HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE PUBLISHED BY THE CALIFORNIA AGRICULTURAL EXPERIMENT STATION

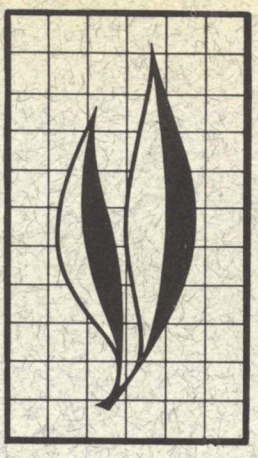
Volume 40, Number 4 · February, 1970

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*

UNIVERSITY OF CALIFORNIA DIVISION OF AGRICULTURAL SCIENCES



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 2½ 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 oviposite 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.

THE AUTHORS:

Frank E. Strong is Associate Professor and Associate Entomologist in the Experiment Station, Davis.

J. A. Sheldahl and P. R. Hughes are graduate Research Assistants, Department of Entomology, Davis.

Esmat M. K. Hussein is a former graduate student, Department of Entomology, Davis, and is now Assistant Professor of Entomology, Alexandria University, Cairo, U. A. R.

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

Reproductive Biology of *Lygus hesperus* Knight[1]

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), parasitism (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 nonreproductive 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 (*Phasiolus 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 provided 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

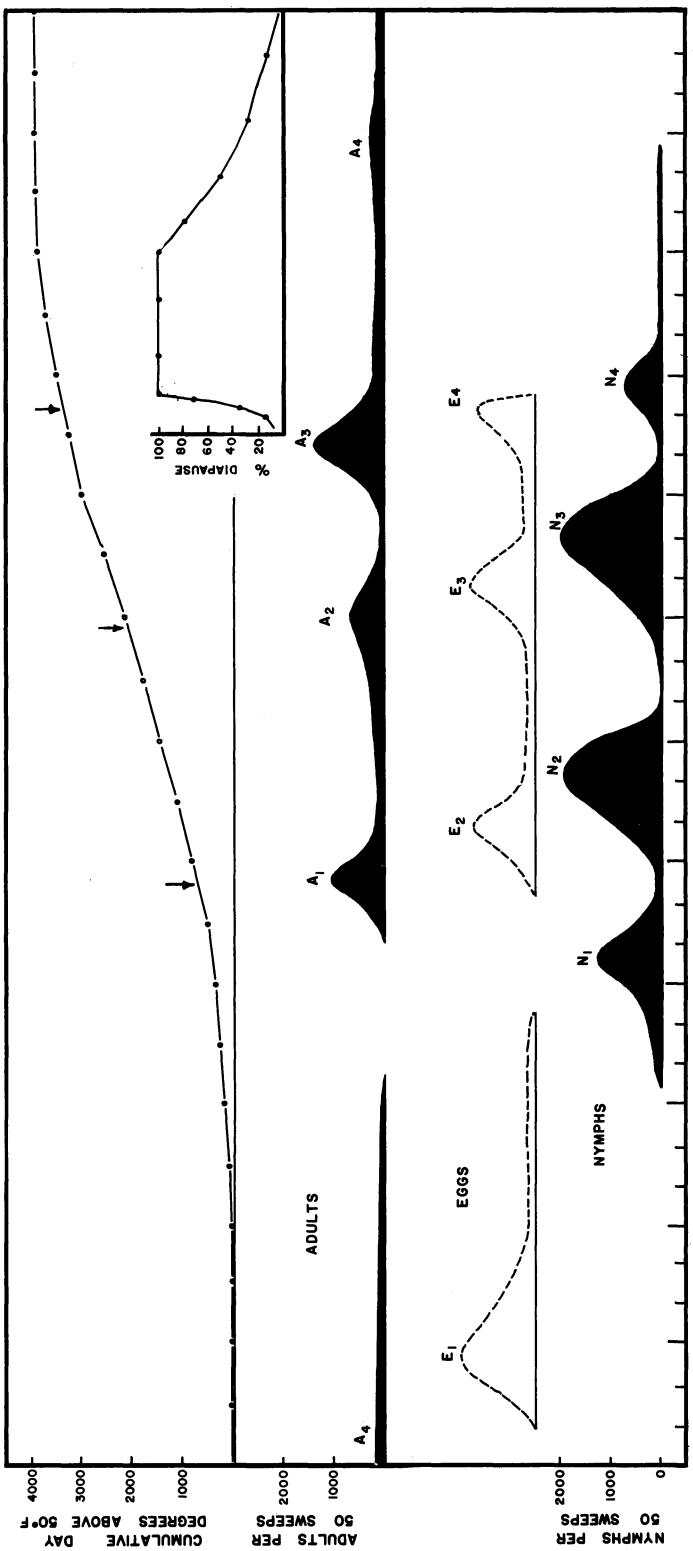


Fig. 1. Seasonal life history of *Lygus hesperus* at Davis, California. The egg curves indicated by dotted lines, are hypothetical. The curve for the cumulative day degrees above 50°F is based on normal Davis temperature records. The arrows indicate the number of day degrees for a complete generation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

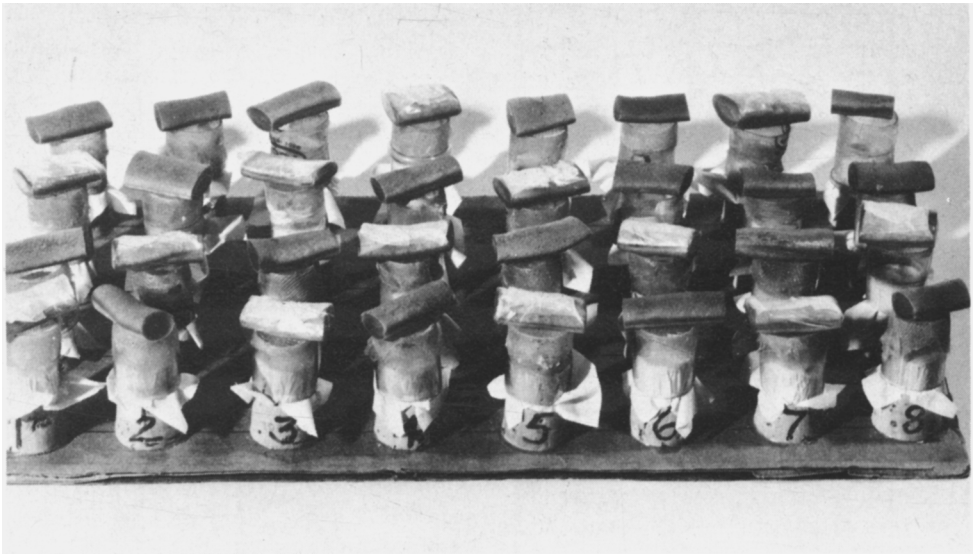


Fig. 2. Palletized individual rearing cages. One bug is placed in each cage. The bean sections are wrapped in parafilm when the females begin ovipositing, to prevent subsequent desiccation of the beans before the eggs hatched.

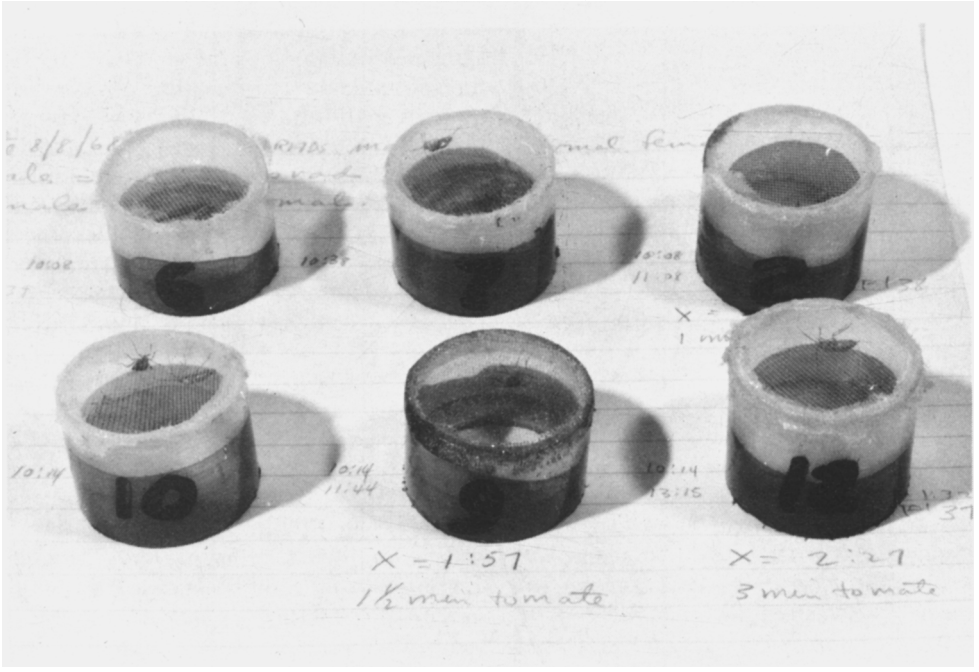


Fig. 3. Cages used for mating *Lygus hesperus*.

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 REPRODUCTION[2]

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.

series of regression analyses of *L. hesperus* populations on time in numerous alfalfa fields in southern Arizona. His results show that during the summer, over a ten-year period, the regression coefficient (b) was 0.0172, which, according to Butler's graphs, is equivalent to a ten-fold increase every 56 days. Fifty-six days under Arizona temperatures conditions are equivalent to 1.23 generations; thus, a net increase of eight times presumably occurred in one generation. Our population simulation studies indicate then, that under the Arizona conditions, the nymphal mortality would be about 92 per cent.

PART II. FIELD BIOLOGY OF *LYGUS HESPERUS* SEX PHEROMONE[3]

Chemical communication between animals is a well documented phenomenon (Marler and Hamilton, 1966). In recent years, insect pheromones have received much attention (Jacobson, 1966). Sex attractants, which constitute a large proportion of the known insect pheromones, have been most thoroughly investigated in holometabolous insects, especially Lepidoptera, with reports of attractants in more than 110 species (Jacobson, 1965). Among the hemimetabolous insects, however, less than a dozen species have been investigated. Doane (1966) was the first to report a sex attractant in Homoptera (the red pine scale, *Matsucoccus resinosae* Bean and Godwin). Subsequent reports demonstrated sex pheromones in the red scale, *Aonidiella aurantii* (Maskell) (Tashiro and Chambers, 1967) and the citrus mealybug *Planococcus citri* (Risso) (Gravitz and Willson, (1968).

The first experimental evidence for a sex pheromone in Hemiptera was published by Scales (1968), when he demonstrated that virgin females of the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), attracted males. During June and July, 1966, at Shafter, the existence of a sex pheromone in *Lygus hesperus* was preliminarily demonstrated. In 26 trapping days a total of 46 males was captured, all in the traps baited with virgin females. No males or females were caught in traps baited with other males, or with beans alone. On the basis of this preliminary experiment, the studies reported herein were initiated at Davis in early 1968.

Methods and Materials

The bugs used in this study were reared collectively or on pallets. Adult bugs less than 24 hours old were placed in the traps for bait. Generally, virgins were used, but occasionally field-collected bugs were tested. Green beans changed twice weekly were always present in all traps regardless of whether bugs were present or not.

The body of the trap, shown in figure 11, was constructed from a half-gallon ice cream carton. Both ends of this carton were fitted with removable funnels made from 16-mesh aluminum window screening; the screen funnels pointed toward the trap's center. The insects used as bait were housed in a half-pint carton, the bottom of which was replaced with screen and the top with a removable screen to permit replacing bugs or beans. A hole the size of the half-pint carton was cut in the side of the large carton, and the small carton which would house bait insects was glued in place. The inner surface of the large container was coated with "Stikem

[3] Taken in part from a thesis submitted in February, 1969, by P. R. Hughes to the Graduate Division, University of California, Davis, in partial fulfillment of the requirements for the Master of Science degree.

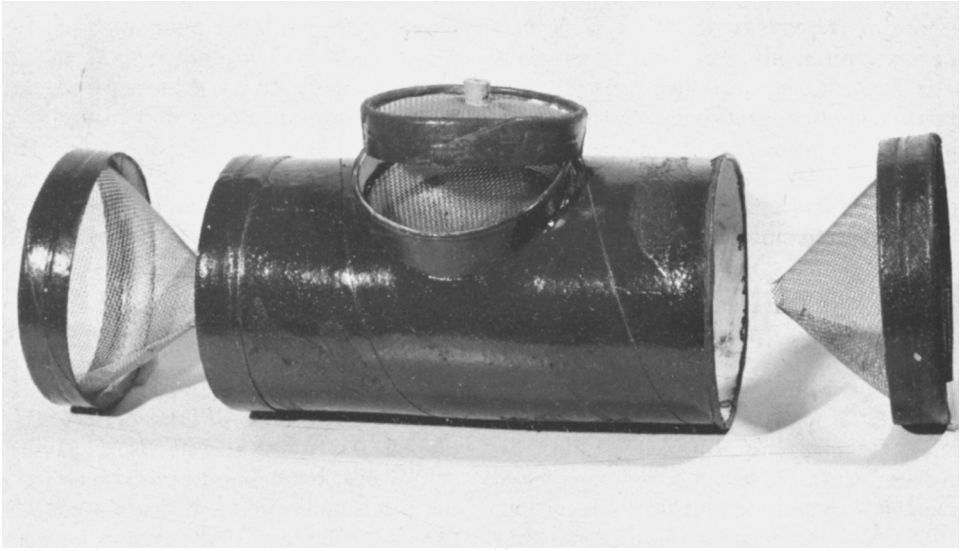


Fig. 11. Trap used for capturing male *Lygus hesperus*. The bait females were placed in the top center compartment, shown with the lid removed. Both ends were removable so that trapped bugs could be recovered. The trap was mounted in the field as shown in figure 12.

Fig. 12. Omni trap used to capture male *Lygus hesperus*. The trap was mounted on a platform capable of weathervaning. The airscoop (see text) was positioned on top of the trap as shown.

Special" (Michel and Pelton Co., Oakland), which entombed trapped bugs and prevented their escape. Without this sticky material, a trapped bug would, after much wandering around, eventually stumble upon the opening of the funnel and escape.

The traps were mounted on metal stakes in two ways. One type of trap, called the omni trap, was provided with a set of ball bearings and a wind vane, so the trap would weathervane and always remain aligned into the wind. The omni traps were also provided with an airscoop which was positioned as shown in figure 12. Thus, air currents would pass over the bait insects and flow out through the downwind end of the trap.

The other traps, called fixed traps, had no ball bearings and were thus incapable of weathervaning. Also they possessed no airscoops. As the winds in Davis are generally either north or south, the fixed traps were oriented in this direction.

In both types of traps, a screen partition was located in the center of the large container, which made it possible to determine which end of the traps the lygus bugs had entered.

All trapping experiments were conducted in alfalfa hay fields, one located on the University of California, Davis campus, and the other 4 miles north, designated the Medlock field. These fields were selected because—although both were being cut on a monthly cycle—each had an acre or so which remained uncut and received no insecticides throughout the season. The traps were placed on east-west lines spaced at 12-foot intervals. The height was periodically adjusted so they remained level with the tops of the alfalfa plants.

All traps were examined one to several times daily, depending on the particular experiment. Captured insects were removed and counted each time the traps were visited. A recording thermograph was maintained adjacent to the traps throughout the study. Also, biweekly sweep net samples were taken in the vicinity of the traps to monitor the field populations.

Confirmation of a sex pheromone in *Lygus hesperus*

The initial experiment in this study was to corroborate the results obtained two years previously at Shafter. Fifteen of the fixed traps were placed in the cut portion of the Campus field. Five were baited with five virgin females, five with five virgin males and five check traps contained only beans. The test was terminated after 14 days. Fifteen additional traps, baited in an identical manner were stationed in the uncut portion for 8 days. All 30 traps were examined daily for the first 4 days and twice daily thereafter.

The results, shown in table 7, indicate

TABLE 7

NUMBERS OF LYGUS BUGS CAPTURED IN TRAPS BAITED WITH MALE OR FEMALE VIRGIN *L. HESPERUS*

Date	Field	Bait	Number of traps	Total catch		Number of bugs in field/50 sweeps*	
				Males	Females	Males	Females
July 11–25	Cut	Check	5	0	0		
		Males	5	0	0	22.4	23.4
		Females	5	38	0		
July 12–26	Uncut	Check	5	0	1		
		Males	5	0	0	110.0	111.6
		Females	5	22	0		

* Sweep net samples represent the average of counts (sex ignored) from July 12 to July 25, 1968.

that the females were releasing something which attracted only males. Although the numbers of males captured were not large, the figures are meaningful considering the competition from the field population. The fact that more bugs were captured in the cut portion (which had a lower native population) probably reflects this competition. Furthermore, males were only captured in traps baited with females. The one female found in a check trap was undoubtedly an accidental capture.

Efficiency of fixed traps vs. omni traps

Data assembled from several of the field experiments demonstrated that the airscoop above the bait insects was the most important single design feature for increasing trapping efficiency. Although omni traps without the airscoops were three times as effective as the fixed traps, they caught on the average 2.5 times fewer males than omni traps provided with airscoops. Without the airscoop the location of the bait insects did not allow adequate air circulation to carry the pheromone through the center of the trap. Male bugs were occasionally seen resting on the top screen of the small carton containing the females. Had they approached the trap toward the funnels, they would have been captured. The airscoop directed the wind flow over the bait insects and out past the funnels. Flying males were then frequently observed approaching from the downwind side directly toward the funnel. After alighting on the screen, they would continue on foot, walking upwind until they happened upon the opening of the funnel.

Influence of temperature and photophase on male catch

Whether a male is captured depends not only on the female producing attractant but also upon conditions suitable for flight. Thus, the conditions under which males fly as well as the conditions suitable for production of the pheromone, were examined.

Ten virgin females were placed in each of eight omnitraps; two additional traps having only beans served as checks. The test was run for 12 days. During 4 of the trapping days the catches were monitored in relation to temperature and the traps were examined every 15–30 minutes from sunrise to sunset. During the remaining 8 trapping days, the traps were examined (with the aid of a flashlight) starting 30 minutes before any visible sign of sunrise and at 30 minute intervals thereafter until 1 hour after sunset. The traps were also occasionally checked at night.

The data from this experiment are summarized in tables 8 and 9. No males were captured at temperatures below 54°F or above 80°F (table 8). In the first 4 days of trapping, a total of 173 males was recovered, 97 per cent of which were captured between 54° and 75°F. The remaining 4 per cent (7 males) were caught on a single morning when the air temperature exceeded 75°F but a cloud cover was present. No bugs were captured when the wind exceeded 12–15 mph, regardless of the temperature.

In the last 8 trapping days, during which time the influence of photophase was studied, 458 males were captured (table 9). About 94 per cent of these were recovered after daybreak and before darkness, but 28 males were caught during darkness when favorable temperatures existed for flight. Thus, males are capable of flying and responding to the pheromone during the night.

The data from this experiment also yielded information indicating that the rate of pheromone production and male responsiveness is probably constant throughout the day. Using the percentage of distribution of males captured at different temperatures (last column in table 8) and the hourly temperature

TABLE 8

MALE *L. HESPERUS* CAPTURED AT VARIOUS TEMPERATURES IN TRAPS BAITED WITH VIRGIN FEMALES

Temperature range	Males trapped on:				Mean
	Aug. 20	Aug. 21	Aug. 22	Aug. 23	
degrees F	*per cent*				*per cent*
Below 54	0	0	0	0	0.0
54-65	95.2	51.7	46.3	70.8	66.0
65-75	4.8	48.3	40.7	29.2	30.8
75-80	0	0	13.0	0	3.2
Above 80	0	0	0	0	
	Number of males caught per day*				Total
	42	29	54	48	173

* In eight traps each baited with ten virgin females.

records for the 4 trapping days, it was calculated that 74.6 per cent of the catch should occur in the early morning hours and 25.4 per cent in the evening hours before darkness. Actually, 76.1 per cent of all males were trapped after sunrise and before the temperature reached 80°F, and 23.9 per cent in the late afternoon after the temperature fell below 80°F and before sunset. If these percentages were widely different from the predicted ones, it would indicate that either the males' responsiveness changed during the day or that the rate of pheromone production was not constant.

Direct observations of flight activity corroborated the observations concerning temperatures. Right at day break, the temperature during August in Davis was normally about 50°–52°F and the wind was generally calm. No males were observed flying at this time. Twenty to 40 minutes later, when a slight breeze was present (1–4 mph) and the temperature had risen to 54°–56°F, lygus bugs were observed flying between alfalfa plants or in level flight even with the tops of the plants. Bugs which were around to 6–8 feet downwind of an omni trap were seen to orient upwind and fly in a reasonably straight

TABLE 9

CATCHES OF MALE *L. HESPERUS* DURING THE DAY AND NIGHT IN EIGHT TRAPS EACH BAITED WITH TEN VIRGIN FEMALES

Date when trapped	Number of males trapped		Trapped during day	Trapped during night
	Daybreak to darkness	Darkness to daybreak		
			per cent	*per cent*
Aug. 24	41	1	97.7	2.3
25	50	0	100.0	0.0
26	48	9	84.2	15.8
27	65	6	91.5	8.5
Sept. 13	100	8	92.6	7.4
14	70	0	100.0	0.0
18	28	2	93.3	6.7
19	28	8	93.3	6.7

path toward the trap. These orientation flights were always slow; the male would occasionally stop its forward progress, hover momentarily, and then proceed slowly upwind. Apparently, once a male detected the pheromone, he never varied from his flight path and invariably reached the trap. Just before alighting on the traps, males would fly back and forth, perpendicular to the airflow. They would often become airborne immediately after landing on the funnel, repeat the sideways flight and re-alight. They would continue the searching on foot until they entered the opening of the funnel and became trapped.

Additional evidence supporting the upwind movement of the flying males was available because of the partitions in the center of the traps. Of 403 males captured during a portion of the temperature and photophase experiments, 399 were found in the downwind end of the traps.

Relationship between age of females and production of sex pheromone

The data in Part I indicate that females do not normally mate until they are 5 or 6 days old, and that this was correlated with the development of the reproductive system. Because sex pheromones are associated with the mating process it was hypothesized that female *L. hesperus* would not produce the attractant during the first 5 days or so of her adult life.

This hypothesis was examined in five field tests. Generally five to ten females, 12–24 hours old, were placed in each trap. The traps were examined daily, and the average age of the females when males were first captured was noted. These tests were terminated at various times depending on weather and field conditions, but some tests ran continuously for 28 days using the same females as bait.

As shown in table 10, the average age of the females when males first were trapped varied between 6 and 9 days; the over-all mean age was 6.6 days. Once released of the attractant was initiated, the females remained attractive for the duration of the tests (up to 28 days). Because of natural mortality of the females, the tests demonstrated only how long they did attract, not how long they could.

The effect of mating on female attractiveness

Lygus bugs are not obligatorily monogamous, for females are capable of multiple matings. However, females

TABLE 10

AGE OF FEMALE *L. HESPERUS* WHEN MALES WERE FIRST ATTRACTED TO THEM JULY AND AUGUST, 1968

Trap number	Average age of females when males were first captured	Trap number	Average age of females when males were first captured
	days		*days*
1	6	8	7
2	7	9	6
3	6	10	6
4	7	11	6
5	6	12	6
6	6	13	9
7	6	14	8

Mean = 6.63 days.

TABLE 11

NUMBERS OF MALE *L. HESPERUS* CAPTURED IN TRAPS BEFORE AND AFTER BAIT FEMALES WERE MATED

Day	Traps baited with females plus males*						Traps baited with virgin females only†					
	Trap number				Males (daily totals)	Percentage of total‡	Trap number:				Males (daily totals)	Percentage of total‡
	1	2	3	4			5	6	7	8		
						per cent						*per cent*
.5...	0	2	0	4	6	21.4	6	1	0	15	22	78.6
1.....	0	0	1	4	5	6.8	28	25	8	7	68	93.2
2.....	0	7	0	3	10	11.4	25	12	14	27	78	88.6
3.....	1	12	6	8	27	28.4	25	3	20	20	68	71.6
4.....	0	11	5	3	19	28.8	19	13	13	2	47	71.2
5.....	7	19	20	5	51	48.6	12	16	9	17	54	51.4
6.....	2	14	3	9	19	31.7	7	11	9	25	41	68.3
7.....	10	13	6	2	31	50.0	10	15	0	6	31	50.0
				TOTAL	168					TOTAL	409	
	(*Females Only*)						(*Females Plus Males*)					
9¶....	0	0	0	1	1	100.0	0	0	0	0	0	0.0
10.....	6	7	2	12	27	90.0	0	0	3	0	3	10.0
11.....	5	11	3	8	27	90.0	0	0	2	1	3	10.0
12.....	5	0	1	1	8	88.9	0	1	0	0	1	11.1
				TOTAL	63					TOTAL	7	

* Males removed at end of the second day.
† Males added at end of the eighth day.
‡ Percentage distribution of males caught between the two groups of cages (Nos. 1, 2, 3, 4 vs. Nos. 5, 6, 7, 8) on any given day.
¶ No data were obtained on the eighth day because of high winds, and those from the ninth day were influenced by an unseasonal rainstorm and low temperature.

could not be induced to mate sooner than 5–6 days after a previous mating and it was questioned if this was associated with pheromone production. Trapping experiments were therefore conducted to ascertain the attractiveness of females before and after mating.

Ten 10-day old virgin females were placed in each of eight omni traps. In traps numered 1, 2, 3, and 4, ten 12-day old males were also placed in the small carton with the females. Forty-eight hours later the males were removed. All eight traps were examined three times daily for 7 days. On the 8th day, ten 12-day old males were placed in traps 5, 6, 7, and 8 to mate with the virgin females. Trapping was then continued for an additional 4 days, to see if the attractiveness of the virgins which had been in traps 5–8 days would suddenly decrease after mating with the males.

The results (table 11), indicate that virgins lose their attractiveness immediately after mating. During the first 7 days, the four traps containing virgins captured a mean total of 58.4 males per day, but the same four traps caught only an average of 1.75 males per day in the 4 days following mating. During the first 3 trapping days, about 90 per cent of all males captured were in the virgin-baited traps (Nos. 5 to 8). This was anticipated because the females in traps 1, 2, 3, and 4 had just mated when the experiment was started. This relationship soon changed, however, and by the 5th day, both sets of traps were capturing approximately equal numbers of males.

From this experiment, it was concluded that mating decreases the attractiveness of females for only a few days. Because of the natural mortality of the females used as bait, the overall attractiveness of the group decreased with

time. This fact, coupled with the increasing attractiveness of the surviving females resulted in a relatively constant catch of males in traps 1, 2, 3, and 4 and a slightly decreasing catch in traps 5, 6, 7, and 8 during the first 7 days.

Seasonal changes in attractiveness of field-collected females

As fall approaches, *L. hesperus* enters an adult diapause characterized by atrophy of the ovaries and cessation of egg laying. This condition begins at Davis in the second week of September and lasts about two months (Beards and Strong, 1966). A field experiment was conducted to examine the relationships between diapause, pheromone production, and the fact whether the overwintering females mated before or after entering diapause.

Beginning on September 9, 1968, ten field-collected females were placed in each of two omni traps. A third fixed trap containing ten laboratory-reared females (which did not enter diapause) was included during the last half of the test and was used to insure that the field males continued to be responsive. The bait females were replaced with females from the same source after 5 trapping days of suitable weather (i.e. above 54°F and light winds). Females removed from the traps were dissected and examined for sperm to see if they had been mated before being placed in the traps for bait. All traps were checked daily and the experiment was continued until October 29. Dissections of field-collected females continued, however, until November 21.

The principal results are shown in figure 13. As the experiment progressed, the number of males trapped per 5-day period decreased sharply beginning September 19 and continuing to October 5. This decrease corresponded closely with the increase of diapausing females during this period. Observations made on the dissected females

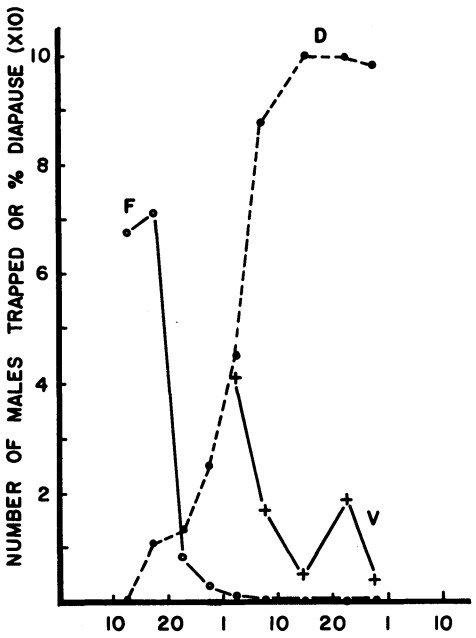


Fig. 13. Relationship between incidence of diapause and the attractiveness of field-collected female *L. hesperus*. F = traps baited with field-collected females; V = traps baited with laboratory-reared virgins; and D = incidence of diapause.

demonstrated that the diapausing females had not mated. More than 150 diapausing females collected from October 15 to November 1 were dissected and all lacked sperm. Reproductive females first reappeared about November 5 when non-diapausing virgins were collected. Two weeks later, several mated reproductive females were collected.

From this study it was concluded that field bugs destined to enter diapause fail to produce sex pheromones upon attaining adulthood and consequently fail to mate at that time.

The sharp decrease in the numbers of males caught from September 19 to October 3 is in part attributable to a seasonal change in male responsiveness. This change in male responsiveness was suspected, when the number of males captured in the trap baited with labora-

tory-reared virgins decreased as the incidence of female diapause increased (see figure 13). In spite of this observation, the above conclusion concerning absence of sex pheromone in diapausing females is still valid, because on October 3 there was an ample supply of responsive males in the field (see figure 13), as evidenced by the catch in the trap baited with laboratory-reared virgins.

Seasonal changes in male responsiveness

Diapause studies in *L. hesperus* have been concerned largely with the females, and scant attention has been paid to the physiological conditions of the male during the fall season. Leigh (1963) mentioned that although the testes appeared unchanged, the fat body was well developed and the seminal vesicles were very small in "diapausing" males as compared with males collected during the summer months. A series of experiments was conducted to further clarify the conditions of the males when females were in diapause, and the effect of this condition on their responsiveness and subsequent matings.

Twenty males were field-collected on October 11, 1968, when 100 per cent of the females were in diapause. In the laboratory, these males were placed individually in mating cages with laboratory-reared virgin females. Each pair was observed constantly for 6–10 minutes, and the response of the males noted. They were then removed, and laboratory-reared males were placed with the virgin females. This was done because none of the field-collected males showed any aggressiveness or attempted to mate, and it was necessary to verify that the laboratory-reared females were themselves attractive. Eighty per cent of the laboratory-reared males attempted to mate with the virgin females within 6 minutes. Coitus was not permitted, however, for as soon as it was evident the female was attractive, she was removed and replaced with her original field-collected partner. Here she remained for 48 hours, then the male was removed. Oviposited eggs were collected for a 3-day period and held two weeks to determine viability. At the end of the 3-day ovipositional period, ten of the 20 females were dissected and examined for sperm; the remaining ten females were again placed with laboratory-reared partners, held 48 hours and allowed 3 days to oviposit after which they were dissected. Finally, all 20 field-collected males were dissected and the condition of their internal organs noted.

This experiment demonstrated that field-collected males are in a physiological state of unresponsiveness when the field females are in diapause. Initially, none of these males showed agressiveness toward the laboratory-reared females. Only one of the ten females caged with the field-collected males for 48 hours possessed sperm, but none of her eggs hatched. In fact, none of the 198 eggs collected in the 3 days from any of the females caged with field collected males hatched, whereas 25 per cent of those eggs laid by females caged with laboratory-reared males hatched. Also, eight of the ten latter females contained sperm.

Dissections of the field-collected males revealed a marked difference between them and the laboratory-reared males. All of the field-collected males contained sperm, but the seminal vesicles in the field males were very slender and contained only a small quantity of sperm compared to the laboratory-reared males. Also, the accessory glands of the field males were reduced in size and nearly empty. Hypertrophy of the fat body was evident in field-collected males and atrophy of the testes appeared in half of those dissected.

There was also an external color difference between the two groups of bugs. Field-collected bugs possessed reddish coloration laterally along the spiracles,

ventrally on both sides of the meson and the tip of the abdomen. The background color of the abdomen was dull cream or tan, whereas laboratory-reared males lacked red coloration and had a green abdomen. Of significance is the fact that the only normally appearing field-collected male was the one which mated.

Discussion

The field experiments reported here demonstrate that the female *Lygus hesperus* produces a sex pheromone attractive only to the males. Preliminary attempts to extract this material have failed, probably for lack of a suitable laboratory bioassay. Several attempts to develop such an assay were made but none resulted in a technique which yielded reproducible data.

The lack of a sensitive laboratory bioassay has been partly responsible for failing to locate the morphological source of the pheromone. The constant correlations of 5–6 days that exist between age of first mating, age of first pheromone release, time lapse between successive mates, time required for the genital pouch to empty, and the first appearance of fully formed eggs in the ovarioles, strongly suggest that the pheromone-producing glands are associated with the reproductive system. The spermatheca would seem like a reasonable organ to suspect of producing pheromones. Davis (1955) has shown that this organ is homologous to the spermatheca of other insects, but in the higher Hemiptera its function is unknown, for it is not associated with sperm transfer. Moreover, while it is glandular, the size of the gland appears too small to be of any significant importance if its secretions are involved with egg maintenance. The spermatheca empties into the center of the genital chamber, not the seminal depository, and therefore it is probably not associated with sperm maintenance.

The field experiments indicate that lygus behavior is not unlike that of many other insects which produce a sex pheromone. A loss of female attractiveness after mating, which occurs in *L. hesperus,* also occurs within 24 hours after mating in the California red scale (Tashiro and Moffitt, 1968), and in the sawfly, *Diprion similis* (Coppel, *et al.,* 1960). Noctuids, however, remained relatively attractive after mating (Shorey, *et al.,* 1968).

The age when an insect mates appears to be associated with the first production of the sex pheromone. In *L. hesperus,* pheromone is first produced when adults are 5–6 days old—generally the minimum age for mating. The tobacco budworm, *Heliothis virescens,* which mates about 3 days after emergence, first produces the sex attractant at that time (Sparks, *et al.,* 1964). Insects which mate shortly after molting are known to initiate pheromone production upon becoming adults (Coppel, *et al.,* 1960; Cleveland and Murdock, 1964). In the silk worm, *Bombyx mori,* the female pupa produces the pheromone, and the male, which emerges first, awaits on top of the cocoon and copulates within minutes after the female emerges (Jacobson, 1965).

The use of sex attractants to regulate population densities has been discussed (Parkes and Bruce, 1961; and Calhoun, 1962). Others have discussed the possibilities of helping insect control programs by using sex pheromones to alter behavior or attract males to self-sterilizing devices (Shorey and Gaston, 1967; Babson, 1963; Wright, 1964). Bacon, Riley and James[4] have developed a sophisticated sampling device baited with extracts from female potato tuber moth, *Phthorimaea operculella* (Zeller),

[4] O. G. Bacon, W. D. Riley, and R. James, personal communication, 1968.

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 relatively 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 potential 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 entomolgists. 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 chromosones 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 journal HILGARDIA is published at irregular intervals, in volumes of about 650 to 700 pages. The number of issues per volume varies.

Single copies of any issue may be obtained free, as long as the supply lasts; please request by volume and issue number from:

Agricultural Publications
University Hall
University of California
Berkeley, California 94720

The limit to nonresidents of California is 10 separate titles. The limit to California residents is 20 separate titles.

The journal will be sent regularly to libraries, schools, or institutions in one of the following ways:

1. **In exchange for similar published material on research.**
2. **As a gift to qualified repository libraries only.**
3. **On a subscription basis—$7.50 a year paid in advance. All subscriptions will be started with the first number issued during a calendar year. Subscribers starting during any given year will be sent back numbers to the first of that year and will be billed for the ensuing year the following January. Make checks or money orders payable to The Regents of The University of California; send payment with order to Agricultural Publications at above address.**