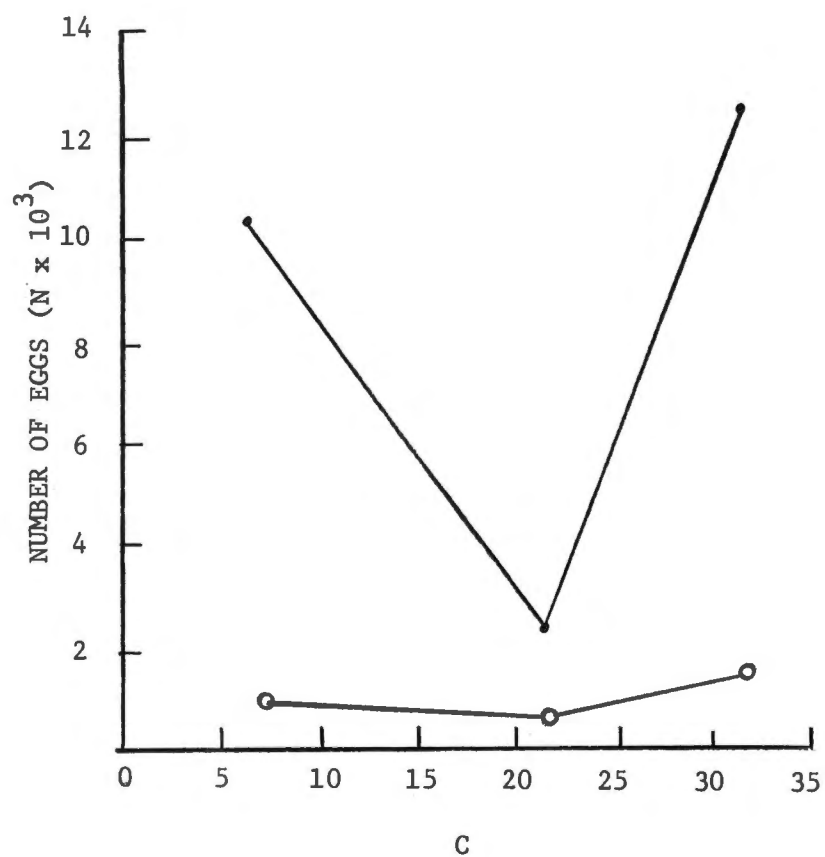


Figure 10. Egg production by females developing from larvae emerging from irradiated eggs: Effect of developmental stage (Stage I (O) and Stage II (●)) and irradiation temperature (pooled data).

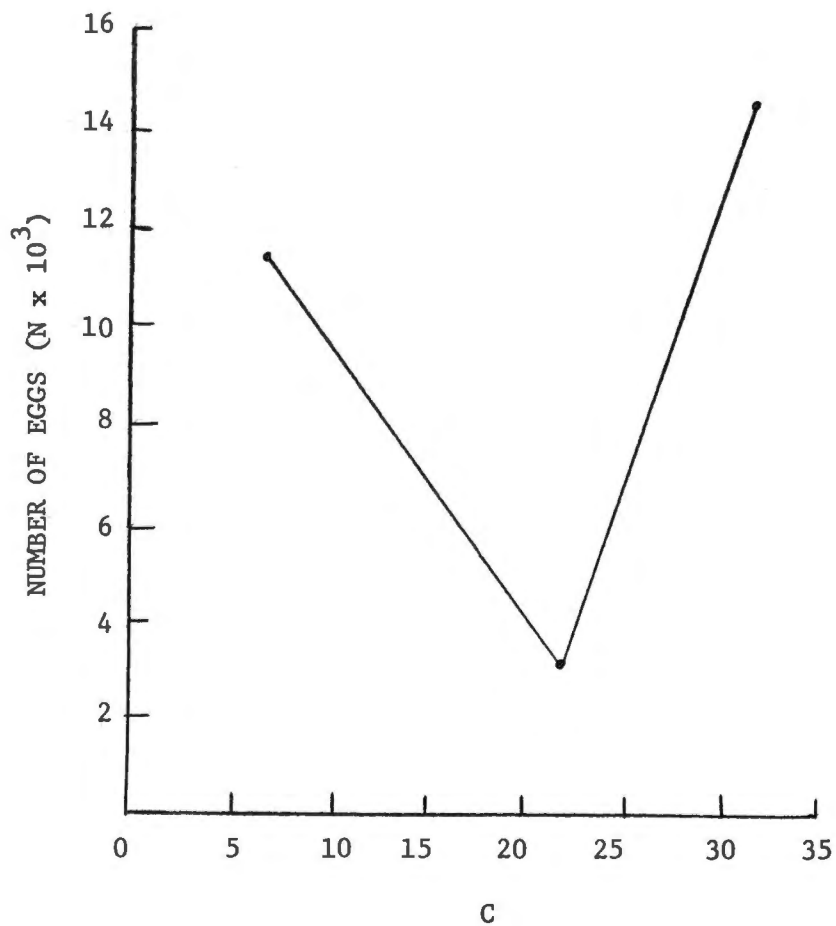


Figure 11. Egg production by females developing from larvae emerging from irradiated eggs: Effect of irradiation temperature (pooled data).

V. RESPONSE OF VARIOUS PLANTS TO INOCULATION WITH
EGGS PRODUCED BY PROGENY OF IRRADIATED EGGS

A. Lycopersicon esculentum var. 'Rutgers'

At 7°C the highest gall size score was observed at 0, 2, and 4 Krads from inoculations with Stage I progeny (Figure 12). The 3 Krad score was significantly lower ($P = 0.01\%$) than the score at 0, 2, and 4 Krads. The score at 1 Krad was intermediate between these extremes. No differences were observed in the gall number scores at 0, 1, 2, and 4 Krads. The gall number score at 3 Krads was lower ($P = 0.05\%$) than those observed at the other treatment levels. At this stage and temperature, treatment level had no observable effect on the size of the egg mass produced. The highest gall size score was observed at 4 Krads. While no difference was noted between the gall size scores at 1 and 4 Krads, the score at 3 Krads was significantly lower ($P = 0.05\%$) than that at 4 Krads. The 0 and 2 Krad scores were intermediate between these extremes. At Stage II 7°C the highest gall size score was observed at 2 Krads. The gall size scores at 1, 3, and 4 Krads were approximately equal and were lower ($P = 0.05\%$) than the 2 Krad score. The 0 Krad score was intermediate between these extremes. The highest gall number score at this stage and temperature was observed at 2 and 4 Krads. The 3 Krad score was lower ($P = 0.05\%$) than that noted at 2 and 4 Krads. The gall number scores for 0 and 1 Krads were intermediate between these extremes. The highest egg mass size score for Stage II 7°C was observed at 2 Krads. The egg mass size score for 1 and 4 Krads was smaller ($P = 0.05\%$) than that noted at 2 Krads. While

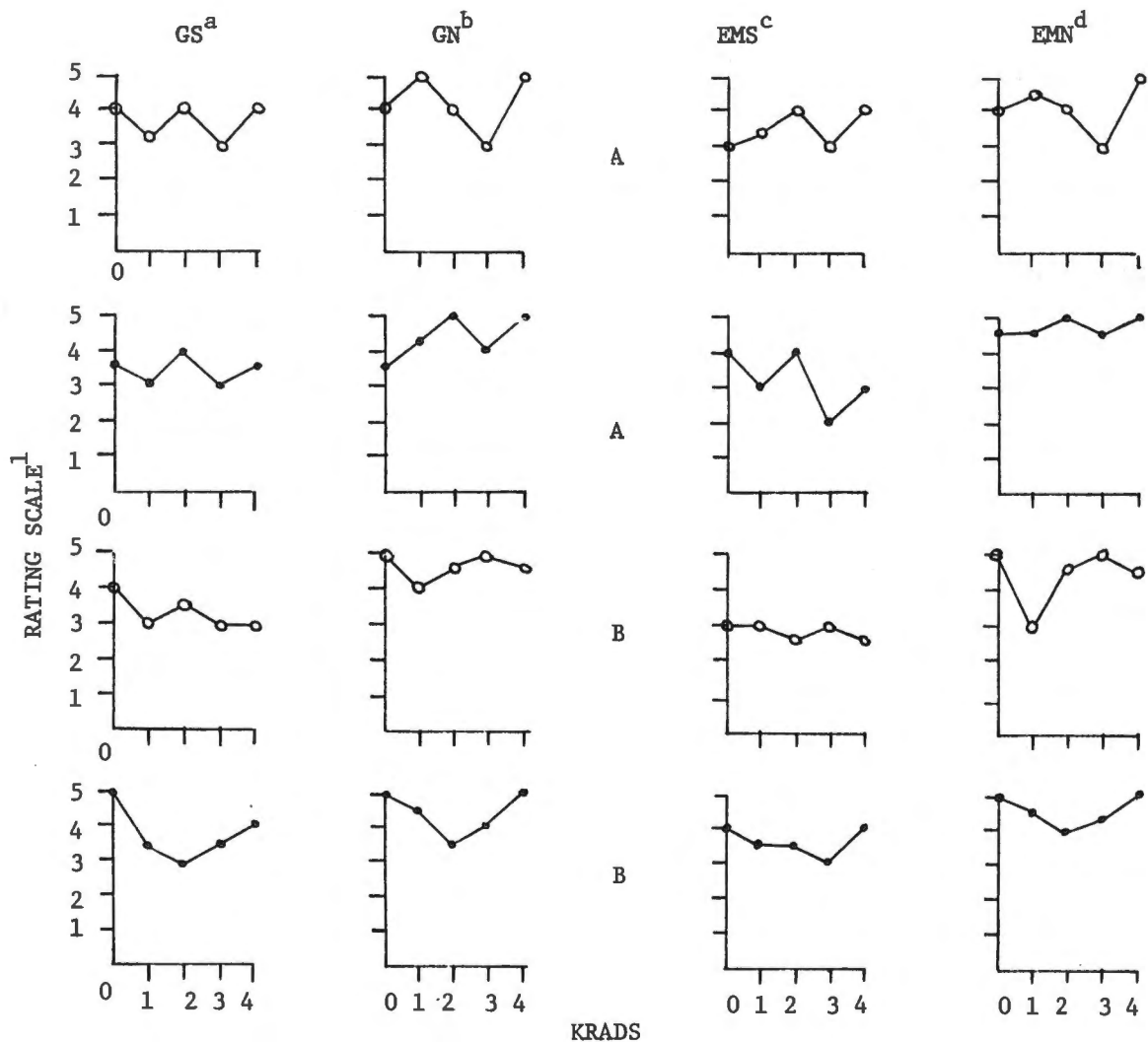


Figure 12. Response of *Lycopersicon esculentum* var. 'Rutgers' to larvae emerging from eggs produced by females developing from larvae emerging from Stage I eggs (○) and Stage II eggs (●) irradiated at 7°C (A) and 32°C (B).

^aGall Size

^bGall Number

^cEgg Mass Size

^dEgg Mass Number

¹Average of five replications.

the egg mass size score for 0 Krads was intermediate between those noted for 2 Krads and for 1 and 4 Krads, the 3 Krad score was lower ($P = 0.05\%$) than that observed at 1 and 4 Krads. Treatment level produced no observable difference in the egg mass number scores for Stage II 7°C.

For Stage I 32°C the highest gall size score was observed at 0 Krads (Figure 12). The scores at 1, 3, and 4 Krads were identical and were lower ($P = 0.05\%$) than that noted at 0 Krads. The gall size score at 2 Krads was intermediate between these two extremes. Treatment level had no effect on gall number or egg mass size at this stage and temperature. The highest egg mass number scores were noted at 0, 3, and 4 Krads. The scores at 1 and 2 Krads were not different and were significantly lower ($P = 0.01\%$) than those observed at the other treatment levels. At Stage II 32°C the highest gall size score was observed at 0 Krads. The scores at 2 and 4 Krads differed from each other ($P = 0.05\%$) and were lower ($P = 0.05\%$) than the score noted at 0 Krads. The gall size scores at 1 and 3 Krads were intermediate between those observed at 2 and 4 Krads. The highest gall number score was observed at 0, 1, and 4 Krads. The scores observed at 2 and 3 Krads were not different and were lower ($P = 0.05\%$) than those noted at the other treatments. No differences were noted in the egg mass size scores. The egg mass number scores followed the same pattern as the gall number scores.

When all treatment level scores are pooled according to developmental stage and irradiation temperature, significant differences

($P = 0.05\%$) in gall size and number are noted between Stage I and Stage II at 7°C . While highly significant differences ($P = 0.01\%$) are noted between these two stages at this irradiation temperature in terms of egg mass number, no significant differences are noted in terms of egg mass size. At 32°C highly significant differences ($P = 0.01\%$) were observed between the two developmental stages in terms of gall size, egg mass size, and egg mass number. No significant differences were noted in gall number.

When developmental stage and treatment level scores are pooled according to irradiation temperature, it is observed that gall number and size and egg mass number and size do not differ significantly in regard to irradiation temperature.

B. *Lycopersicon esculentum* var. 'Wonder Boy'

At Stage I 7°C the highest gall size score was noted at 0 Krads (Figure 13). This score differed ($P = 0.05\%$) from that observed at 2 Krads and both scores were higher ($P = 0.05\%$) than those at 1, 3, and 4 Krads. This same pattern was followed by the gall number scores. However, the differences in the gall number scores were much more significant ($P = 0.01\%$) than those observed in the gall size scores. Egg masses were produced only at the 2 Krad level, giving rise to highly significant differences ($P = 0.01\%$) in both egg mass size and number scores. The Stage II 7°C larvae followed the same behavior pattern as did the Stage I 7°C larvae.

No galls or egg masses were produced in either stage irradiated at 32°C .

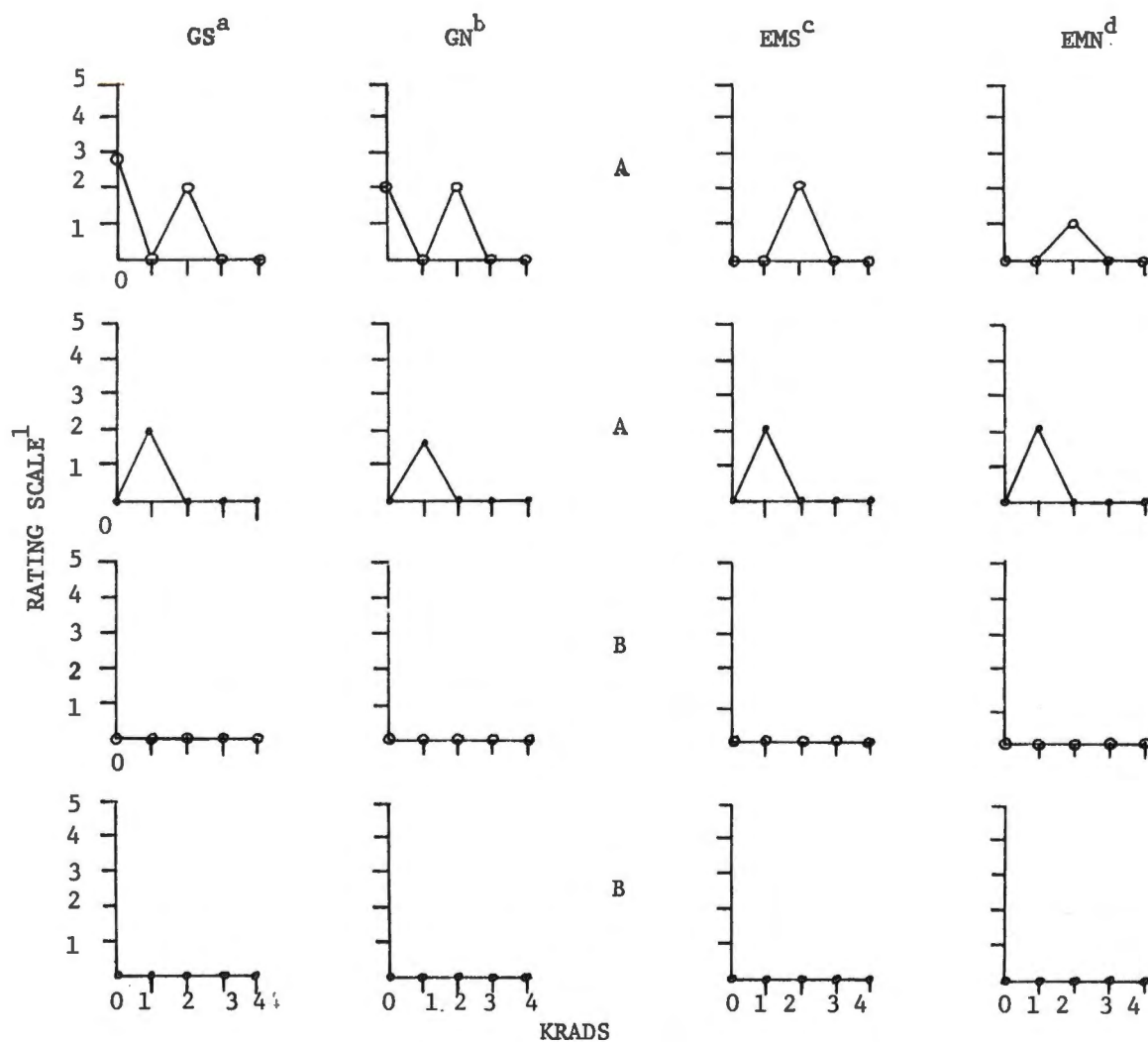


Figure 13. Response of *Lycopersicon esculentum* var. 'Wonder Boy' to larvae emerging from eggs produced by females developing from larvae emerging from Stage I eggs (O) and Stage II eggs (●) irradiated at 7°C (A) and 32°C (B).

^aGall Size

^bGall Number

^cEgg Mass Size

^dEgg Mass Number

¹Average of five replications.

When all treatment level scores are pooled according to developmental stage and irradiation temperature, highly significant differences ($P = 0.01\%$) are observed in gall and egg mass production at 7°C .

C. *Ipomea batatas* var. 'Puerto Rico'

No gall or egg mass production was observed.

D. *Arachis hypogaea* var. 'Floor Runner'

No gall or egg mass production was observed.

E. *Capsicum frutescens* var. 'California Wonder'

At Stage I 7°C the highest gall size score was observed at 2 and 4 Krads (Figure 14). All other treatment levels had identical scores and were significantly lower ($P = 0.01\%$) than the scores noted at 2 and 4 Krads. The highest gall number score was observed at 1 Krad. The score at 4 Krads was significantly lower ($P = 0.05\%$) than the 1 Krad score. All other treatments had scores intermediate between the 1 and 4 Krad scores. The highest egg mass size scores for this stage and temperature were observed at 1 and 4 Krads. These scores were significantly higher ($P = 0.01\%$) than the score at 0 Krads. All other scores were intermediate between these extremes. No significant differences were noted in egg mass number at Stage I 7°C . For Stage II 7°C the gall size scores followed the same pattern exhibited at Stage I 7°C . The highest gall number score for Stage II 7°C was observed at 4 Krads and was significantly higher ($P = 0.01\%$) than the scores at the other treatments. The highest egg mass size score was observed at 1 and 4

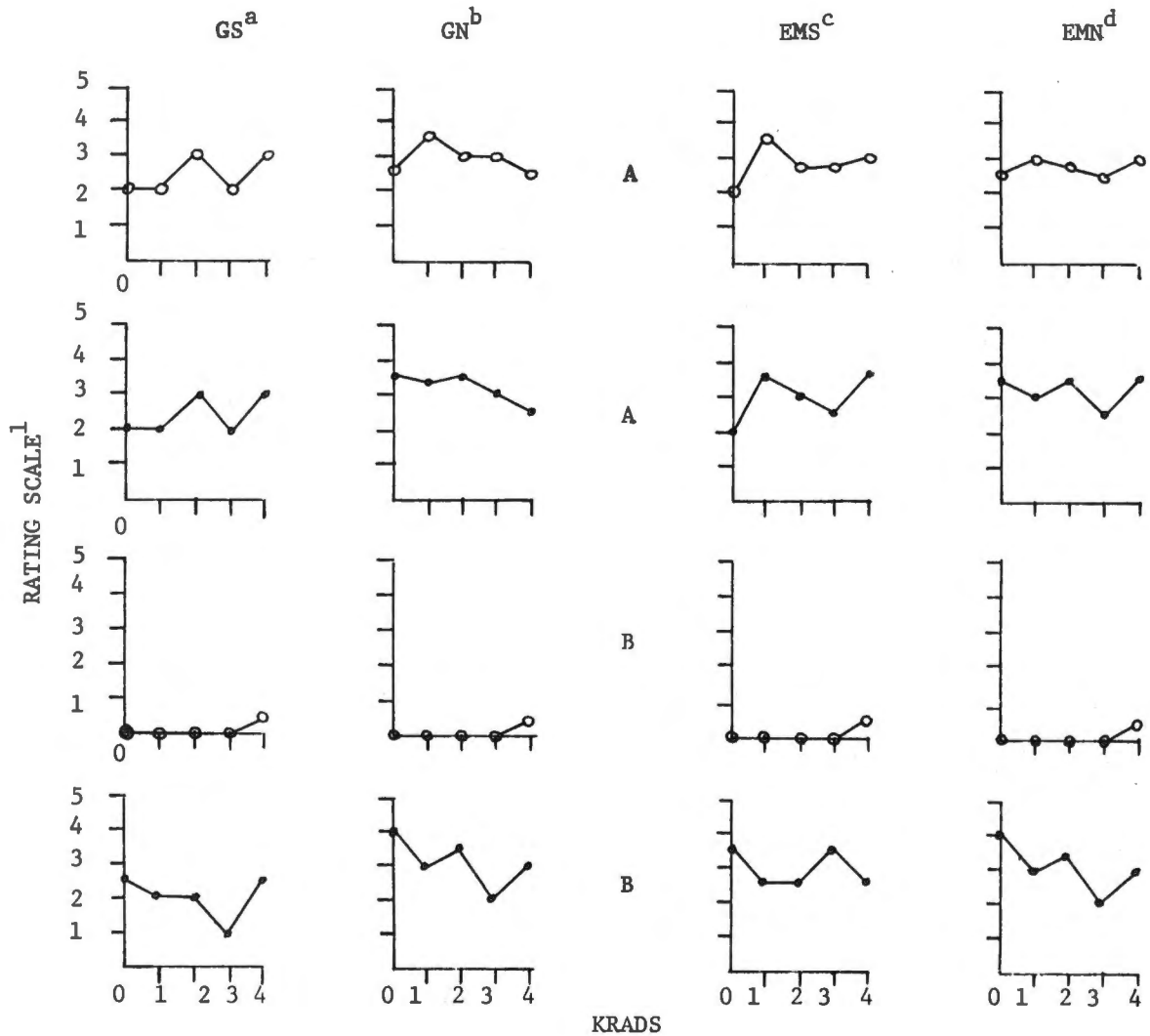


Figure 14. Response of *Capsicum frutescens* var. 'California Wonder' to larvae emerging from eggs produced by females developing from larvae emerging from Stage I eggs (O) and Stage II eggs (●) irradiated at 7°C (A) and 32°C (B).

^aGall Size

^bGall Number

^cEgg Mass Size

^dEgg Mass Number

¹Average of five replications.

Krads. The 0 Krad score was lower ($P = 0.05\%$) than that noted at 1 and 4 Krads. The remaining scores were intermediate between these two extremes. Treatment level at Stage II 7°C did not significantly affect egg mass number.

For Stage I 32°C the highest gall size score was observed at 4 Krads (Figure 14). The next highest score was noted at 0 Krads and was significantly lower ($P = 0.01\%$) than that at 4 Krads. No galls were produced at any other treatment level. This same pattern was observed for gall number. Egg mass production was observed only at the 4 Krad level. At Stage II 32°C the highest gall size score was observed at 0 and 4 Krads. The scores at 1 and 2 Krads were not different and were lower ($P = 0.05\%$) than those noted at 0 and 4 Krads. The 3 Krad score was lower ($P = 0.05\%$) than those at the other treatment levels. The highest gall number score was observed at 0 Krads. The scores at 2, 3, and 4 Krads were different ($P = 0.05\%$) from each other and lower ($P = 0.05\%$) than that noted at 0 Krads. The 1 Krad score was intermediate between the scores for 2 and 3 Krads. The highest egg mass size score for Stage II 32°C was observed at 0 and 3 Krads. The scores at 1, 2, and 4 Krads were not different and were significantly lower ($P = 0.01\%$) than those at 0 and 3 Krads. The highest egg mass number score was observed at 0 Krads. The scores at 1, 2, and 4 Krads were not different and were lower ($P = 0.05\%$) than the 0 Krad score. The 3 Krad score was lower ($P = 0.05\%$) than the other scores.

When all treatment level scores were pooled according to developmental stage and irradiation temperature, no significant differences

between the two stages are observed at 7°C. However, highly significant differences ($P = 0.01\%$) are noted in both gall and egg mass production for both stages at 32°C.

When all treatment level and developmental stage scores are pooled according to irradiation temperature, highly significant differences ($P = 0.01\%$) are noted between 7 and 32°C in terms of both gall and egg mass production.

F. *Vigna sinensis* 'Breeding Line M57-13N'

At Stage I 7°C gall and egg mass production occurred only in the 3 and 4 Krad treatments (Figure 15). Both treatments had identical scores. For Stage II 7°C gall and egg mass production occurred only in the 1 and 2 Krad treatments. In both cases the highest scores were observed at 2 Krads with the 1 Krad score being significantly lower ($P = 0.01\%$).

For Stage I 32°C the highest gall size score was observed at 4 Krads (Figure 15). All remaining scores, with the exception of the 2 and 3 Krad scores, were different ($P = 0.05\%$) from each other. All treatments differed from the 4 Krad treatment. The highest gall number score was observed at 4 Krads. The 2 and 3 Krad scores and the 0 and 1 Krad scores formed two pairs of almost identical scores that were significantly different ($P = 0.01\%$) from each other and the 4 Krad score. The highest egg mass score was also observed at 4 Krads. All other scores were different ($P = 0.05\%$) from each other and the 4 Krad score. The egg mass number scores followed the same pattern. In

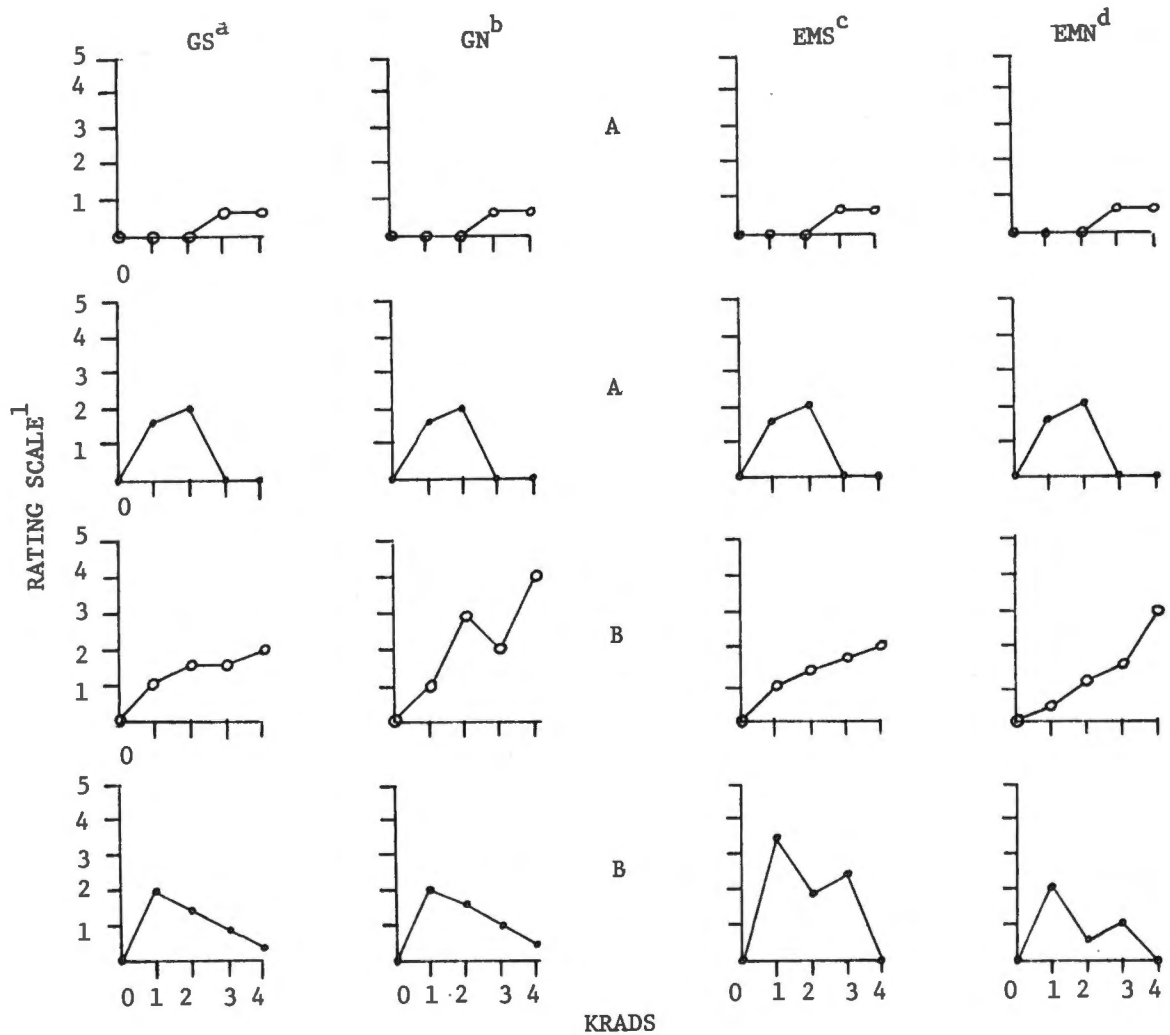


Figure 15. Response of *Vigna sinensis* 'Breeding Line M57-13N' to larvae emerging from eggs produced by females developing from larvae emerging from Stage I eggs (○) and Stage II eggs (●) irradiated at 7°C (A) and 32°C (B).

^aGall Size

^bGall Number

^cEgg Mass Size

^dEgg Mass Number

¹Average of five replications.

Stage II 32°C no gall or egg masses were produced at 0 Krads. The highest gall size score was observed at 1 Krad. All other scores were less ($P = 0.05\%$) than the 1 Krad score. This same pattern was followed by the gall number scores. The highest egg mass score was observed at 1 Krad. The scores at 2 and 3 Krads did not differ and were significantly different ($P = 0.01\%$) from all the other scores. The egg mass number scores followed a similar pattern.

When all treatment level scores are pooled according to developmental stage and irradiation temperature, a definite pattern emerges. At 7°C the highest scores for both gall and egg mass production are found in Stage II. This situation is reversed at 32°C. In both cases the differences between the two stages are highly significant ($P = 0.01\%$).

When developmental stage and treatment level scores are pooled according to irradiation temperature, the higher gall and egg mass scores are observed to occur at 32°C. The differences between the two irradiation temperatures is highly significant ($P = 0.01\%$).

G. *Gossypium hirsutum* var. 'DPL-16'

At Stage I 7°C no gall or egg mass production was noted (Figure 16). At Stage II 7°C no gall or egg mass production was noted at 0, 1, and 3 Krads. The highest gall size and number scores were noted at the 4 Krad level. The scores at 2 Krads were significantly lower ($P = 0.01\%$) than those at 4 Krads. Egg mass production was observed only at the 2 Krad treatment.

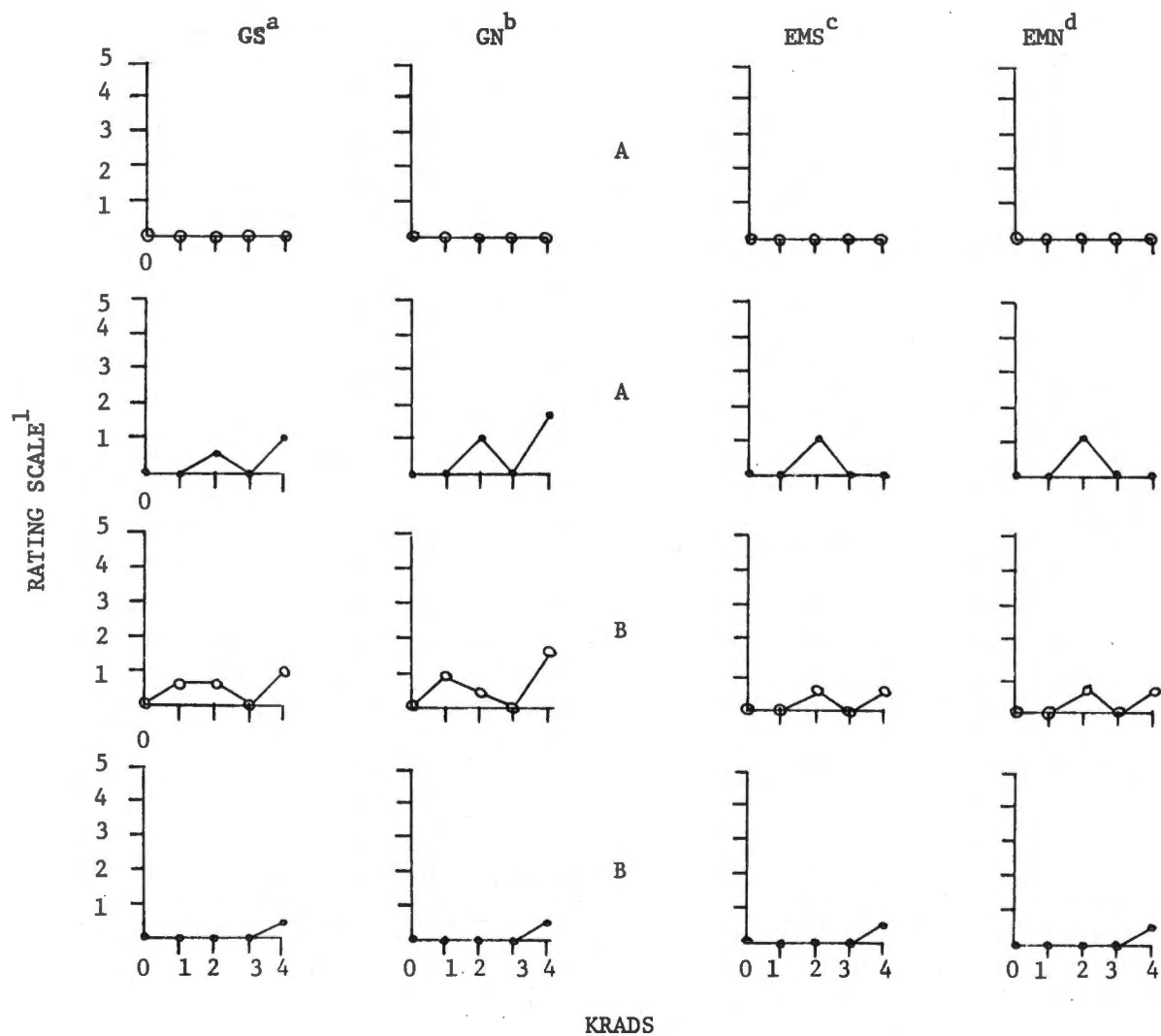


Figure 16. Response of *Gossypium hirsutum* var. 'DPL-16' to larvae emerging from eggs produced by females developing from larvae emerging from Stage I eggs (○) and Stage II eggs (●) irradiated at 7°C (A) and 32°C (B).

^aGall Size

^bGall Number

^cEgg Mass Size

^dEgg Mass Number

¹Average of five replications.

In Stage I 32°C no galls were produced at 0 or 3 Krads (Figure 16). The highest gall size score was observed at the 4 Krad level. The gall size score for 1 and 2 Krads was identical and was significantly different ($P = 0.01\%$) from the scores at the other treatment levels. The highest gall number score was noted at 4 Krads. All other gall number scores differed ($P = 0.05\%$) from each other and the 4 Krad score. No egg masses were produced at the 0, 1, or 3 Krad treatments at this stage and temperature. The highest egg mass size score was observed at 2 Krads. The score at 4 Krads was significantly lower ($P = 0.01\%$). The egg mass number score at 2 Krads was identical to that at 4 Krads. No gall or egg mass production was observed at 0, 1, 2, or 3 Krads at this stage and temperature. The scores at 4 Krads were therefore highly significant ($P = 0.01\%$).

When all treatment level scores are pooled according to developmental stage and irradiation temperature, a pattern of gall and egg mass production similar to that observed for Vigna sinensis (Cowpea) was noted. At 7°C the highest scores are observed at Stage II. At 32°C they are noted at Stage I. The significant difference between the two irradiation temperatures was quite high ($P = 0.01\%$).

When treatment level and developmental stage scores are pooled according to irradiation temperature, no significant differences are noted in egg mass production. However, the gall scores at 32°C are higher than those at 7°C ($P = 0.01\%$).

H. Zea mays var. 'Minnesota A-401'

At Stage I 7°C the highest gall size and number score was observed at 0 Krad (Figure 17). The score noted at 3 Krads was no different from that observed at 0 Krads. The scores at 1 and 2 Krads were identical and differed significantly ($P = 0.01\%$) from all other scores. No galls were produced in the 4 Krad treatment. The highest egg mass size score was observed at 2 and 3 Krads. The scores at 0 and 1 Krads were different ($P = 0.05\%$) from those observed at all other treatment levels. The egg mass number scores for 0, 1, 2, and 3 Krads were all identical. No egg mass production was observed at the 4 Krad level for this stage and temperature. At Stage II 7°C the highest gall and egg mass scores were observed at 2 Krads. No gall or egg mass production was observed at 4 Krads. All other gall and egg mass scores were intermediate between these two extremes.

At Stage I 32°C no gall production occurred in the 1, 2, and 3 Krad treatments (Figure 17). The highest gall size and number scores were observed at 4 Krads, the lowest at 0 krads. Egg mass production was observed only in the 4 Krad treatment. Differences between the 4 Krad scores and all other scores were highly significant ($P = 0.01\%$). No gall or egg mass production was noted in any treatment at Stage II 32°C.

When all treatment level scores are pooled according to developmental stage and irradiation temperature, the scores at Stage II 7°C are observed to be higher than those at Stage I 7°C. The significant difference for all scores except gall number was high ($P = 0.01\%$). At

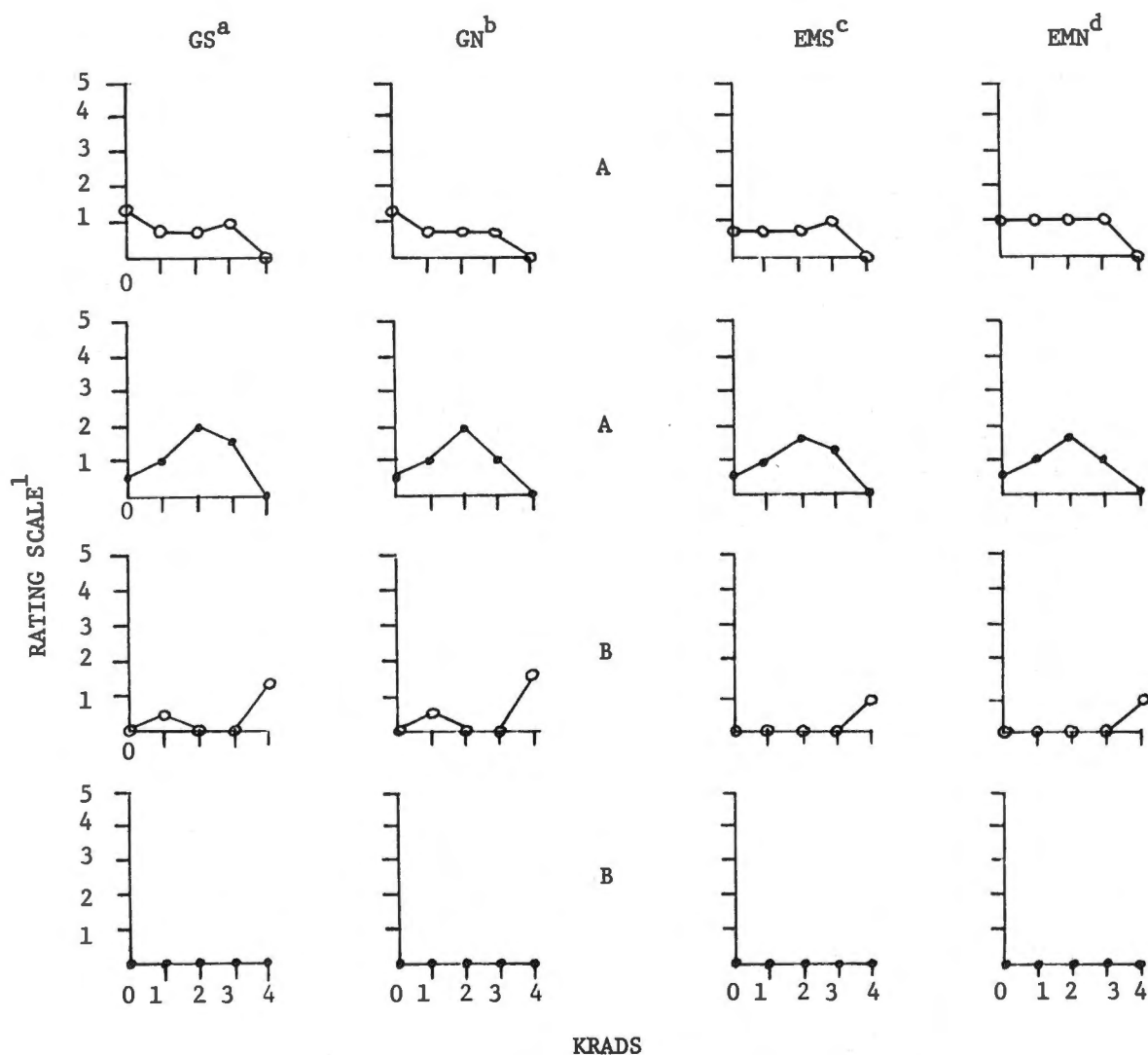


Figure 17. Response of *Zea mays* var. 'Minnesota A-401' to larvae emerging from eggs produced by females developing from larvae emerging from Stage I eggs (○) and Stage II eggs (●) irradiated at 7°C (A) and 32°C (B).

^aGall Size

^bGall Number

^cEgg Mass Size

^dEgg Mass Number

¹Average of five replications.

32°C this trend is reversed. The significant difference at this irradiation temperature is high ($P = 0.01\%$) for all scores.

When treatment level and developmental stage scores are pooled according to irradiation temperature, the 7°C scores are observed to be much higher ($P = 0.01\%$) than the 32°C scores.

I. Citrus vulgaris var. 'Charleston Grey'

At Stage I 7°C the patterns for gall size and egg mass size were identical (Figure 18). In both cases the highest score was observed at 0 and 4 Krads. The 1 Krad score was no different from that at 0 and 4 Krads. No gall or egg mass production occurred at 3 Krads. The 2 Krad score was intermediate between these extremes. The patterns for gall number and egg mass number were also quite similar. In both cases the highest scores were observed at 0 and 4 Krads. All differences observed at this stage and temperature were highly significant ($P = 0.01\%$). At Stage II 7°C the highest gall size score was observed at 0 Krads. The lowest score was noted at 2 Krads and was highly different ($P = 0.01\%$) from the 0 Krad score. All other gall size scores were intermediate between these two extremes. The highest gall number score was observed at 4 Krads. The 0 and 1 Krad scores and the 2 and 3 Krad scores formed two groups of scores highly different ($P = 0.01\%$) from each other and the 4 Krad score. The highest egg mass size score was observed at 1, 3, and 4 Krads. The lowest score was noted at 2 Krads and was highly different from all other scores ($P = 0.01\%$). The 0 Krad score was intermediate between these extremes. The pattern for the

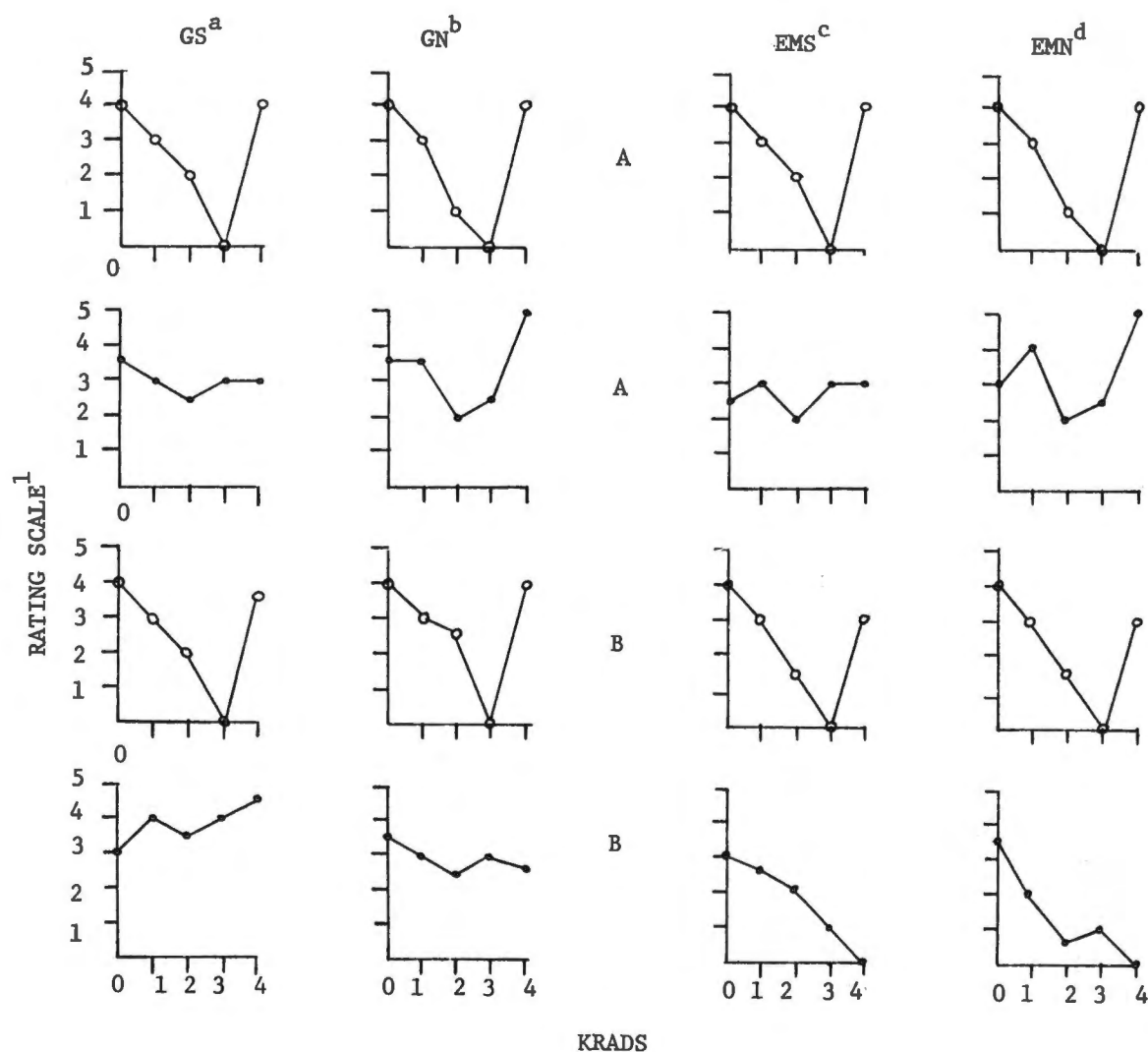


Figure 18. Response of *Citrus vulgaris* var. 'Charleston Grey' to larvae emerging from eggs produced by females developing from larvae emerging from Stage I eggs (O) and Stage II eggs (●) irradiated at 7°C (A) and 32°C (B).

^aGall Size

^bGall Number

^cEgg Mass Size

^dEgg Mass Number

¹Average of five replications.

egg mass number scores was identical to that observed for egg mass size except that the level of significance was lower ($P = 0.05\%$).

At Stage I 32°C the gall size and gall number patterns were almost identical (Figure 18). In both cases the highest score was observed at 0 Krads, the lowest at 3 Krads. All other scores were intermediate between these two extremes. All scores were different ($P = 0.05\%$). The egg mass scores were quite similar to the gall scores. The highest egg mass size and number scores were observed at 0 Krads. The scores at 1 and 4 Krads were no different from the 0 Krad score. The scores at 2 and 3 Krads were different ($P = 0.05\%$) from these scores. At Stage II 32°C the highest gall size score was observed at 4 Krads with the 1 Krad score being no different. All other scores were lower ($P = 0.05\%$) than the 0 Krad score. For egg mass size and number, the highest score was observed at 0 Krads. No egg masses were produced at 4 Krads. All differences in egg mass size at this stage and temperature were significant ($P = 0.05\%$); those in egg mass number were highly significant ($P = 0.01\%$).

When all treatment level scores are pooled according to developmental stage and irradiation temperature, the scores for Stage I 7°C and Stage II 7°C are observed to differ significantly ($P = 0.05\%$) in gall size, highly significantly ($P = 0.01\%$) in gall and egg mass number, and not significantly in egg mass size. At 32°C Stage I and Stage II differed highly ($P = 0.01\%$) in gall size, egg mass size, and egg mass number. No differences were noted in regard to gall number.

When developmental stage and treatment level scores are pooled according to irradiation temperature, highly significant differences ($P = 0.01\%$) are noted between 7 and 32°C in terms of gall size and egg mass size and egg mass number. No differences were noted in gall number.

J. *Nicotiana tabacum* var. 'North Carolina 95'

At 7°C no gall or egg mass production was noted at the 0, 1, 3, or 4 Krad treatment levels with Stage I progeny inoculations. Gall and egg mass production occurred only at the 2 Krad treatment (Figure 19). Thus, highly significant differences ($P = 0.01\%$) were noted. At Stage II 7°C gall and egg mass production occurred only in the 1 and 3 Krad treatments. Again differences were highly significant ($P = 0.01\%$).

No gall or egg mass production was observed in Stage I 32°C (Figure 19). At Stage II 32°C gall and egg mass production was observed only in the 1 Krad treatment. Differences were again highly significant ($P = 0.01\%$).

When all treatment level scores are pooled according to developmental stage and irradiation temperature, Stage I is observed to be significantly higher ($P = 0.01\%$) than Stage II in terms of gall and egg mass production. This pattern holds at both irradiation temperatures.

When all treatment level and developmental stage scores are pooled according to irradiation temperature, highly significant ($P = 0.01\%$) differences are noted between 7 and 32°C in terms of gall size

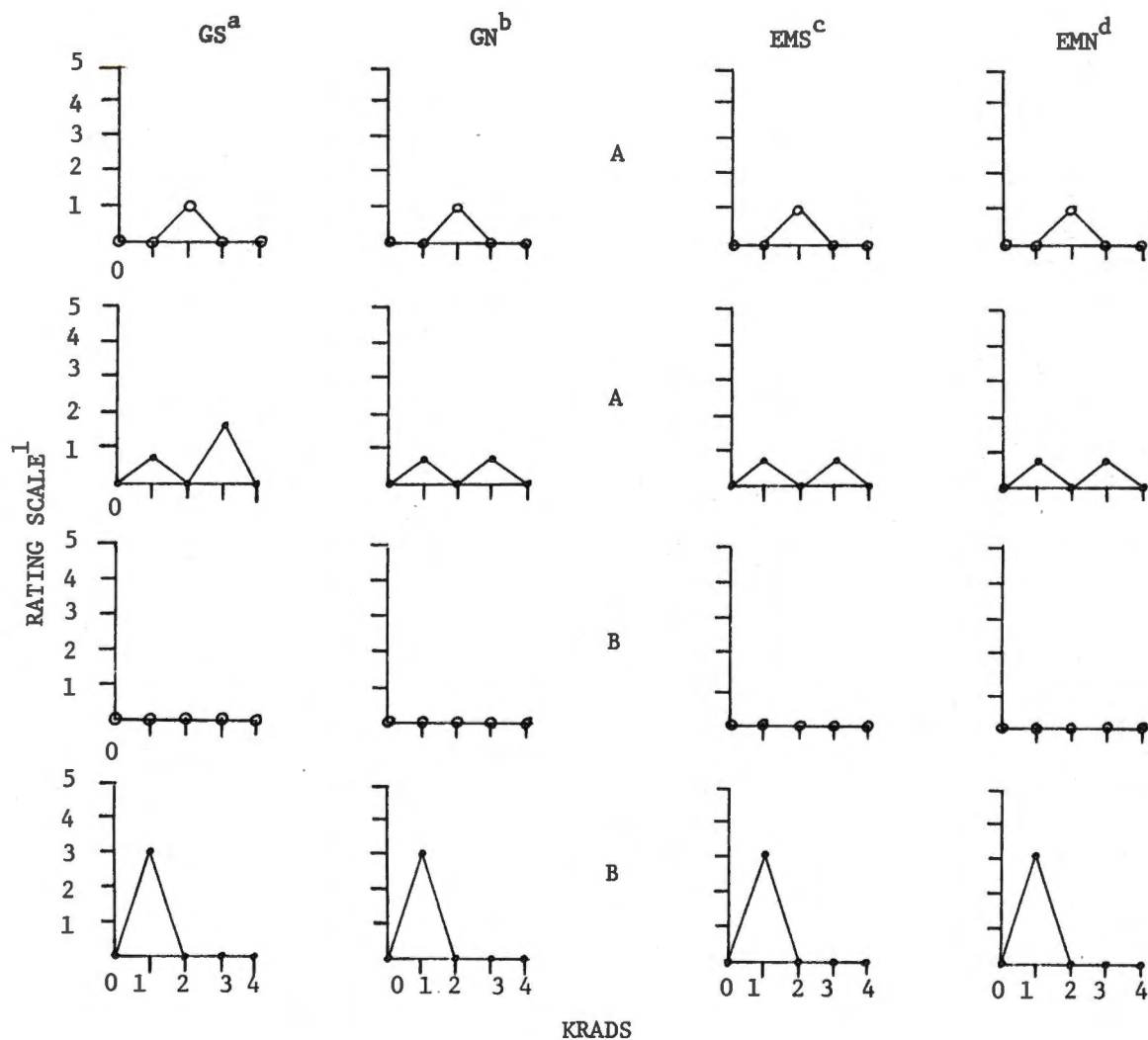


Figure 19. Response of *Nicotiana tabacum* var. 'North Carolina (NC) 95' to larvae emerging from eggs produced by females developing from Stage I eggs (○) and Stage II eggs (●) irradiated at 7°C (A) and 32°C (B).

^aGall Size

^bGall Number

^cEgg Mass Size

^dEgg Mass Number

¹Average of five replications.

and egg mass size. In both cases the 7°C scores were higher than the 32°C scores. No differences were noted in terms of gall number and egg mass number.

CHAPTER V

DISCUSSION

Since this is the first report pertaining to the effects of fast neutron irradiation on phytoparasitic nematodes and, as far as is known, the first paper dealing with the use of radiation to bring about the formation of a new pathotype of a phytoparasitic nematode, the work involved has raised far more questions that it could ever hope to answer.

However, several important phenomena were observed. One such observation was that the temperature at which irradiation is carried out affects the emergence of larvae from the irradiated eggs and the production of galls, eggs, and egg masses by females developing from these larvae. When eggs were irradiated at 7°C, more Stage II eggs hatched than Stage I eggs. This same pattern was observed at 22°C. However, at 32°C this pattern was reversed.

The production of galls, egg masses, and eggs was affected by irradiation temperature interacting with treatment levels. At all three irradiation temperatures egg production was not observed to differ with developmental stage. However, the pattern of egg production at 7°C was quite different from that observed at 22 and 32°C. In many cases significant ($P = 0.05\%$) and highly significant ($P = 0.01\%$) interactions between irradiation temperature and developmental stage, irradiation temperature and treatment level, and irradiation temperature,

developmental stage, and treatment level were observed. However, irradiation temperature does not appear to affect the number of such interactions that might occur.

That irradiation temperature affects the effects of irradiation on a biological system is not new (Lea, 1955; Casarett, 1968). While it is usually accepted that the radiosensitivity of biological systems decreases with decreasing temperature (Dertinger and Jung, 1970), exceptions to this rule are known (Mickey, 1938). Since fewer irradiated Stage I M. incognita eggs hatched at 7°C than at 22 or 32°C, it might be that this nematode is such an exception.

Another important observation was that the radiosensitivity of the nematode egg was a function of the egg's stage of embryological development. This phenomenon has been previously reported for Heterodera rostochiensis (Fassuliotis, 1958; Evans, 1970), Meloidogyne incognita (Ishibashi and Taguchi, 1965A), and Meloidogyne arinaria (Lapp, 1970). The phenomenon is fairly common and has been well documented for both plants (Sparrow, Moses, and Steele, 1952; Auerbach, 1963) and animals (Auerbach, 1963; Cervini and Giavelli, 1965; Murakami and Teulade, 1969; Murakami and Miki, 1972). It was established some time ago that the degree of radiation-induced change differs with various stages of the cell cycle (Dewey and Humphrey, 1964; Okada, 1970). In certain organisms, such as the silkworm moth, Bombyx, the cell cycle has been worked out and the point in time at which the cell is most vulnerable can be pinpointed (Murakami and Teulade, 1969). However, while the embryology of nematodes is well documented (Chitwood,

1950; Bird, 1970) and a great deal of work is being done on nematode cytology and cytogenetics (Triantaphyllou, 1971), very little is known about the cell cycle of these organisms.

A third observation involves the stimulation of the emergence of larvae (hatching) from Stage II eggs at 7 and 22°C. This has been reported by Ishibashi and Taguchi (1965A) for Meloidogyne incognita and by Lapp (1970) for Meloidogyne arenaria. While Lapp (1970) makes no attempt to explain this stimulation, Ishibashi and Taguchi (1965A) attribute it to a radiation-induced enzymatic change. While this probably is the cause, other possibilities should not be overlooked.

One possibility involves changes in the composition and/or permeability of the membranes surrounding the embryo and making up the egg shell. These membranes are quite complex and are known to be involved, in some manner, in hatching (Chitwood, 1950; Lee, 1965; Arthur and Sanborn, 1969; Bird, 1971; Shepherd and Clarke, 1971). It is also known that radiation can induce changes in the permeability and composition of biological membranes (Casarett, 1968; Dertinger and Jung, 1970; Okada, 1971). These two facts make a radiation-induced membrane change a possibility as the mechanism involved in this stimulation of hatch. This hypothesis is somewhat substantiated by the fact that radiation is known to specifically alter the permeability of the cell membrane to potassium (K^+), calcium (Ca^{++}), and sodium (Na^{++}) ions (Tobias, 1974). Each of these ions has been associated with the hatching mechanism of certain nematodes (Shepherd and Clarke, 1971).

A second possibility involves the radiation-induced formation of

peroxides or free radicals within the egg. Peroxides and free radicals are often formed in biological systems exposed to radiation and have extensive biological properties (Adler, 1963; Kotin, 1963; Walling, 1963; Casarett, 1968; Dertinger and Jung, 1970). Many lipids break down to form organic peroxides when irradiated. Water may be converted into hydrogen peroxide. Nematode eggs contain a high percentage (60-70%) of lipid (Krusberg, Hussey, and Fletcher, 1973) which, along with the free and bound water within the egg, would be available for radiation-induced peroxide formation. It has been demonstrated that hydrogen peroxide can induce hatching in many types of nematode eggs (Arthur and Sanborn, 1969; Webster, 1969). However, Meloidogyne incognita eggs do not appear to be affected by hydrogen peroxide treatment (Webster, 1969).

A fourth observation was that irradiation of M. incognita eggs does not appear to affect the number of males developing from the larvae emerging from those eggs. This tends to confirm the belief that the endogenous factors governing sexual determination in this nematode are not functioning during this stage of the organism's life cycle (Bird, 1971).

The final, and perhaps most important, observation involves the radiation-induced formation of a new biotype of Meloidogyne incognita. A "biotype" is an organism or a population of organisms that is morphologically identical to but biologically different from other members or populations of its species. This biological difference is often physiological in nature. If, as in the case of Meloidogyne

incognita, the organism is parasitic or pathogenic, the biotype is referred to as a "pathotype." Several naturally-occurring pathotypes of M. incognita are known to exist. While it is not possible to morphologically distinguish one pathotype of M. incognita from another, they can be separated by employing a "host differential test." In such a test various plants known to be susceptible or resistant to certain pathotypes are inoculated with the suspected organism. Certain pathotypes can parasitize certain plants while others cannot. Thus, the pathotype can be identified by its dietary restrictions.

Pathotypes of Meloidogyne incognita are important due to the nematodes' wide distribution and economic importance. While it can be controlled by chemical treatment, the preferred method of control for M. incognita is the cultivation of crop varieties resistant to the nematode. However, if the same resistant variety is cultivated in the same area for several seasons, the pathotype originally in the field will have become adapted to the resistant variety and a new pathotype will have developed. Thus, work is constantly being carried out to develop new more resistant varieties of crops. However, crop breeding is a long and expensive process. Any information that would increase our rather scanty knowledge of the origin and nature of pathotypes and our ability to control them would be greatly desirable.

This was the main reason that this research was conceived and carried out. It was hoped that by artificially inducing the development of a new pathotype through the use of a mutagenic agent, in this case fast neutron irradiation, we might possibly gain useful information regarding pathotypes.

The majority of plants used in the host differential tests were extremely to moderately susceptible to most populations of M. incognita and exhibited gall and egg mass production not much different from that encountered at the control level. Two extremely resistant plants, peanut (Arachis hypogaea var. 'Floor Runner') and sweet potato (Ipomea batatas var. 'Puerto Rico'), showed no gall or egg mass production. However, three plants--one susceptible and two resistant--gave very interesting results. 'Charleson Grey' watermelon (Citrulus vulgaris) is normally extremely susceptible, but tolerant, to M. incognita. However, all watermelon plants inoculated with the 3 Krad treatment of Stage I at 7 and 32°C died within 3-4 weeks after inoculation. When the roots of these plants were examined, no galls were found. It appears that the larvae emerging at this treatment level had undergone an increase in virulence so great that they overwhelmed their hosts and killed them before they could mature and reproduce. It is interesting to note that while gall and egg mass production at the 1 and 2 Krad treatments was significantly lower than that observed at the 0 Krad treatment, gall and egg mass production at the 4 Krad treatment was equal to that at the 0 Krad treatment.

Changes were also observed in both developmental stages at both irradiation temperatures when the nematode was cultured on 'Breeding Line M57-13N', a normally resistant variety of cowpea (Vigna sinensis). In Stage I 7°C the changes were "small" and were observed only in the 3 and 4 Krad treatments. In Stage II eggs irradiated at the same temperature, the changes were more drastic and occurred at the 1 and 2

Krad levels. Extensive changes were noted at the 1, 2, 3, and 4 Krad levels for both developmental stages irradiated at 32°C.

At Stage I 7°C the larvae emerging in the 2 Krad treatment were able to successfully parasitize 'North Carolina 95 (NC-95)', a normally resistant variety of tobacco (Nicotiana tabacum). At Stage II 7°C the same change was observed at 1 and 3 Krads. While no changes were noted at Stage I 32°C, the Stage II 1 Krad treatment exhibited drastic changes. These results, along with those obtained from the cowpea, are extremely important because both varieties were selectively bred for resistance to Meloidogyne incognita.

These changes, at least those involving the cowpea, can be assumed to involve genetic material. The Stage II 7°C population has retained its new host range through eight successive generations.

Since the same physiological changes appear to be occurring at several radiation levels regardless of developmental stage or irradiation temperature, it can be assumed that the same portion of the genetic material is being altered each time. At the present time there is no way of knowing if the same part of the genetic material is involved in the changes observed in all three host responses.

Regardless of what is involved, a new pathotype of Meloidogyne incognita has been developed through radiation treatment of viable eggs. While it has been known for some time new pathotypes could be developed through selective breeding (Riggs and Winstead, 1959; Triantaphyllou and Sasser, 1960; Winstead and Riggs, 1963), the process takes up to a year or more to complete. In this study the time was reduced to slightly over two months.

It is appropriate that this new pathotype be given a name. I propose that it be designated as the "nidhogg" or "N" pathotype. Nidhogg, a giant serpent in Norse mythology, helped to bring the world to an end by constantly gnawing on the roots of Yggdrasill, the World Tree (Davidson, 1964).

CHAPTER VI

CONCLUSIONS

1. Radiosensitivity of Meloidogyne incognita eggs is a function of developmental stage.
2. Emergence of larvae from irradiated eggs is influenced by the temperature at which the eggs were irradiated.
3. The temperature at which the eggs were irradiated appears to affect gall, egg mass, and egg production by the adult females developing from the eggs.
4. A stimulation in larval emergence was noted at the 2 Krad level in Stage II eggs irradiated at 7°C.
5. Irradiation of eggs does not affect the development of males.
6. Radiation-induced mutations have affected the ability of this "strain" of Meloidogyne incognita to parasitize 'Charleston Grey' watermelon, 'M57-13N' cowpea, and 'NC-95' tobacco. The changes affecting the nematode's ability to parasitize watermelon are not influenced by irradiation temperature but do appear to be restricted to the Stage I material. The most extreme change in gall and egg mass production was observed at the 3 Krad level. In regard to 'M57-13N' cowpea the changes were observed to occur in both developmental stages at both irradiation temperatures but were most pronounced in Stage II 7°C and Stage I and II 32°C. For Stage II 7°C and 32°C the most drastic changes occurred at 1 and 2 Krads. At Stage II 32°C drastic changes were

observed at 1, 2, 3, and 4 Krads. The changes affecting the ability to parasitize 'NC-95' tobacco occurred in Stage I 7°C and Stage I and II 32°C. At Stage I 7°C the change was observed at the 2 Krad level. At Stage II 7°C the changes were noted at the 1 and 3 Krads. At Stage II 32°C the changes were limited to the 1 Krad level.

7. Because the changes affecting the nematode's ability to parasitize the cowpea were observed at several treatment levels at both developmental stages and irradiation temperatures, it is assumed that the same portion of the genetic material was altered each time.

8. Because of these changes it is proposed that a new pathotype of Meloidogyne incognita, designated as the "N-pathotype," be erected.

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VITA

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