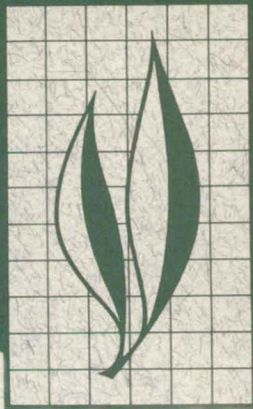


HILGARDIA

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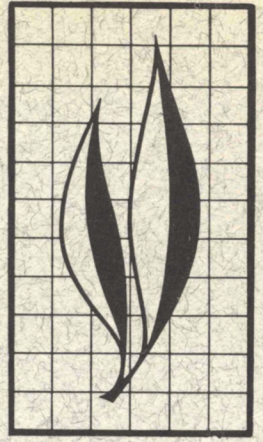
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Reproductive Biology of *Lygus* *hesperus* Knight

Frank E. Strong, J. A. Sheldahl, P. R. Hughes and
Esmat M. K. Hussein

- I. Laboratory Studies on *Lygus* Reproduction
- II. Biology of the *Lygus* Bug Sex Pheromone
- III. Modification of Reproduction in *Lygus hesperus*



Studies on the reproduction biology of *Lygus hesperus* demonstrated that most adults first mated when they were 8 days old. The mating act lasted about $2\frac{1}{2}$ minutes. Males could mate once per day for 6 consecutive days, but females only mated three times at 6-day intervals. One mating enables a female to oviposit viable eggs for the remainder of her life, which lasted an average of 38 days.

Virgin females began to produce a male-attracting sex pheromone as eggs matured within her ovaries; this first occurred when the adult female was about 6 days old. Pheromone release ceased immediately after mating, but was reinitiated 6 days later. In the fall, when the bugs entered diapause (characterized by atrophied ovaries) the pheromone was not released until diapause was terminated. During the period of diapause, males did not respond to the sex pheromone.

L. hesperus can be sterilized effectively by exposing the males to 5,000 rads of gamma radiation. Increased exposures affects the mating behavior by reducing male aggressiveness. The offspring from irradiated parents inherited a high degree of sterility; thus, a large proportion of the F_2 generation was sterile.

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Reproductive Biology of *Lygus hesperus* Knight¹

INTRODUCTION

BUGS IN THE GENUS *LYGUS* are primarily pests of cotton, coffee, alfalfa, beans, carrots, and other crops grown for seed. The present control programs for these insects have been developed from detailed studies of their life history and behavior. When *L. hesperus* was first recognized as a pest of alfalfa seed 40 years ago, its life history in the Western United States was thoroughly described by Schull, *et al.*, 1934; Sorenson, 1939; Stitt, 1940; and King and Cook, 1932. Next damage studies were reported, records were published of numerous new hosts, and the destructive capabilities of *L. hesperus* and related species were described (Addicott and Romney, 1950; Baker, *et al.*, 1946; Davis, *et al.*, 1963; Carlson, 1964). Much research on *Lygus* spp. in the decade following World War II dealt with insecticidal control. In the last ten years, however, problems caused by chemical control have prompted studies on biology, ecology and physiology (Beards and Leigh, 1960; Leigh, 1963; Champlain and Butler, 1967), resistance to insecticides (Bacon, *et al.*, 1964), diapause (Beards and Strong, 1966), feeding behavior (Flemion, *et al.*, 1954; Landes and Strong, 1965; Strong and Landes, 1965), physiology of damage (Jeppson and MacLeod, 1946; Strong and Kruitwagen, 1968), nutrition (Auclair and Raulston, 1966; Strong and Kruitwagen, 1969; Vanderzant, 1967), para-

sitism (Clancy and Pierce, 1966; Clancy, 1968), flight activity (Stern and Mueller, 1968), new control methods with strip cutting (Stern, *et al.*, 1964) and host plant resistance (Lindquist, *et al.*, 1967).

No reports have appeared on the dynamics of lygus populations. The ultimate goal of any control program is to manipulate the population, not the individuals. Yet for most insects, including lygus bugs, the factors responsible for population performance are virtually unknown.

In reviewing the behavioral and physiological events leading to an increase of *L. hesperus* populations, we recognized a gross lack of understanding. For example, the simple act of mating, which in July occurs probably thousands of times daily in each acre of alfalfa, had only been observed three times by as many persons in the past 30 years. This act, essential for population maintenance and increase, had essentially escaped observation.

At Davis, *L. hesperus* has three generations per growing season. The relative numbers of insects per generation are shown in figure 1. These data have been compiled from several sources but stem mainly from sweep-net samples taken twice a week from portions of alfalfa hay fields left uncut for the entire 1968 season. When the remaining portions of the fields were harvested,

¹ Submitted for publication February 3, 1969.

the number of adults increased sharply the following day in the uncut portion. This increase is not reflected in figure 1 because it only lasted for a few days, and the plotted data in figure 1 are to represent the relative changes in numbers of *L. hesperus* in the absence of mass immigrations.

By mid-January all overwintering adults of *L. hesperus* are reproductive and the females begin egg laying. Although the data are incomplete, there is sufficient evidence to indicate that only the last group of eggs deposited by these females hatch, producing the spring nymphs labeled N1 in figure 1. This result is supported by Champlain and Butler (1967): their data indicate that the minimum developmental temperature for *L. hesperus* is 49°F, and that 225 day degrees (DD) are required for egg hatch. Eggs incubated at 49°F for 8–10 weeks failed to hatch which indicates that all eggs laid before February 10th would die, and those laid after this date would hatch around April 10–15, when 225 DD had accumulated. The spring nymph population gives rise to the first adult generation (A1), which in turn produces two additional complete generations. Nymphs hatching on or about August 20th are destined to become diapausing adults (Beards and Strong, 1966). Thus, about one-fourth of the individuals of the adult peak labeled A3 are nonrepro-

ductive and probably do not contribute to the overwintering adult population because they do not live long enough. The overwintering adults arise from nymphs produced by the reproductive members of A3. All surviving nymphs resulting from the A3 adults enter diapause upon reaching maturity. Diapause terminates during December after which the females become sexually mature, produce sex pheromones (see Part II), mate, and on warm days begin to lay eggs. Their eggs, upon hatching, give rise to the spring nymphs, completing the yearly cycle.

The time and temperature requirements for the complete development of *L. hesperus* (i.e., from egg to first egg) is about 945 DD (32 days at 80°F) but 1,380 DD are required for the completion of a generation. This discrepancy is caused by the change in rate of egg deposition as the females age (see the shape of the egg laying curve, figure 10). The mean age of all reproductive females in one generation was determined and compared to that of the next generation. When this was done, an indicated generation time of 45 days at 80°F, (or the equivalent of 1,380 DD) was obtained. As shown by the arrows on the day-degree curve in figure 1, increments of 1,350 DD correlate closely with the observed peaks of field-collected adults.

METHODS

Stock colonies of *L. hesperus* were maintained on fresh green beans (*Phaseolus vulgaris* L.) after the method of Beards and Strong (1966). Beans, grown locally during the summer or purchased from local groceries in the winter, were washed with detergent, rinsed, and patted dry before being used to replenish the bugs' supply. Fresh beans were placed in the cages twice weekly.

Oviposition cages were made from

five-gallon cartons, each fitted with a screened lid, a window, and a sleeved entry port. Fresh beans were supplied twice weekly to provide food and oviposition sites. The entire stock colony was maintained at room temperatures (73°–80°F) and humidities (45–65 per cent RH) under 16 hours of light per day, supplied by cool white fluorescent lamps.

Bugs selected for the experiments were in the 5th instar stage; each bug

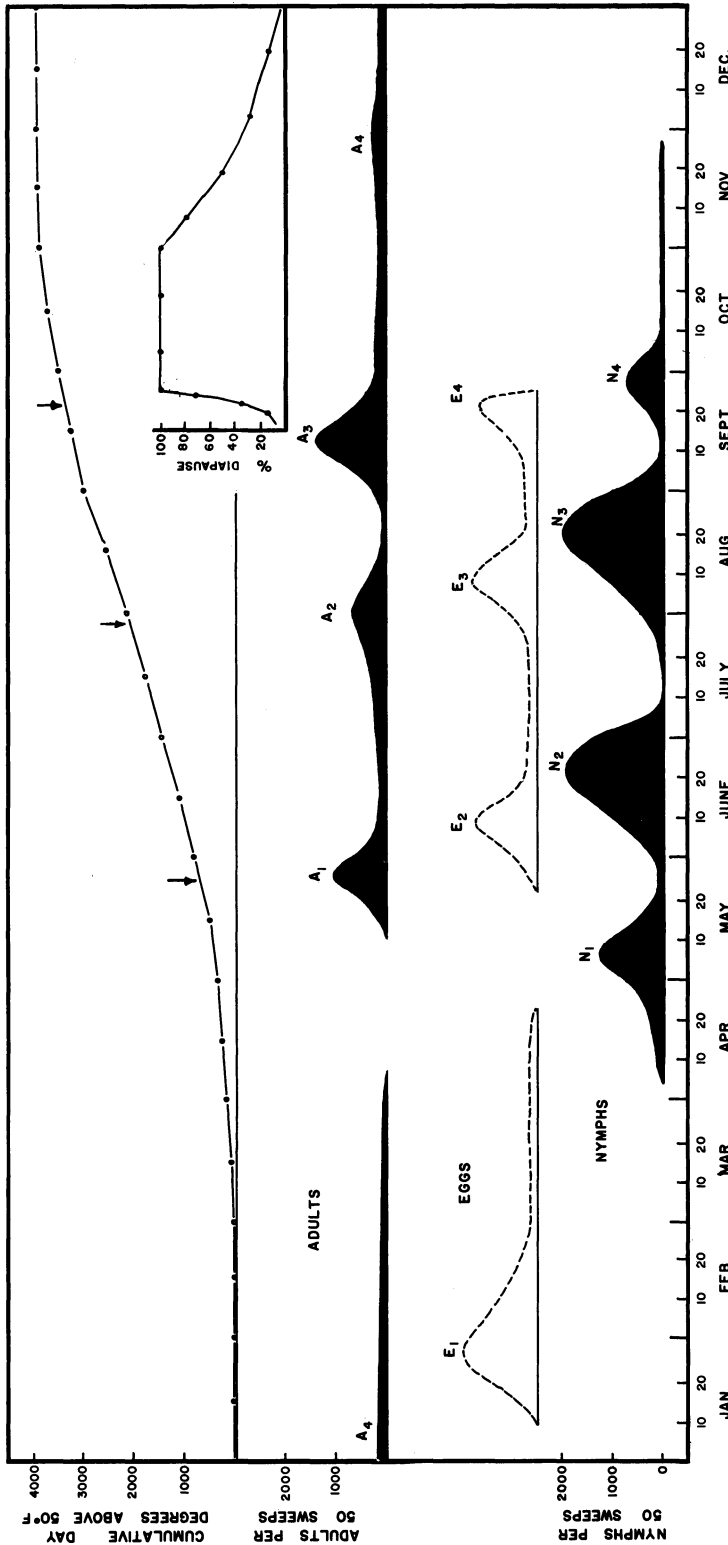


Fig. 1. Seasonal life history of *Lygus hesperus* at Davis, California. The egg curves indicated by dotted lines, are hypothetical. The curve for the cumulative day degrees above 50°F is based on normal Davis temperature records. The arrows indicate the number of day degrees for a complete generation as determined from laboratory studies.

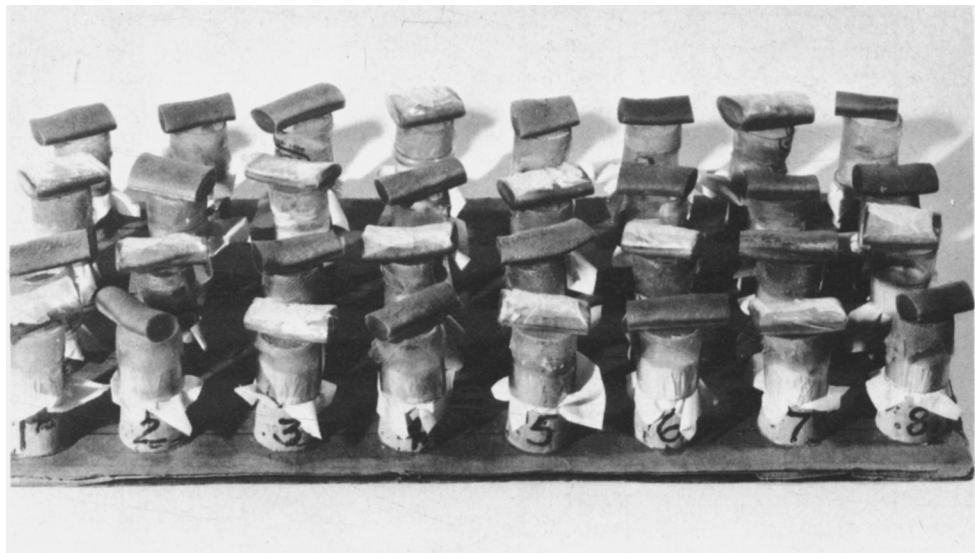


Fig. 2. Palletized individual rearing cages. One bug is placed in each cage. The bean sections are wrapped in parafilm when the females begin ovipositing, to prevent subsequent desiccation of the beans before the eggs hatched.

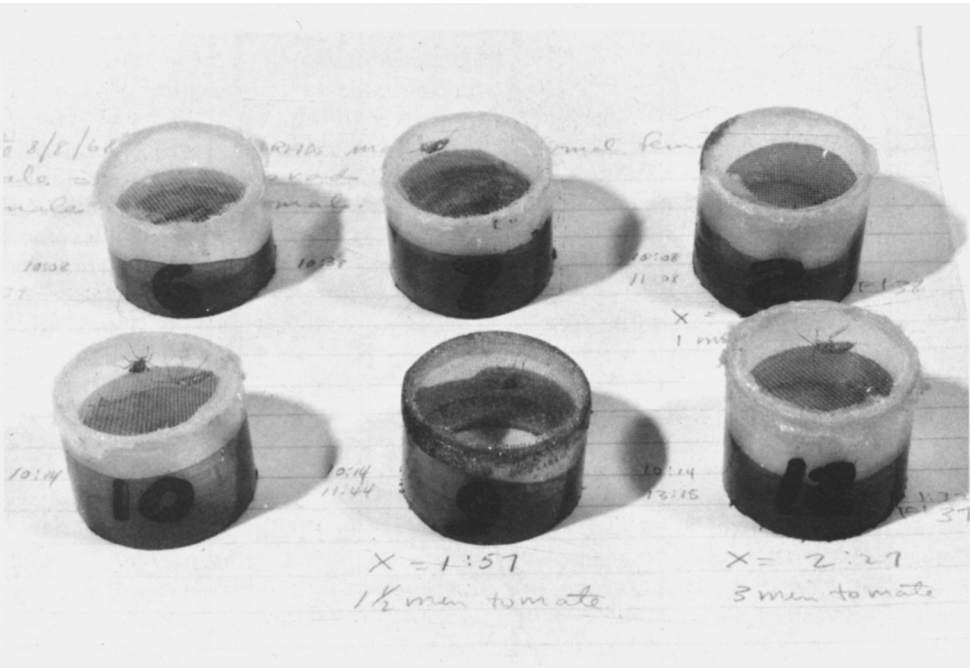


Fig. 3. Cages used for mating *Lygus hesperus*.

was placed in one of 32 individual cages glued on a 8-inch by 5-inch cardboard pallet. The cages were similar to those described by Landes and Strong (1965). Frequently several pallets were prepared simultaneously permitting detailed records on 200–300 bugs of similar age and sex. Pallets containing bugs were placed in an incubator at 80°F, 75 per cent RH, and 16 hours of light per 24 hours. Palletized bugs were fed fresh sections of green beans which were changed daily. These beans also served as oviposition sites permitting an accurate recording of the fecundity of the individual females. Figure 2 shows a pallet of individual rearing cages.

The mating cages were made from 1-inch sections of plastic test tubes (1-

inch ID) whose inside surfaces had been abraded to provide a foothold for the bugs. The tops were covered with nylon hose while the bottoms were left open. Figure 3 illustrates several pairs of bugs in the mating cages.

For morphological studies etherized bugs were dissected under diluted Belar's (3:2) saline (Breland, 1961). Living sperm were observed with a phase-contrast microscope. Stained sperm were prepared with aceto-orcein following Breland's (1961) method. Histological preparations were made using the technique described by McManus and Mowry (1960). Tissues were embedded in Paraplast^R and cut at 10 μ . Sections were stained with Harris's hematoxylin and counterstained with eosin.

I. LABORATORY STUDIES ON *LYGUS* REPRODUCTION²

Leigh (1963) and Champlain and Butler (1967) have adequately reported the general laboratory biology and life history of *L. hesperus*. Their studies, however, lack the details on reproduction *per se* that are crucial to studies on population dynamics. Therefore, this section presents information germane to the act of reproduction.

Mating behavior

When a male and a female of the appropriate age were caged together they were very active for 10–20 seconds, moving about haphazardly. Soon, however, they became quiet and remained motionless for a few seconds. Each partner then initiated a slow walk, and if the male touched the female with both antennae, an aggressive behavioral pattern immediately followed. This was characterized by a vertical jerking and quivering motion of his abdomen. If the male was on the female's right side he would move closer, bend his abdomen

under the right side of the female's and attempt copulation. The male always directed the tip of his abdomen toward the right-hand side of the base of the female's ovipositor. If the female was receptive, copulation occurred. If not, she displayed an evasive behavior and eluded him. The male would then often repeat his aggressive courtship and occasionally the female would accept him.

Frequently, the male would mount the female and extend his abdomen but fail to do so from her right side. The female always rejected this pattern, as well as attempts to mount from the front or left side. If the male approached the female from the rear or her right side and she was receptive, they would simultaneously rotate toward each other until the tips of their abdomens touched. The male would then curl his abdomen under hers, enabling the genitalia to make contact at the base of the ovipositor.

² Taken in part from a thesis submitted in December, 1968, by J. A. Sheldahl to the Graduate Division, University of California, Davis, in partial fulfillment of the requirements of Master of Science degree.

to monitor economic levels of this pest. A review of the biology of *L. hesperus* makes it appear unlikely that the sex pheromone could be advantageously used in suppressing the populations. The pheromone is presumably not exceedingly potent, because the catches of males were insignificant compared to the number of males present in the field. Lygus bugs live in a relatively compact niche dictated by the height of their host, which in alfalfa is seldom over 2 feet. The plants and foliage are rela-

tively thick which restricts long-distance air movements, and the bugs normally exist in close association with one another. Such an ecosystem obviates an exceedingly potent, long-range pheromone, such as is found in some of the Hymenoptera and Lepidoptera associated with forest systems (Jacobson, 1966). Deployment of lygus pheromone in traps to monitor field populations is possible but perhaps not practical considering the ease of sampling with the sweep net.

PART III. MODIFICATION OF REPRODUCTION IN *LYGUS HESPERUS*⁵

Use of Gamma Irradiation

Reproduction in insects can be modified or influenced either environmentally or genetically. The selection of host plant can materially affect reproduction (House, 1961; Lewis and Taylor, 1967), and is probably related to nutritional adequacy of the host. In fact, Strong and Kruitwagen (1969) have shown that egg production in *L. hesperus* is directly related to the amino acid content of their diets. Harcourt (1963) and Harcourt and Cass (1966) have reported on the change in fecundity of the diamond back moth, *Plutella maculipennis* (Curt.), in relation to seasonal changes. In some insects, fecundity is directly related to density (Watt, 1960; Fujita, 1954).

In the above cited references, the responses of the populations are related to environment; the gene pool has probably not changed. Changes in reproductive capacity are often natural regulatory processes. If, however, the genetic complement of individuals within the population is suddenly changed, abnormal conditions exist which can drastically reduce the reproductive poten-

tial of the species. Such changes can be affected with radiation or radiomimetic chemicals.

Today, the sterile-male technique for insect control is a familiar term to entomologists. The extensive literature in this field has been reviewed by Grosch (1962) and LaChance, *et al.*, (1967). The majority of insect sterilization studies have been performed with the Lepidoptera and Diptera. Only one Hemipteran (*Rhodnius prolixus* Stahl) has been sterilized with radiation (Baldwin and Shaver, 1963). Because of this, and as part of the over-all study on lygus reproduction, studies were initiated on the changes in reproduction of *L. hesperus* using gamma radiation and chemosterilants.

The factors influencing the amount of radiation required to sterilize an insect have been discussed by Nelson (1968). Lepidoptera, whose chromosomes have diffused centromeres (Virkki, 1965) show a high degree of radioresistance to the induction of sterility (North and Holt, 1968b). Thus, 30–40 kilorads (KR) are required to sterilize adults of

⁵ See footnote 2, page 109.

the codling moth, *Carpocapsa pomonella* L. (Proverbs and Newton, 1962), European corn borer, *Ostrinia nubilalis* (Hubner) (Walker and Brindley, 1963), the cabbage looper, *Trichoplusia ni* (Hubner) and the tobacco budworm, *Heliothis virescens* F. (North and Holt, 1968a). One hundred KR are needed to effect sterility in the Indian meal moth, *Plodia interpunctella* (Hubner) and the Angoumois grain moth, *Sitogroga cerealella* (Olivier) (Cogburn, et al., 1966).

Diptera, which have a localized centromere, require relatively low dosages to induce male sterility. For example, the screwworm fly, *Cochliomyia hominivorax* (Coquerel) is sterilized with 4–5 KR, as is the Mexican fruit fly, *Anastrepha ludens* (Loew) Rhodes, et al., 1961) and the house fly *Musca domestica* L. Hemiptera, which have the diffuse centromere (Halkka, 1959) are apparently intermediate in their degree of radioresistance. The only available report is that of Baldwin and Shaver (1963) who stated that *Rhodius* required 17.5 KR for sterilization.

Methods and Materials

All bugs used in irradiation experiments were reared from 5th instars on pallets. The bugs were palletized without anesthesia and sexed after becoming adults. Studies were performed with bugs of two ages; "young bugs" were irradiated as 2-day old adults (range 1–3 days), and "old bugs" as 8-day old adults (range 6–9 days). The average ages of the bugs and the dosages received in all experiments are shown in table 12.

The gamma rays were from a Co⁶⁰ source operated by the Department of Pomology, University of California, Davis. The bugs were exposed while caged on individual pallets, each of which held 32 bugs. After treatment, the pallets were placed in an incubator at 80°F and 70 per cent RH. Each bug was given a fresh section of bean daily for food and oviposition.

At various times, but not until the minimum adult age was 7 days, matings were made between treated and non-treated individuals. To assess the effects

TABLE 12
AGE, DOSAGE AND SEX OF *L. HESPERUS* TREATED IN
STERILITY EXPERIMENTS. 1968

Date of treatment	Sex	Dosage (KR)	Number adults treated	Mean age at time of treatment
				days
June 21.....	Male	5	30	2.0
June 21.....	Male	10	32	2.0
June 21.....	Male	20	30	2.0
June 24.....	Male	30	27	2.0
June 24.....	Male	40	19	2.0
June 24.....	Male	50	28	2.0
June 24.....	Male	60	26	2.0
July 20.....	Male	2	31	1.5
July 20.....	Male	4	32	2.0
July 20.....	Male	6	32	1.5
July 20.....	Female	5	17	1.0
July 20.....	Female	10	13	1.0
July 20.....	Female	20	10	1.5
August 5.....	Male	5	31	7.5
August 5.....	Male	10	32	7.0
August 5.....	Male	20	30	8.5
August 5.....	Female	5	29	8.5
August 5.....	Female	10	26	8.5
August 5.....	Female	20	20	6.0

TABLE 13
INFLUENCE OF IRRADIATION ON THE ABILITY OF
L. HESPERUS TO MATE WITH NORMAL PARTNERS

Partner treated	Dosage	Number of pairs	Successful matings	Duration	
				Precopulation	Copulation
	KR		per cent	minutes	minutes
Neither.....	0	36	72	—*	3:23
Young males.....	2	16	87	2:54	2:37
	4	15	80	6:32	2:50
	5	31	80	2:45	2:55
	6	21	57	19:40	2:43
	10	30	43	20:00	3:34
	20†	—	—	—	—
Old males.....	5	16	50	4:00	2:33
	10	22	28	5:37	2:27
	20	6	16	7:00	3:10
Young females.....	5	9	55	7:00	3:33
	10‡	—	—	—	—
Old females.....	20‡	—	—	—	—
	5	15	53	8:21	2:03
	10	24	54	13:41	3:16
	20	15	21	33:56	2:42

* See table 1.

† 20 ♂'s were so unresponsive that no matings were attempted.

‡ Young 10 ♀'s and 20 ♀'s never matured eggs; mating behavior was not investigated.

of the irradiation, records were maintained on adult behavior, longevity, egg production, embryonation, egg hatch, and fertility of the F_1 individuals. All unhatched eggs were examined to see if they had begun to embryonate. This was done by removing the operculum and squeezing out the contents. When definite evidence of cellular organization was present the egg was counted as one which got fertilized but died before hatching.

For brevity, the following notations have been employed to describe irradiated and nonirradiated bugs: A "normal" bug received no irradiation and is designated N ♂ or N ♀ for a normal male or a normal female. The symbol 5 ♂ indicates a male which received a dose of 5 KR. Likewise 20 ♀ stands for a female which received 20 KR, and 10 ♂ × N ♀ refers to a mating between a male treated with 10 KR and an untreated (or normal) female.

Results

General Behavior. If a cage containing normal adult *L. hesperus* is dis-

turbed, the bugs become very active and run inside the cage for a few seconds. Young caged males, exposed to 2, 4, or 5 KR, responded like normal males when disturbed, but those exposed to 6 or more KR were considerably less responsive. Young males treated with 10–20 KR were slow and deliberate in their movements and practically unresponsive to cage tapping. Old males irradiated with 5, 10, or 20 KR did not show a loss of responsiveness; they remained as active as the normal males throughout their lives.

No differences in regard to general responsiveness were observed among females, either young or old, after exposure to 5, 10, or 20 KR. Both groups were equally responsive to cage tapping.

Effect of radiation on mating aggressiveness and receptiveness. Two criteria were used to determine what effects radiation had on mating behavior of both males and females: 1) The percentage of attempted matings successfully completed, and 2) length of the

precopulation time. In all tests, the irradiated individuals were given an opportunity to mate with a normal partner.

The data, shown in table 13, indicate that both male aggressiveness and female receptiveness decreased as the dosage was increased. Young males exposed to 2–5 KR were not appreciably influenced, for the percentage of successful mating and the precopulation times (table 13) were about the same as for the controls. Young males receiving more than 6 KR, however, demonstrated a lack of interest in their mates commensurate with their generally slow, unresponsive behavior. Only 43 per cent of the attempted matings were successful by males treated with 10 KR, and their average precopulation time was more than 19 minutes. No young males treated with 20 KR or more could be induced to mate; they displayed no aggressiveness and were totally unresponsive.

Old males irradiated with 5 or 10 KR were somewhat less aggressive sexually than young ones treated with the same dose. However, one (out of 6) of the old 20 ♂'s did mate successfully, a phenomenon not observed among young males treated with 20 KR.

Young females exposed to 10 KR or more never produced any eggs, so we did not investigate their ability to mate. In contrast to this, young females exposed to only 5 KR did produce some eggs, and 55 per cent of those treated mated successfully.

Old females showed a significant reduction in ability to mate as the exposure to irradiation increased. Only 21 per cent of the 20 ♀'s successfully mated, and their precopulation times averaged almost 34 minutes. The data in table 13 also indicate that regardless of the exposure, once a pair engaged in copula the time in copulation was normal.

Longevity of irradiated bugs.

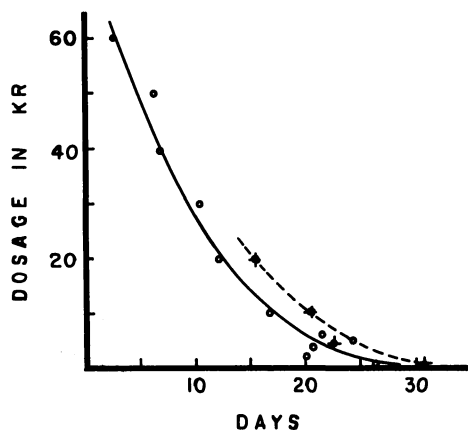


Fig. 14. Relationship between exposure to gamma radiation (in KR) to adult longevity. Solid line is for young males and broken line is for young females.

Figure 14 shows the relationship between dosage and longevity of irradiated young males and young females. The curves for the treated old males and females have been omitted, because they were not substantially different from those shown. There was no difference in the longevity between young males which received 0, 2, 4, 5, or 6 KR but a significant reduction occurred as the exposure exceeded 10 KR.

Another difference was observed in regard to longevity. Normal young bugs and those receiving less than 5 KR remained very active until the day of their deaths. Young bugs exposed to 6 or more KR, however, became inactive a few days after treatment, but did not die for several additional days. This general weakening and loss of activity several days before death is undoubtedly associated with somatic damage, for treatment of old males did not produce this response.

Oviposition, embryonation, and hatch. The ovipositional records and associated data for all normal and treated females mated to normal or treated males are recorded in table 14. Young females, irradiated with only 5 KR, produced an average of only 17 eggs. No eggs were produced by irradi-

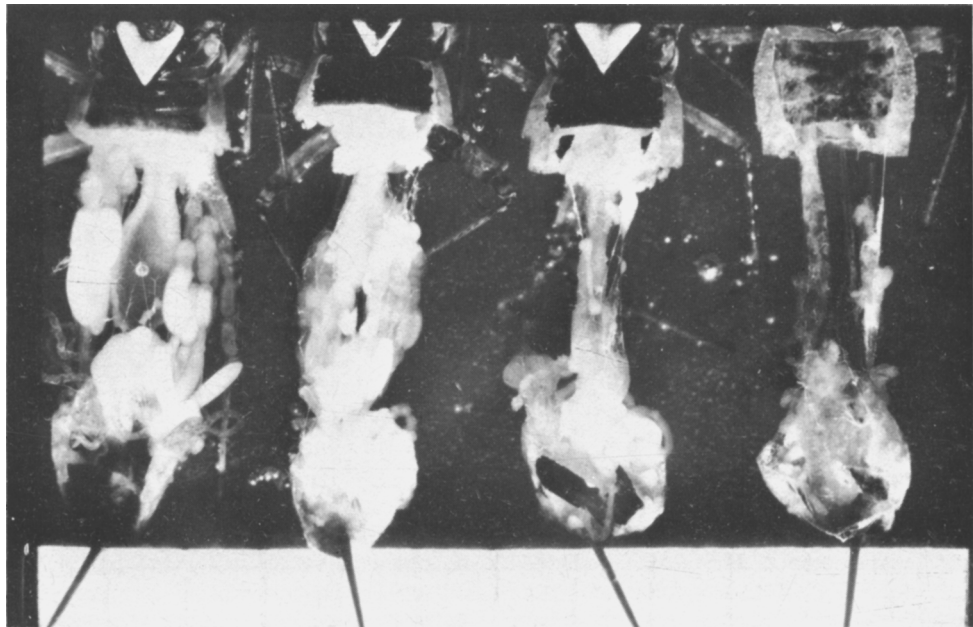


Fig. 15. Ovaries of 21-day-old *L. hesperus* exposed to 0, 5, 10, or 20 KR (left to right) when adults were 2 days old.

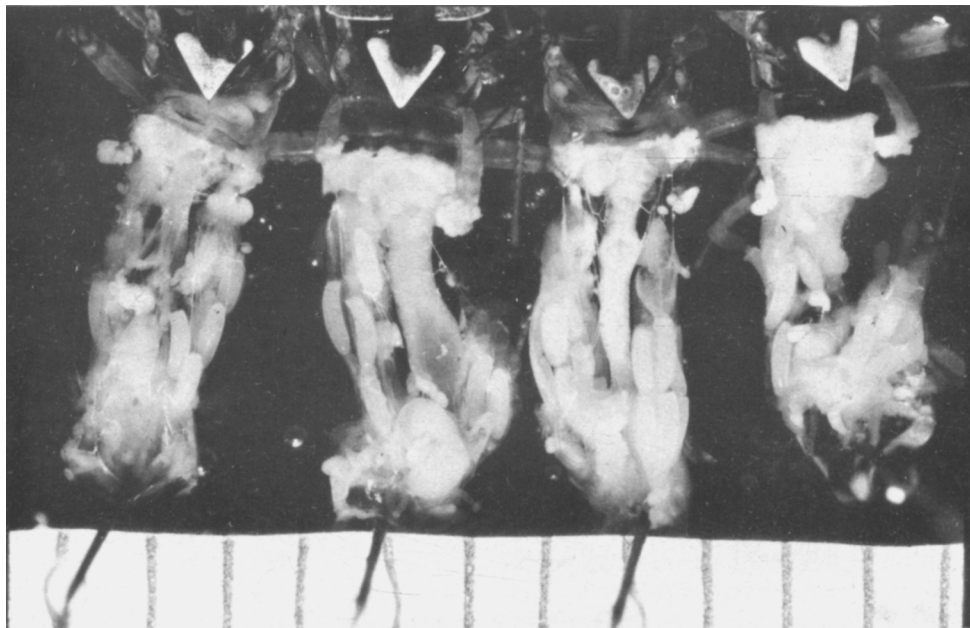


Fig. 16. Ovaries of 12-day-old *L. hesperus* exposed to 0, 5, 10 or 20 KR (right to left) when adults were 8 days old.

TABLE 14
EFFECTS OF GAMMA IRRADIATION ON ECLOSION OF EGGS
PRODUCED BY TREATING *L. HESPERUS*

Treated partner	Dosage	Number of pairs	Mean number of eggs/female	Initial embryonation	Ultimate hatch
	<i>KR</i>			<i>per cent</i>	<i>per cent</i>
Neither.....	0	25	212	45.2	43.1
Young males.....	2	13	115	34.8	21.5
	4	11	115	36.4	16.1
	5	22	146	44.4	21.9
	6	10	171	27.1	13.9
	10	7	134	3.6	1.5
Old males.....	5	8	102	33.3	19.2
	10	7	135	31.2	10.4
	20	1	21	0.0	0.0
Young females.....	5	5	17	0.4	0.0
	10	12	0	0.0	0.0
	20	8	0	0.0	0.0
Old females.....	5	8	84	1.6	0.0
	10	13	17	0.0	0.0
	20	7	1	0.0	0.0

ated young females when the dosage was increased to either 10 or 20 KR. This was not unexpected, for as shown in Part I, 1-3-day-old females were just initiating oogenesis. Exposure of young females to 10 KR of radiation at this age totally destroyed the germarium and prevented egg production. Young females irradiated with various dosages when 2 days old and dissected 21 days later were found to have a progressive lack of ovaries, as shown in figure 15.

Females treated with 5, 10, or 20 KR when they were 8 days old oviposited an average of 84, 17, and 1 eggs each, respectively, after being treated. Dissections of similarly treated females revealed that the average female had approximately the same number of eggs in her body 4 days after being irradiated regardless of the dosage (figure 16). Thus it is apparent that the oviposition behavior of irradiated females was materially affected, because the females that received the higher dosages withheld their eggs rather than oviposit them.

Regardless of the female's age at time of treatment, none of the eggs from treated females X N ♂'s ever hatched (see table 14). This fact is im-

portant when considering the possibility of using sterilized individuals for a control program: if the females of a species can be sterilized with a lesser dose than the males, it is unnecessary to sex individuals to be used in a sterile-release program.

The data in table 14 indicate a significant reduction in the hatch of eggs produced by normal mothers mated to treated males. Compared to the controls, there was about a 50 per cent reduction in the hatch of eggs fertilized by young males treated with either 2, 4, or 5 KR. Ten KR sufficiently sterilized young males so only 1.5 per cent of eggs from N ♀ × 10 ♂ hatched. The age of the male treated had no effect on the ultimate percentage of hatch of eggs they fertilized. This was expected, for gonadal development of the males is completed during the last nymphal instar.

Dissection of treated males, both young and old, 10-20 days after treatment revealed no gross differences in the reproductive organs that could be associated with increasing dosages. If the dosage was at least 20 KR, however, the testes appeared abnormal; they were amorphous and the individual tubules were not distinct. Regardless of

TABLE 15
ECLOSION OF EGGS PRODUCED BY F₁ *LYGUS HESPERUS* WHOSE
MALE PARENTS WERE EXPOSED TO 5 KR GAMMA IRRADIATION

Cross	Number of pairs	Mean number of eggs/female	Initial embryonation	Ultimate hatch
			per cent	per cent
F ₁ female x normal male.....	7	85.1	28.7	9.4
F ₁ male x normal female.....	6	106.3	4.8	0.7

the dosages tested, both pairs of accessory glands, as well as the seminal vesicle, appeared normal in every respect.

Induced sterility of males may manifest itself in several ways. If the sperm are killed outright, no fertilization occurs and no embryonation results. If dominant lethality is induced, fertilization may occur, and embryonation begins but the embryo will die before hatching. Lastly, if irradiation results in fracturing some of the chromosomes, inherited sterility may result, especially if the species has chromosomes with diffuse centromeres (North and Holt, 1968b).

A critical evaluation of male sterility by observing egg hatch depends on knowing the hatch from matings by normal males. In all the sterility experiments, only 43 per cent of all eggs from untreated bugs hatched. Use of bean sections as oviposition sites is somewhat undesirable, for if the small sections desiccate, a high egg mortality results.

By comparing the last two columns in table 14 with the results for the controls, it is possible to determine the type of sterility induced. For example, young 10 ♂'s inseminated normal females with nonviable sperm, for only 3.6 per cent of the eggs from these females began to embryonate. Young males treated 2, 4, or 5 KR and mated to N ♀'s apparently had some living sperm, but approximately half of these sperm carried a dominant lethal which

exerted its effect before egg hatch: only about half of the eggs from these matings which began to embryonate ultimately hatched. Lastly, those eggs resulting from the latter matings which did hatch were fertilized with sperm carrying fractured chromosomes, for as shown below, a significant proportion of the F₁ nymphs from these eggs were themselves sterile.

Inherited sterility in F₁'s. All progeny that hatched from eggs produced by mating 5 ♂'s x N ♀'s were reared collectively on fresh beans. None of these nymphs showed any abnormal characteristics; no indication of malformations were detected. When they became 5th instars, they were transferred to individual cages on pallets and sexed after becoming adults. At the appropriate adult age (8–12 days) both F₁ males and females were allowed to mate with normal partners produced by untreated parents. The number of F₂ eggs produced and the associated hatch was recorded individually for each mated pair.

The results of mating the F₁'s are shown in table 15 and indicate that both sexes of the F₁ offspring of treated parents have inherited a high degree of sterility. F₁ females (whose fathers were exposed to 5 KR) mated to N ♂'s produced an average of only 85 eggs each and only 9.4 per cent of these hatched. As 43 per cent of the control eggs hatched, this is equivalent to 78.2 per cent inherited sterility. Normal fe-

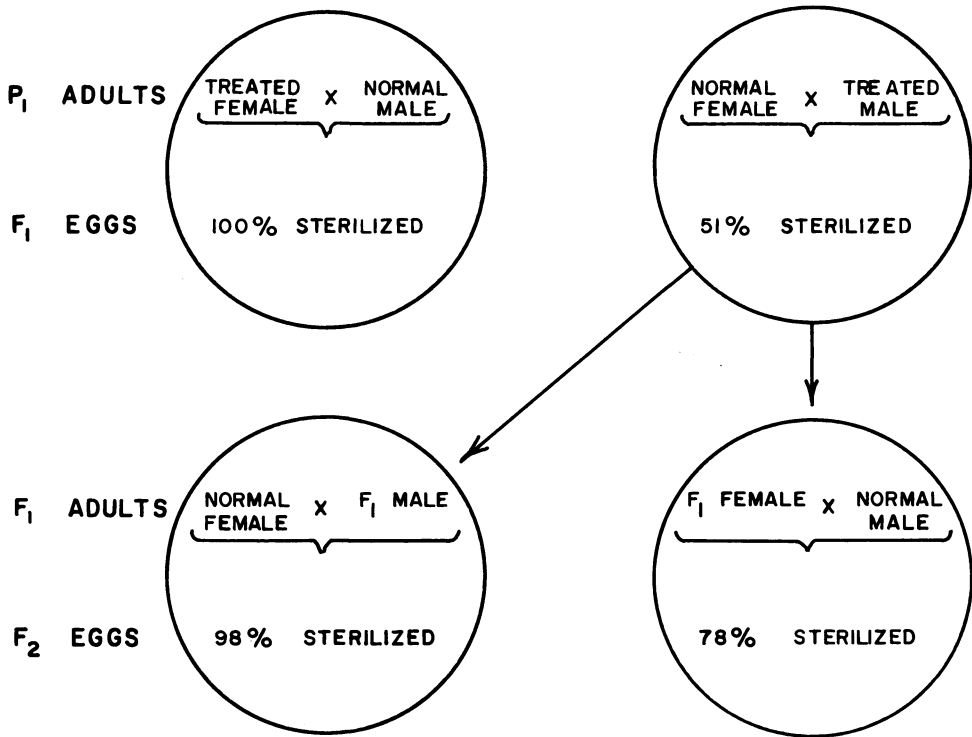


Fig. 17. Summary diagram of *L. hesperus* sterility achieved by exposing young adults to 5 KR of gamma radiation.

males mated to F₁ males whose fathers received 5 KR irradiation produced 106 eggs each but only 0.7 per cent of these hatched, which is equivalent to 98.4 per cent inherited sterility.

This phenomenon of induced (or inherited) sterility is discussed by North and Holt (1968b). Its significance and impact on a control program utilizing released sterile males is not difficult to visualize, for if F₁ males competed equally with normal males in the field, the total number of irradiated individuals needed to achieve control would be considerably reduced.

Because of the manner in which the F₁ experiments were conducted, it is not possible to compare the mean number of eggs laid by N ♀'s mated to irradiated males, as shown in tables 14 and 15, to the number of eggs laid by normal females mated to normal males. The primary purpose of mating irradi-

ated males was to see what degree of sterility they possessed, as measured by subsequent egg hatch. Therefore, we did not rigidly standardize the age of the normal females when they were mated to the F₁ males. Thus, the figures shown in tables 14 and 15 represent only those eggs laid after mating; eggs laid by these females before being mated are not included, which makes it appear as though the N ♀'s x T ♂'s produced fewer eggs than those from N ♀'s x N ♂'s.

A summary diagram depicting the major results of the gamma irradiation experiments is presented in Fig. 17. This diagram includes only the results of exposure to 5 KR irradiation, for this exposure appears to be the most practical for control purposes. Treatment of males with 10 KR resulted in more sterilization of eggs than the 5 ♂'s but an exposure of 10 KR materi-

ally affects mating behavior (see table 13). Males exposed to 5 KR appeared not to be so affected. Therefore, considering mating aggressiveness and steri-

lization of both the P_1 and F_1 generation, it was concluded that 5 KR of gamma irradiation would be adequate.

Use of Chemosterilants⁶

Chemosterilants cause sexual sterility in one of three principal ways. They may totally destroy the germarium so no sperm or ova are produced. They may cause the death of sperm or ova which were produced prior to treatment. Lastly, these compounds may induce dominant lethal mutations, so that, although the gametes remain alive, the zygotes do not grow to mature individuals. An excellent review on chemosterilants, their mode of action, biochemistry, and theory of population reduction using chemosterilants has been authored by LaBrecque (1968).

The experiments reported in this section were performed before the details of lygus reproduction presented in Part I were known. Thus, they are only preliminary, but are included in this report because they do demonstrate a means of significantly modifying reproduction of *L. hesperus*.

Methods and Materials

Two alkylating agents, metepa⁷ and HMPA⁸ were the chemosterilants used. Three dosages of metepa, 3.75, 7.5, and 11.25 $\mu\text{g}/\text{insect}$ were tested; only 11.25 $\mu\text{g}/\text{insect}$ of HMPA was used. The materials were dissolved in acetone and 0.75 μl of test solution was applied topically to the prothorax of each test insect. Controls received 0.75 μl of acetone. The bugs were immobilized by chilling before treatment.

The bugs, reared individually from the third instar in a manner similar to

the pallet method were 1-day old adults when treated. Twenty-five adult *L. hesperus* of each sex were treated with each dosage; after treatment the bugs were held individually overnight before being caged with their mates. No data on mating behavior were obtained. The treated bug and its untreated mate were simply caged together for the duration of the experiment. A fresh section of bean was supplied daily for food and ovipositional sites.

The effects of the chemosterilants were determined by recording fecundity, subsequent egg hatch and longevity of the F_1 nymphs. Also general observations were made on the treated individuals' reproductive systems and on some histological preparations of the testes and ovaries of treated bugs.

Results

The results of the chemosterilant experiments are summarized in table 16. Three major conclusions emerged from this study. First, in regard to egg production and eclosion, treatment of the male parent imparted a higher degree of egg sterility than did treating the females. This difference was especially pronounced at the lower dosages, for application of 3.75 μg of metepa to males subsequently induced 78 per cent sterility whereas this same dose applied to the females resulted in only 43 per cent sterile eggs. At the highest dosage, induced sterility for both metepa and HMPA was about 85 per cent regardless of which sex was treated.

⁶ Taken in part from a thesis submitted in January, 1966, by Esmat M. K. Hussein to the Graduate Division, University of California, Davis, in partial fulfillment of the requirement for the Ph.D. degree.

⁷ *tris* (1-(2-methyl) aziridiny) phosphine oxide.

⁸ hexamethylphosphoramide.

TABLE 16
EFFECTS ON FECUNDITY, ECLOSION AND LONGEVITY OF
L. HESPERUS CAUSED BY TREATMENT WITH METEPA OR HMPA

Parents	Dose	Mean eggs/ female	Mean hatch	Sterility*	Mean longevity of treated partner	Mean longevity of F ₁ nymphs
	$\mu\text{g}/\text{bug}$		per cent METEPA	per cent	days	days
Treated males x normal females.....	3.75	76.00	17.1	78.0	24.6	8.6
	7.50	48.2	9.7	87.5	19.9	8.3
	11.25	76.9	6.9	91.5	19.8	6.6
Treated females x normal males.....	3.75	42.4	43.4	43.0	19.1	6.4
	7.50	52.3	30.3	60.8	19.8	7.7
	11.25	56.7	12.7	83.7	19.7	6.6
Controls.....	0.0	115.3	77.8		42.0(σ^7) 34.8(φ)	32.7
			HMPA			
Treated males x normal females.....	11.25	57.8	9.8	87.2	19.7	5.5
Treated females x normal males.....	11.25	41.8	13.2	83.0	19.3	9.5

* Calculated by comparing the reduction in egg hatch between eggs from treated and nontreated parents.

Secondly, a significant reduction occurred in the number of eggs laid by treated females. Also females caged with treated male partners laid fewer eggs. (This reduction was attributed to contamination of the female from the treated male.) The dissections and histological studies showed that metepa acted on the females by temporarily blocking oocyte formations. Ovaries of 7-day old adults possessed no oocytes and were similar in appearance to the ovaries of 1-day old untreated bugs. By the 8th day, oocytes began to form, and the treated females began to oviposit about 6–7 days later. This delay of 6–7 days, coupled with the fact that the longevity of the treated bugs was only one-half to two-thirds of the controls, accounted for the observed decrease in egg production.

Dissections and sections cut from testes of treated males revealed that metepa had a more permanent effect on the males than the females. Metepa not only destroyed sperm which had been formed before treatment, but also initiated general testicular atrophy. This became evident about 8 days after

treatment. At this time, no sperm were present in the lower regions of the testicular tubules and over-all cellular organization was lacking; the testes appeared nonfunctional.

Thirdly, F₁ nymphs which emerged were all malformed, and survived only 7–8 days. None of the F₁ nymphs attained adulthood. Malformed nymphs had greatly reduced prothoracics, short, thick antennae and the coxae were compressed posteriorly.

In comparing the end results of the two methods of inducing sterility (irradiation vs. chemosterilization), the use of gamma rays is preferred. Oviposition was permanently stopped with irradiation, but only temporarily interrupted with metepa. Those few eggs produced by irradiated females were 100 per cent sterile, whereas only 43 to 84 per cent of eggs from chemosterilized females were sterile. The initially induced sterility of F₁ eggs from matings involving metepa-sterilized males was, depending on dosage, generally lower than that from irradiated males. However, the phenomenon of inher-

ited sterility which is missing in chemo-sterilized progeny, more than compen-

sates for the lower level of initial egg sterility.

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