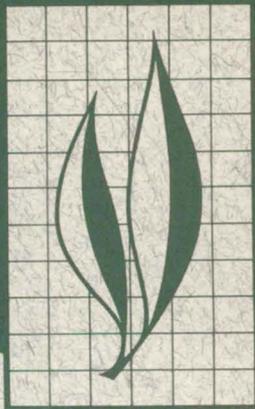


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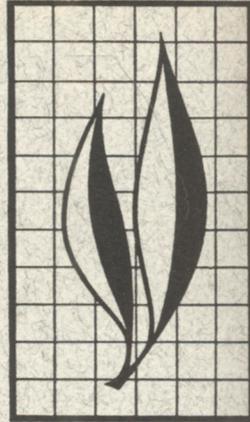
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Photoperiod in Relation to Diapause in *Lygus hesperus* Knight

George W. Beards

and

Frank E. Strong



Lygus hesperus Knight, a common polyphagous insect pest of certain widely grown California crops, undergoes a seasonal facultative diapause. Experiments described here were conducted upon both field-collected and laboratory-raised *L. hesperus* populations and have demonstrated the critical effects of photoperiod and temperature upon the inception, termination, nullification, and intensity of diapause in this species.

THE AUTHORS:

George W. Beards is a graduate student, Department of Entomology, Davis. Frank E. Strong is Associate Professor of Entomology, Davis.

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INTRODUCTION

Lygus hesperus Knight is a common polyphagous insect found throughout the Rocky Mountain and Pacific states. It is primarily a pest of cotton and alfalfa, beans, carrots, and of sugar beets grown for seed (Metcalf and Flint, 1951). In California, *L. hesperus* has consistently been rated among the most destructive insect pests on cultivated crops; numerous investigations have dealt with economic considerations of this pest.

Detailed life history and behavioral studies of *Lygus hesperus* under field conditions have been reported by Schull (1933) and Sorenson (1939); and under laboratory conditions by Leigh (1963), Jeppson and MacLeod (1946), and Strong and Landes (1965). This species may have three to five generations per year depending upon climatic conditions. It overwinters as an inactive adult among leaf trash and litter or, in warmer climates, feeds actively upon weed hosts and cultivated crops. Observations by Leigh and Beards (unpublished data) suggest that *L. hesperus* may undergo a facultative diapause rather than merely becoming inactive under low winter temperature conditions. The studies reported herein were conducted at Davis, California, to investigate the supposed facultative diapause. These studies concern natural and artificial environmental conditions, principally photoperiod, which were

found to influence diapause in local populations of *L. hesperus*.

Many plants and animals react to seasonal differences in lengths of the daily cycles of light and dark. Each cycle is termed a photoperiod; the light and dark portions are termed the photophase and scotophase, respectively. The term diapause refers to a state of arrested growth or reproduction which is typical of hibernating or aestivating arthropods. Although development may be delayed by a multitude of adverse conditions, most investigators tend to distinguish quiescence (or hibernation) from diapause, because certain physiological mechanisms are present in the diapausing insect which are absent in the quiescent insect.

Because there are borderline cases, no completely adequate criteria have been established to distinguish all the types of arrested development which may occur between quiescence and diapause. However, a commonly accepted definition of diapause has been proposed by Simmons (1948) who considers it to be a state ". . . in which a reduction of growth processes or maturation occurs which is not necessarily caused by immediate environmental influences, does not depend for its continuation on unsuitable conditions, and is not easily or quickly altered by change to a more favorable environment. However, once the state of diapause comes to an end,

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TABLE 1
 CLASSIFICATION OF FEMALE *LYGUS HESPERUS*
 ACCORDING TO REPRODUCTIVE STATE
 (Specimens field-collected at Davis, California, 1962 to 1964)

Date collected	Number collected	Visual classification*				Total Nonreproductive
		Reproductive	Intermediate	Nonreproductive	Nonreproductive: diapause	
1962:						
Jul. 30.....	85	74	0	11	0	<i>per cent</i> 12.9
Sept. 21.....	103	87	1	0	14	13.7
Sept. 27.....	103	28	1	0	73	71.5
Oct. 4.....	102	2	0	0	100	98.0
Oct. 19.....	104	2	2	0	100	98.0
Oct. 30.....	119	15	9	0	86	85.1
Nov. 5.....	112	26	11	0	64	71.1
Nov. 13.....	126	42	21	0	42	50.0
Nov. 30.....	101	61	6	0	28	31.4
Dec. 14.....	139	71	28	0	12	14.4
1963:						
Jan. 16.....	17	16	0	0	1	6.0
Feb. 5.....	7	7	0	0	0	0.0
Mar. 15.....	0
Apr. 23.....	0
May 24.....	16	16	0	0	0	0.0
Jun. 28.....	110	103	3	3	0	3.7
Jul. 27.....	108	104	1	3	0	2.8
Aug. 22.....	101	98	1	2	0	2.0
Sept. 9.....	125	120	1	4	0	3.2
Sept. 16.....	83	45	0	0	38	45.8
Sept. 23.....	117	70	4	0	43	38.1
Oct. 2.....	102	0	0	0	102	100.0
Oct. 8.....	97	0	0	0	97	100.0
Oct. 20.....	102	0	0	0	102	100.0
Oct. 24.....	114	5	4	0	105	95.5
Oct. 28.....	107	17	8	0	82	82.8
Nov. 1.....	99	12	0	0	87	87.8
Nov. 11.....	77	16	8	0	53	76.8
Nov. 17.....	77	27	17	0	33	55.0
Nov. 26.....	91	45	13	0	33	42.3
Dec. 12.....	51	30	3	0	18	37.5
1964:						
Jan. 8.....	54	54	0	0	0	0.0
Feb. 20.....	32	32	0	0	0	0.0
Mar. 20.....	17	17	0	0	0	0.0
Apr. 14.....	0
May 6.....	23	23	0	0	0	0.0
Jun. 5.....	73	70	1	2	0	2.7
Jun. 29.....	64	63	0	1	0	1.5
Jul. 15.....	103	98	2	3	0	2.9
Aug. 1.....	94	90	1	3	0	3.2
Aug. 20.....	106	99	0	7	0	6.6
Sept. 1.....	87	86	0	1	0	1.1
Sept. 11.....	78	77	0	1	0	1.3
Sept. 17.....	103	92	0	0	11	10.7
Sept. 24.....	86	12	1	0	73	85.9
Oct. 1.....	87	3	0	0	84	96.5
Oct. 8.....	100	0	0	0	100	100.0
Oct. 20.....	97	0	1	0	96	100.0

* See text for a description of the various classifications.

normal growth and development are resumed." In the light of recent developments, perhaps a more precise definition is that given by Beck (1962) who considers that diapause is "... a state of arrested development in which the arrest is enforced by a physiological mechanism rather than by concurrently unfavorable environmental conditions. Although not maintained directly by environmental factors, diapause is apparently induced, and in many species also terminated, in response to environmental stimuli."

The stage of development in which diapause occurs varies in different insect species: some diapause in the egg stage, while others diapause during the larval, pupal or adult stages. In immature stages, diapause is manifested by arrested growth or development. In the adult, it may be expressed by failure to show reproductive activity; or mature sex cells may degenerate, while relatively immature reproductive tissue is preserved (Brazzel and Newsom, 1959; de Wilde and de Boer, 1961). Lees (1955) cited failure of reproductive organs—particularly the ovaries—to enlarge, and hypertrophy of the fat body as morphological criteria of diapause in adult insects.

In most insect species having a facultative diapause, the principle stimulus for the onset of diapause is photoperiod. Photoperiod is the only environmental factor that changes with mathematical precision, being not at all affected by changes in weather and climate, but only by changes in latitude and season of year. The critical photoperiod is defined as that photoperiod consisting of the correct proportions of daylight to darkness which initially educes a maximal diapause response in the majority of individuals in a population.

Considerable evidence exists to support the assumption that diapause has been independently evolved among species and even among strains of the same

species which occupy different geographic locations. Danilyevsky (cited by de Wilde, 1962) found that northern and southern strains of the moths *Acronycta rumicis* (Linnaeus) and *Spilosoma menthastri* Esper differed in the critical photoperiod and ecological characteristics of the diapausing pupa, e.g., cold hardiness. Beck and Apple (1961) demonstrated significant differences in the critical photoperiod among different geographic populations of the European corn borer, *Ostrinia nubilalis* (Hubn.). In general, at higher latitudes local insect populations enter diapause in response to longer light phases and shorter dark phases than those which would induce diapause in lower latitude populations.

Under natural conditions, diapause eventually terminates and morphogenesis resumes. The physiological processes that must be completed before normal development resumes occur on a biochemical level and were given the paradoxical name "diapause development" by Andrewartha (1952). Andrewartha defined diapause development as "the physiological development, or physiogenesis, which goes on during the diapause stage in preparation for the active resumption of morphogenesis." Schneiderman and Horwitz (1958) use the term "diapause-ending process" in a similar sense. Regardless of which terminology is preferred, both authors are simply referring to the factors responsible for the termination of diapause.

The intensity of diapause, as determined by the length of time required for diapause termination, varies considerably among the species which have been studied. It may last only a few days, as is true for *Eurydema ornatum* Linnaeus, or for several months, as in the case of the codling moth, *Carpocapsa pomonella* (Linnaeus). Furthermore, the intensity of diapause may also vary among individuals of the same

species, depending on how long each has been exposed to diapause-inducing conditions. Yothers and Carlson (1941) observed that healthy hibernating codling moth larvae may fail to transform the season following hibernation and may, under certain conditions, transform to moths two seasons later.

Since Marcovich (1924) reported photoperiodic effects in insects, extensive work on diapause and related phenomena has been published. For detailed selected coverage, we refer the reader to reviews by Andrewartha (1952), Lees (1955, 1956) de Wilde (1962), and Beck (1962).

FIELD OBSERVATIONS ON THE NATURAL INDUCTION OF DIAPAUSE

At Davis, *Lygus hesperus* has several generations per year and overwinters as an adult. Studies were initiated in 1962 to determine whether all generations were continually reproductive or if one generation entered diapause.

The first problem was to identify diapausing bugs. The criteria proposed by Lees (1955)—failure of the reproductive organs to enlarge, and hypertrophy of the fat body—were used to identify diapausing individuals.

From July, 1962, to October, 1964, adult bugs were collected periodically from local alfalfa hay fields with a sweep net. Within six hours after being collected, the females were killed in 70 per cent alcohol, pinned through the scutellum, positioned dorsal side up with the head pointed forward, and dissected under water in a wax-bottom dissecting dish at 10× magnification. They were dissected by grasping the tip of the abdomen with a sharp forceps, and pulling backward until the abdomen came apart sufficiently to expose the ovaries. The bugs were then classified into one of four categories:

1. Reproductive females—these had a general green coloration except that they could be somewhat yellowish in November and December. The ovaries contained at least one egg in which the operculum, or egg cap, could be distinguished. The operculum gave a truncate appearance to the anterior part of the elongated egg (fig. 1).

2. Intermediate females—those as-

signed to this group had the same general coloration as the reproductive insects. Although the ovaries contained eggs in various stages of vitelligenesis, no operculum was apparent.

3. Nonreproductive females—these were teneral or had a general greenish coloration. The ovaries were not discernibly developed; this category represented young bugs apparently in a pre-ovipositional state.

4. Nonreproductive: diapause females—those placed in this category had a general yellowish coloration. The ovaries were undeveloped and the fat body was hypertrophied.

A diapausing and a reproductive bug are shown in figure 1.

Records obtained from periodic dissections of field-collected females are shown in table 1. Bugs classified as nonreproductive: diapause consistently appeared first about the middle of September and were found in this state through December. Although a small percentage of nonreproductive bugs was found in the field throughout the summer, these were still in a pre-ovipositional state rather than in diapause. The number of young adult bugs collected varied considerably in relation to the maturity of the alfalfa field in which they were collected—that is, fields cut recently had a higher preponderance of young adults than did fields approaching the blooming stage. This was probably because when the fields were cut, many of the mature bugs

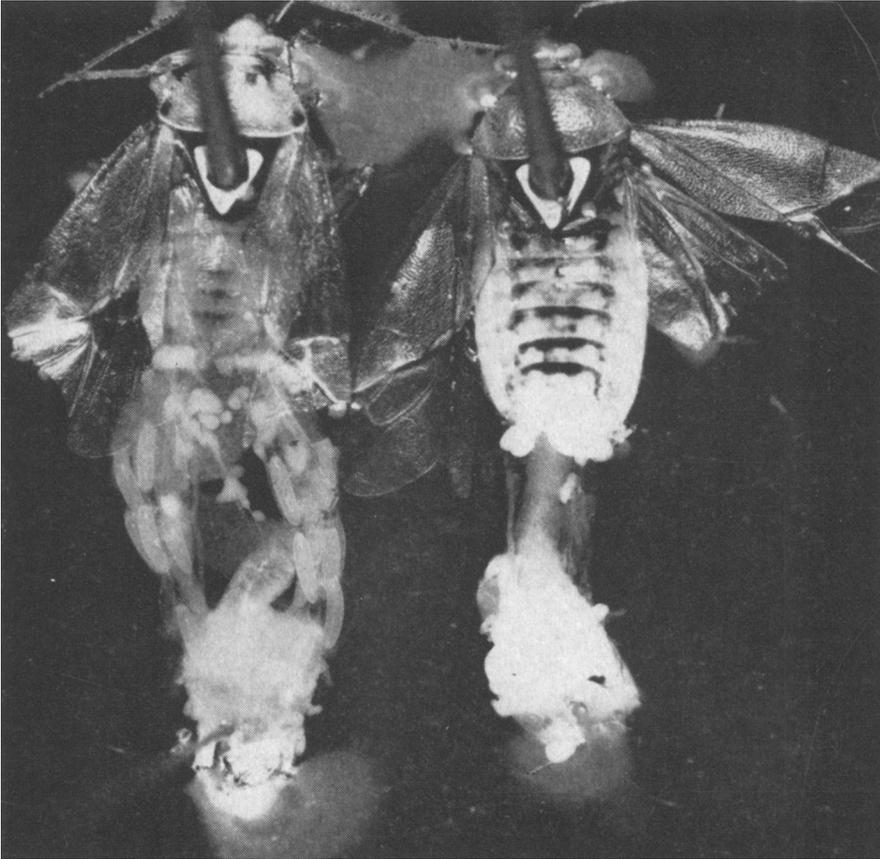


Fig. 1. Left and right: reproductive and nonreproductive (diapausing) *Lygus hesperus*. Note hypertrophied fat body of diapausing bug. (Actual size: 5.0 to 6.0 mm.)

migrated to surrounding fields, leaving behind all the nymphs, which soon become young adults.

As shown in figure 2, and summarized in table 2, diapause induction under natural conditions followed a similar pattern in September of 1962, 1963, and 1964, and is well correlated with hours of daylight. Since seasonal timing of diapause induction was markedly uniform over this three-year period, diapause apparently is induced by environmental factors which are themselves seasonally consistent. Photoperiod is the only environmental factor that remains constant annually and which could, in species having a facultative diapause, consistently evoke the diapause-inducing process in the sensi-

tive stadia at the same time, year after year.

Estimation of the critical photoperiod

Adult response. Figure 2 and table 2 indicate that the critical photoperiod occurred about September 15 of each year, when the photophase was about 12.5 hours.

This abrupt decrease in reproduction around September 15 raised the question of whether the reproductive bugs, prevalent before that date, would themselves respond to the apparent critical photoperiod. To study this, reproductive females were periodically field-collected from the latter part of August through September, 1964. On each

collection date, a sample of ten bugs was dissected to substantiate visual classification as reproductives. All other collected bugs were given an opportunity to feed and oviposit in beans placed in cartons (see p. 352). The cartons were

placed outside in an area protected from the direct rays of the sun, but were subjected to natural photoperiod and temperature.

Adults classified as reproductives when collected did not enter diapause but appeared to simply exhaust their egg-laying capacity and die. Since the photoperiod had no diapause-inducing effect after the bugs were reproductive, it was concluded that adults themselves did not enter diapause, but must have received the appropriate stimulus before the adult stage was attained, and that the apparent critical photoperiod of about 12.5 hours was not the true critical photoperiod.

Nymphal response. A minimum of 60 third- through fifth-instar nymphs was collected periodically during the month of September, 1964. In addition, eggs were obtained from field-collected adults between August 22 and 24 by allowing field-collected adults to oviposit in green bean pods. As before, the nymphs and eggs were held outdoors in cartons.

Approximately 90 per cent of the third- to fifth-instar nymphs field-

TABLE 2
CORRELATION OF DIAPAUSE INCIDENCE AMONG FEMALES OF FIELD-COLLECTED *LYGUS HESPERUS* AND ASTRONOMICAL DAYLENGTH (Davis, California, 1962 to 1964)

Dates	Mean astronomical daylength*		Diapause incidence†
	hrs.	min.	
August:			
20-25.....	13	24	0.0
26-31.....	13	10	0.0
September:			
1-5.....	12	58	0.0
6-10.....	12	45	0.0
11-15.....	12	33	0.0
16-20.....	12	20	28.2
21-25.....	12	08	45.9
26-30.....	11	56	71.5
October:			
1-5.....	11	40	98.1
6-10.....	11	29	99.3

* Source for astronomical daylength: Department of Agricultural Engineering, University of California, Davis.
† Average of 1962, 1963, 1964.

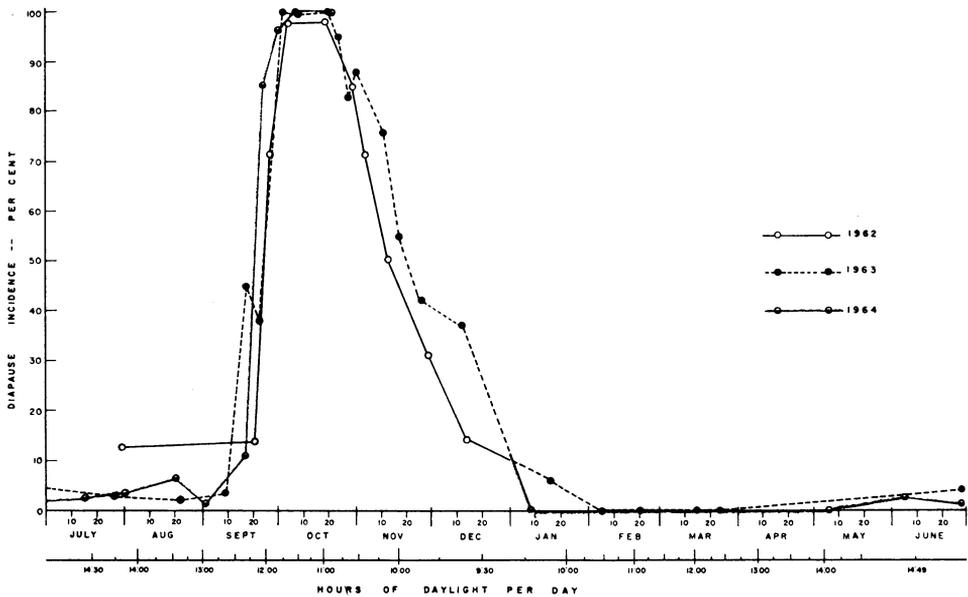


Fig. 2. Correlation of daylength and diapause incidence in *Lygus hesperus* field-collected at Davis, California 1962-1964.

collected on September 1 and 11 entered diapause. All third- to fifth-instar nymphs collected during the latter part of September entered diapause. Visual observations of the eggs deposited August 22 to 24, 1964, and reared under natural conditions showed that the majority of nymphs emerged from the eggs about September 4 and that most of the emerged nymphs became adults about October 3, after a total nymphal period of about 29 days. Dissections showed that all of these bugs entered diapause.

The results suggested that the nymphal stage was the one sensitive to the diapause-inducing stimulus, and that the critical photoperiod occurred before September 1 each year.

Nymphal development, of course, varies with temperature, but in general, from mid-August to mid-September, it requires about 30 days. Nondiapausing adults, collected September 9 to 11 were therefore produced from eggs hatched about August 10, which has an astronomical day length of 13 hours and 50 minutes (see fig. 2). Diapause was first apparent among adults collected September 16 to 21 (table 2); these adults were young nymphs about August 17, when the astronomical day length is 13 hours and 35 minutes. From these observations, it was concluded that the photophase of the critical photoperiod for diapause induction was about 13.5 hours.

Estimation of the critical photoperiod undoubtedly could be improved upon by more accurate measurements of nymphal growth rate under natural conditions. However, it is probably not possible to determine the exact day length responsible for diapause induction under field conditions because of the unknown influences of fluctuating temperature, and the light before sunrise and after sunset.

The precision of the time-measuring

effect of the photoperiodic induction process is indicated by the steepness of the photoperiodic response curve. It could be expected that the natural induction curve as shown in figure 2 for *Lygus hesperus* would be even steeper except for the activity of mature reproducing bugs which prevails through September.

Under natural conditions, diapause terminates in *Lygus hesperus* in a relatively short time. Diapause is broken in some bugs after about three weeks, and in approximately 50 per cent of the population by the middle of November. Apparently the bugs mate after emerging from diapause and die after depositing eggs, since adults become scarce during March and April.

Under mild winter temperature conditions, a few nymphs may be found in the field through November. (In Davis, nymphs generally disappeared completely by early December and were not collected again until the following April, when first instars became evident.)

Lees' (1955) morphological criteria for diapause include not only failure of reproductive organs to enlarge, but also hypertrophy of the fat body. Studies by Bacon *et al.* (1964) included a fat analysis of *Lygus hesperus* field-collected over a period of May to October. Their seasonal data on fat content show rather consistently low values from May to mid-September, and a sharp rise in fat content from mid-September to early October. This increasing fat content is associated with the color change from greenish to yellowish, and is well correlated with the induction of diapause.

The hypertrophied fat body, steep induction curve, and failure of the reproductive organs to initially mature in the overwintering generation of female *Lygus hesperus* indicate that a facultative adult diapause under photoperiodic control does exist in this species.

LABORATORY STUDIES OF PHOTOPERIODIC RESPONSE

General methods

A laboratory population of *Lygus hesperus* was established and maintained on green bean pods according to the general methods of Beards and Leigh (1960). Bugs were reared in temperature-controlled ($\pm 1^\circ\text{C}$) chambers lighted by one to three 15-watt fluorescent lamps wired to an automatic timer. Thus, it was possible to set any desired photoperiod in a 24-hour cycle. Relative humidity could not be controlled, and varied from about 40 per cent to 85 per cent. All bugs were reared in one-gallon ice cream cartons (fig. 3). A $\frac{1}{8}$ -inch mesh wire screen, fitted about 1 inch above the bottom of the carton, provided adequate escape room for young nymphs, many of which might otherwise have been crushed inadvertently when the bean supply was changed. The stock culture was maintained at a 16-hour photophase at 27°C .

Photoperiodic response curve

Photoperiodic response curves were obtained by exposing experimental populations to different photoperiods throughout the sensitive stage. Different proportions of the population go into diapause depending on the length of the applied photoperiod. This proportion is expressed as the diapause incidence; when it is plotted against the photoperiods under which the insects were reared, the response curve obtained shows a characteristic critical photoperiod. In species with a facultative diapause, each individual possesses the faculty of entering or not entering diapause, depending upon whether the stimulus falls above or below its response threshold. In obtaining photoperiodic response curves, populations rather than individuals are considered.

Methods. In order to avoid ambiguous results due to varying tempera-

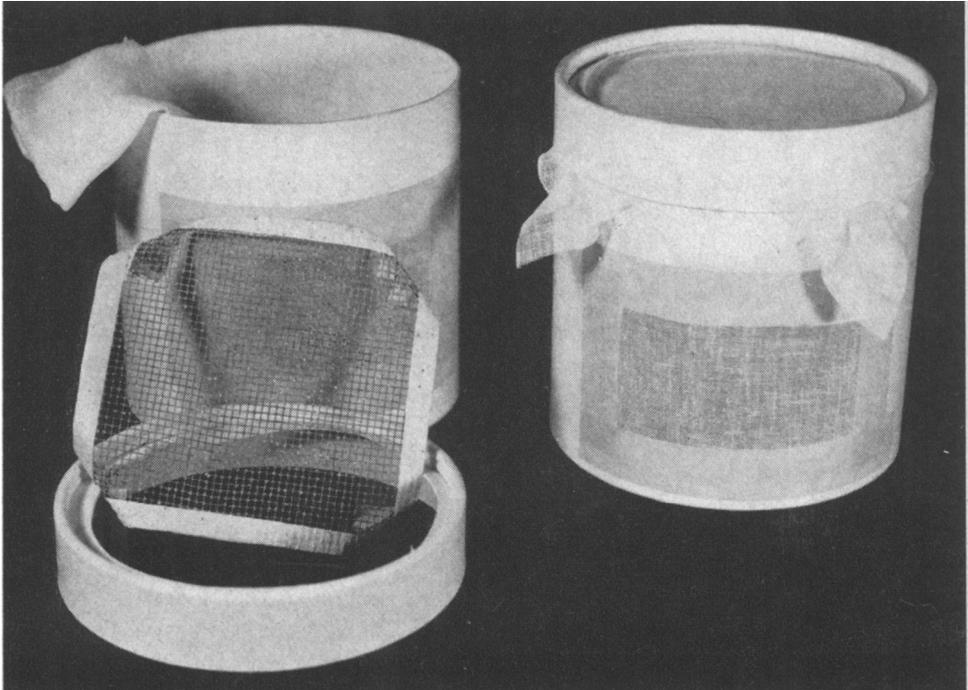


Fig. 3. Cartons used to rear *Lygus hesperus* in the laboratory.

tures, and to obtain a response curve which could be applied most appropriately to field observations, a temperature of 27°C was selected for this experiment. This temperature is well within the normal ecological temperature limits encountered by *Lygus hesperus* in the Davis area.

The photoperiodic response was obtained by rearing the bugs from eggs no more than 48 hours old to adults 44 days old, under continuous exposure to the desired photoperiod. Preliminary experiments indicated that bean pods which were placed in the available stock culture for approximately 48 hours had enough eggs deposited in them to insure that at least 50 females would be available for dissection after 44 days. This 44-day rearing time under a constant photoperiod was selected on the basis of preliminary experiments: earlier dissections of samples of 30 females reared at a 16-hour photophase for 34, 44, and 54 days at 27°C showed that after 34 days, the ovaries of many bugs were only intermediate in egg formation; after 44 days, the ovaries of all the bugs contained some eggs having a discernible operculum; but after 54 days, the increasing mortality rate of the culture counteracted the probable increasing validity of the photoperiodic response. An experimental control with a 16-hour photophase at 27°C was conducted simultaneously with each group of experiments.

After 44 days' exposure to the desired photoperiod, females were dissected and classified as reproductive or nonreproductive. Intermediate forms were not found after this period of time and nonreproductive bugs were considered to be in diapause. The difference in coloration between green reproductive bugs and yellowish diapause bugs, which was so marked in field-collected specimens, was also evident under artificial conditions, although to a lesser degree.

Results. The effects of various photoperiods on the incidence of diapause at

a rearing temperature of 27°C are shown in table 3. These data are shown graphically in figure 4. The shape of the curve depicted is not unique for *Lygus hesperus*, but is typical for long-day insects—those which enter diapause in response to short photophases.

Under the conditions tested, the effective range of photophases was between 6 and 13 hours, with maximum diapause incidences occurring with photophases of 7, 7.5, 8, 10, and 11 hours. Intermediate diapause incidences were obtained with 6-, 12-, and 13-hour photophases. No diapause occurred under continuous light or continuous darkness.

The sensitivity of the time-measuring ability of *Lygus hesperus* was demonstrated by complete absence of diapause under a 13.5-hour photophase, while a 13-hour photophase evoked 20 per cent diapause. Under constant temperature and time conditions at least some of the population apparently were able to discern absolute time differences as short as 30 minutes. Because the experimental populations were reared under constant conditions of exposure to the desired photophase, and natural populations

TABLE 3
EFFECT OF PHOTOPERIOD ON
INCIDENCE OF DIAPAUSE IN
LYGUS HESPERUS AT 27° C

Photoperiod program		Number of insects tested	Diapause per cent
Photophase	Scotophase		
hours	hours		
0	24	71	0
5	19	63	0
6	18	71	34
7	17	97	100
7.5	16.5	55	100
8	16	78	98
10	16	100	100
11	13	62	100
12	12	96	84
13	11	88	20
13.5	10.5	93	0
16	8	100	0
20	4	76	0
24	0	93	0

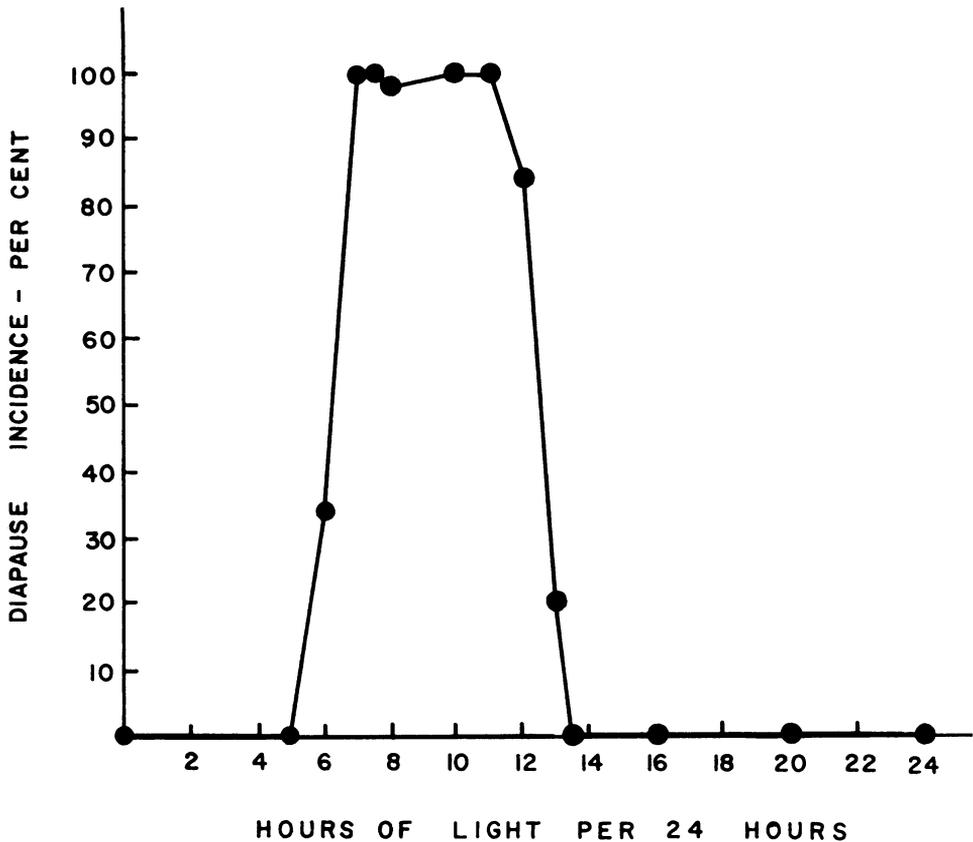


Fig. 4. Photoresponse curve, showing the relationship between photophase and diapause incidence among 44-day-old *Lygus hesperus* females laboratory reared at 27°C.

were subjected to daily decrements in daylength, the experimental and natural photoperiods could not be directly compared. However, there was a reasonably close correlation between the experimentally obtained critical photoperiod and the estimated critical photoperiod that was proposed for the natural population in the previous section. An experimental population continuously exposed to a 13-hour photophase had a diapause incidence of 20 per cent; an estimated photophase of 13.5 hours induced 28 per cent diapause in the natural population (see p. 351). The 12-hour photophase induced diapause in 84 per cent of the experimental population while an estimated photophase of 13 hours induced almost 100 per cent diapause in the natural population.

Although further experiments using a wider range of temperatures would be desirable, it is felt that the position of the critical photoperiod which was obtained at 27°C gave a good indication of what the critical photoperiod might be in the natural environment.

Temperature effect on diapause induction

Temperature affects the induction of diapause. In general, high temperatures tend to avert diapause in long-day insects which normally enter diapause in response to a short photophase. Way and Hopkins (1950) reported that diapause in *Diataraxia oleracea* Linnaeus was prevented if the larvae were reared in darkness at temperatures of 30° to 34°C, although diapause was induced

in darkness at 24°C. Dickson (1949) found that while diapause in *Grapholitha molesta* (Busek) was induced at 24°C, it was prevented at either 30°C or 12°C. Conversely, high temperatures may promote diapause in some species (such as *Bombyx mori*) which enter diapause in response to a long photophase.

Since the geographical range of *Lygus hesperus* extends throughout areas where either high or low temperatures may prevail through August and September when diapause is induced, an experiment was conducted to determine whether temperature had any influence on the induction of diapause in this species.

Methods. *Lygus* bug eggs were obtained from the stock culture over a period of 48 hours and subjected continuously to two photoperiodic regimes at three different temperature conditions. Photophases of 16 and 10 hours were selected, for the photoresponse curve demonstrated that these photophases elicited minimal and maximal diapause-induction at 27°C. Diapause response at 27°C was compared with that obtained at 21°C and 32°C. Rearings attempted at 38°C were unsuccessful due to heavy mortality which occurred at about the fourth instar. The beans in the 16-hour photophase rearing cartons were examined periodically for eggs. The female bugs in related long and short photophase treatments were dissected when eggs were being produced by bugs reared under the long photophase treatment.

Results. The effects of three temperatures on diapause induction are shown in table 4. Results at 27°C confirmed results previously obtained. Bugs reared at 21°C and a 10-hour photophase were considered to be in diapause even though the fat body was not appreciably developed. A small percentage of bugs reared at 21°C and a 16-hour photophase was nonreproductive, but considering that 73 days passed before eggs

TABLE 4
EFFECT OF TEMPERATURE ON
DIAPAUSE INDUCTION IN
LYGUS HESPERUS

Temperature	Photo- phase	Days exposed (egg to adult)	Bugs dissected	Nonrepro- ductive
°C	hours	no.	no.	per cent
21.....	10	73	81	100.0
	10	73	73	100.0
	16	73	66	3.3
	16	73	64	5.6
27.....	10	44	54	100.0
	10	44	87	100.0
	16	44	81	0.0
	16	44	93	0.0
32.....	10	30	80	90.0
	10	30	82	92.8
	16	30	66	0.0
	16	30	48	0.0

were observed in abundance, it is probable that this test represented a case of delayed development rather than one of diapause. Bugs reared at 32°C matured rapidly and contained many eggs 30 days after hatching. All bugs reared at 32°C and a 16-hour photophase were reproductive, whereas 91 per cent of those reared at 32°C and a 10-hour photophase were in diapause.

The results indicate that under constant conditions, the diapause incidence caused by a 16-hour photophase is similar at 21°C, 27°C, and 32°C. The response to a 10-hour photophase is similar at 21°C and 27°C, but the diapause-inducing effect of a 10-hour photophase may be partly cancelled at 32°C, for only 91 per cent of the populations entered diapause.

Nullification of diapause-inducing photoperiods

The results of experiments by many workers have demonstrated that insects' sensitivity to photoperiod never extends to the whole life cycle. Furthermore, evidence shows that the effects of a diapause-inducing photoperiod can be nullified by subsequently exposing the sensitive stages of development to a

photoperiod which normally does not induce diapause. In general, photoperiods which promote diapause are easier to nullify than those which are nondiapause-inducing (de Wilde, 1962).

The available evidence presented thus far indicates that diapause in *Lygus hesperus* is a function of photoperiod and that, under natural conditions, the photoperiod apparently exerts its diapause-inducing influence during the sensitive nymphal stage. It seemed desirable to augment these observations with experiments designed to nullify the effects of the photoperiods on the sensitive nymphal stages. Experiments were conducted to determine the degree of nullification that could be obtained with bugs reared under laboratory conditions and under field conditions.

Nullification experiments on laboratory-reared bugs

Two groups of bugs were reared at 27°C from the egg stage through the fifth instar at either a 16-hour photophase (nondiapause-inducing) or a 10-hour photophase (diapause-inducing). Examination of the photoresponse curve (fig. 4) shows that these photophases elicit an all-or-none effect on diapause induction. After the bugs had reached the fifth instar, 60 of them were placed individually in vials provided with a piece of green bean for food. The bugs were observed daily, and within 24 hours after becoming adults, 15 were switched from the 16-hour photophase to the 10-hour photophase. Conversely, 15 young adults were switched from the 10-hour to the 16-hour photophase. A control group of 15 bugs remained continuously at the original rearing photophases of 10 and 16 hours. The transferred bugs were held at the new photoperiods until each was 18 days old; they were then dissected and evaluated for diapause.

Results. The results (table 5) indicated that 20 per cent of the young adults transferred from a 16-hour to

TABLE 5
EFFECT OF PHOTOPERIOD CHANGE
ON DIAPAUSE IN
LYGUS HESPERUS ADULTS*

Adults transferred from:		Bugs dissected	Diapause
1st photophase†	2nd photophase‡		
hours	hours	no.	per cent
16.....	10	15	20
16 (control)...	16	15	0
10.....	16	15	0
10 (control)...	10	15	100

* Adults were less than 24 hours old when transferred to second photoperiod.

† Bugs were raised at first photophase to adult stage.

‡ Bugs were held at second photoperiod for 18 days.

a 10-hour photophase had entered diapause. However, in contrast to this, all adults transferred to a 16-hour photophase, after being reared as nymphs on a 10-hour photophase, were nondiapausing. These results agree with the general observations made by many workers that diapause-inducing photoperiods are more easily nullified than photoperiods which promote uninterrupted development.

Nullification of photoperiodic effects among field collected bugs

Bell and Adkisson (1964) reported that larval diapause in the pink bollworm, *Pectinophora gossypiella* (Saunders), which was induced by exposure of the early larval instars to long nights, could be cancelled for the most part by subsequent exposure of the late instars to short nights, but that an 8-hour scotophase was much less effective than a 10-hour scotophase in reversing induction caused by a 12-hour scotophase. Furthermore, the intensity of diapause, in part, appeared to be dependent on the previous photoperiodic experiences of the test insects.

A series of experiments was conducted to determine what influence field-environmental conditioning experienced by nymphal *Lygus hesperus* had on subsequent diapause-inducing photoperiods.

August 17 (daylength 13.5 hours) represents the demarcation point between nondiapause and diapause-inducing photoperiods for that proportion of the population having a low response threshold. September 2 (daylength: 13 hours) represents the critical date after which all the nymphs enter diapause. Therefore, young nymphs in the field developing after August 17 should have been subjected to an increasingly potent diapause-inducing stimulus—one which should be increasingly more difficult to nullify by subjecting the nymphs to nondiapause-inducing photoperiods. In treating field-collected nymphs, it was felt that rather than selecting clearly antagonistic photoperiods (10- and 16-hour photophases), the use of somewhat borderline photophases with later-instar field-collected bugs might give a more discriminating indication of the degree of induction sustained by the early instars in the field. The photoperiodic response curve (fig. 4) shows that 13.5- and 12-hour photophases are somewhat borderline in eliciting a differential response between diapause and nondiapause bugs.

Also, some measure of the intensity of the diapause stimulus experienced by the early instars in the field should

be provided by subsequent exposure of the older instars to the 13.5 hour photophase, which favored nondiapause development, and the 12-hour photophase, which favored diapause induction.

Methods. Approximately 100 third- to fifth-instar nymphs, periodically collected from the field between July 17 and October 22, 1964, were placed in rearing cartons and exposed to either a 12-hour or a 13.5-hour photophase at 27°C. The females which matured from these nymphs were dissected 20 days after becoming adults and evaluated for diapause.

Results. The influence of previous natural environmental conditioning experienced by early nymphs on the incidence of diapause induction or nullification as determined by subsequent exposure of the field-collected nymphs to a 12- or 13.5-hour photophase treatment is shown in table 6.

Third- to fifth-instar nymphs collected on July 17 (daylength: 14 hours, 34 minutes—nondiapause inducing) continued nondiapause development irrespective of subsequent exposure to either a 12- or 13.5-hour photophase. However, among nymphs collected on August 21 (daylength: 13.5 hours) the 12-hour photophase induced diapause

TABLE 6
EFFECT OF DIAPAUSE-PROMOTING AND DIAPAUSE-INHIBITING PHOTOPHASES ON EARLY INSTARS OF FIELD-COLLECTED *LYGUS HESPERUS* NYMPHS*

Date collected	Instars collected	Daylength on collection date	Diapause induced by photoperiod of:			
			12-hour photophase (diapause-promoting)		13.5-hour photophase (diapause-inhibiting)	
			no. of bugs	Diapause	No. of bugs	Diapause
		<i>hrs. min.</i>		<i>per cent</i>		<i>per cent</i>
1964:						
July 17.....	3-5	14 : 34	49	0	38	0
Aug. 21.....	3-5	13 : 24	64	53	52	0
Sept. 11.....	3-5	12 : 33	70	91	60	73
Sept. 17.....	3-5	12 : 20	66	100	84	62
Sept. 23.....	3-5	12 : 08	53	100	44	32
Oct. 7.....	5†	11 : 27	59	100	45	51†
Oct. 22.....	3-5	10 : 56	65	89	72	22

* This experiment was designed to show the influence of natural environment conditioning on early-instar *L. hesperus* nymphs in terms of diapause induction or nullification under laboratory-controlled photophases.

† Only fifth instars collected on this date.

in about 50 per cent of the bugs, whereas no diapause resulted among nymphs exposed to the 13.5-hour photophase. This probably means that some inclination toward diapause had been induced in the early nymphal instars, and this inclination was augmented by exposing the later instars to the diapause-favoring 12-hour photophase. Data obtained from the nymphs collected on September 11 (daylength: 12.5 hours) indicated that although the subsequent 12-hour photophase treatment induced about 90 per cent diapause, a 13.5-hour photophase was not able to completely cancel the natural diapause-inducing stimulus these nymphs had received, for about 70 per cent diapause incidence resulted from the 13.5 hour photophase treatment. This indicated that the first- to third-instar nymphs had received a strong natural diapause-inducing stimulus after August 21 and prior to September 11. Supporting data from the collection of September 17 (daylength: 12 hours, 20 minutes) and September 23 (daylength: 12 hours, 8 minutes) indicated that whereas the 12-hour photophase retained a maximum diapause-inducing effect of 100 per cent, the incidence of diapause obtained by a 13.5-hour photophase markedly declined from about 60 per cent diapause for the September 17 collection to about 30 per cent for the September 23 collection. This decline suggests that the environmental conditioning of natural diapause-promoting photoperiods upon the early instars clearly influenced the degree of cancellation obtainable with a 13.5-hour photophase. The influence of the natural diapause-inducing photoperiod probably persisted into early October, as shown by the data from the collection of October 7. Although only

fifth-instar nymphs were collected on this date, 100 per cent diapause was still obtainable by the 12-hour photophase treatment. However, there was a decrease in cancellation with the 13.5-hour photophase since the diapause incidence increased from only about 30 per cent for the September 23 collection to about 50 per cent for the fifth instars collected on October 7. Since only fifth instars were collected, this increase in diapause incidence may have been caused by the relatively longer exposure of all the bugs in this collection to a natural diapause-inducing photoperiod. Data obtained from the October 22 (daylength: 11 hours, 5 minutes) collection showed an overall decrease in diapause incidence since only about 90 per cent of the bugs entered diapause in response to the 12-hour photophase. Furthermore, the 13.5-hour photophase treatment elicited only about 20 per cent diapause, which indicated a high degree of reversibility; thus, it seems probable that those nymphs which are found in the field during late October and November do not enter diapause.

Although it was not determined which nymphal instar was the most sensitive to the diapause-inducing stimulus, it was apparent that although first- to third-instar nymphs were receptive to diapause induction by August 21, subsequent treatment with a 13.5-hour photophase completely reversed the natural induction. Furthermore, the continued effect of partial nullification in the older instars by the 13.5-hour photophase suggested that the effect of the stimulus received by the later nymphal instars was more critical than that received by the earlier nymphal instars.

DIAPAUSE TERMINATION

In many species, low temperature is commonly acknowledged as being conducive to diapause termination, and temperature ranges favorable for diapause termination may or may not coincide with the temperature ranges favorable for morphogenesis (Lees, 1956). The optimum temperature range for diapause termination appears to be related to whether the species is from a warm or cold climate. Species from cold climates usually have a low optimum temperature for diapause termination whereas species from warmer climates usually have a correspondingly higher optimum range.

Studies on termination of photoperiodically-induced diapause have demonstrated that some species may retain their responsiveness to photoperiod while in a diapause state. McLeod and Beek (1963) found that larval diapause in the European corn borer, *Ostrina nubilis*, could be terminated by a long photophase regime and that no exposure to low temperature was necessary. De Wilde *et al.* (1959) reported that diapause in *Leptinotarsa decimlineata* Say was photoperiodically reversible shortly after the adults displayed diapause behavior, but three or four days later, the reversal was no longer possible.

Termination of naturally-induced diapause

During the course of these studies, it was observed that if diapausing bugs were brought from the field into the laboratory, diapause would eventually be terminated. An experiment was performed to elucidate the effect of photophases on diapause termination.

Methods. Diapausing bugs were collected from the field on October 8, 1964, and held in rearing cartons at 27°C under the following light conditions:

TABLE 7
EFFECT OF DIFFERENT PHOTO-
PERIODS ON TERMINATION OF
NATURALLY-INDUCED DIAPAUSE
IN *LYGUS HESPERUS**

Photophase	Bugs	Reproductive
<i>hours</i>	<i>no.</i>	<i>per cent</i>
24.....	63	65.1
16.....	79	65.8
13.5.....	90	45.6
12.....	94	3.2
7.....	99	1.0
0.....	81	1.2

* All bugs were collected on Oct. 8, 1964, and dissected after 15 days exposure to the photophases listed.

continuous light, 7-, 12-, 13.5-, 16-hour photophases; and continuous dark. Female bugs were dissected after 15 days exposure and evaluated for diapause.

Results. Results obtained with the different photoperiodic treatments are shown in table 7. The results indicate that the bugs were capable of terminating the naturally-induced diapause when exposed to a long photophase. Since bugs collected on October 8 and held at 27°C throughout the experiment were in early diapause (see table 1), a low temperature treatment is apparently not necessarily a crucial factor for diapause development.

Comparison of the data in tables 3 and 7 shows that photoperiods which are apparently equally effective in preventing diapause induction under experimental conditions may vary greatly in their effectiveness in terminating the natural diapause. Both continuous light and a 16-hour photophase completely prevent diapause and appear to be equally effective in terminating diapause. A 13.5-hour photophase, which appeared to prevent diapause induction as efficiently as either continuous light or a 16-hour photophase, had con-

siderably less effect upon the termination of diapause (table 7). Continuous darkness, which completely prevented diapause induction, had little influence on diapause termination. A 12-hour photophase, which partially induced diapause, appeared to be only slightly more effective in terminating diapause than a 7-hour photophase, which completely induced diapause.

Termination of experimentally-induced diapause

Several attempts were made to terminate diapause which had been induced by rearing bugs from eggs to adults at 27°C and 10-hour photophase for 40 days. None of these attempts was successful. When 40-day-old diapausing bugs were subjected to a 16-hour photophase, 27°C, they simply suffered a pro-

gressive mortality until all were dead after 23 days. Interposing various cold treatments after diapause induction and before the bugs were exposed to the 16-hour photophase also had no effect in diapause termination.

For successful termination of an experimentally-induced diapause, the quantity and quality of the inductive stimulus before diapause termination may be of critical importance. It is conceivable that the diapause intensity induced by a 10-hour photophase over a period of 40 days might be of considerably greater magnitude than that experienced under natural conditions. Since termination of naturally-induced diapause can proceed at 27°C, it is evident that the diapause induced in laboratory-reared bugs was not similar in intensity to that of field-collected bugs.

ECOLOGICAL CONSIDERATIONS

The termination of insect diapause is usually synchronized with the appearance of a favorable season or otherwise suitable developmental conditions. At Davis, however, diapause in *Lygus hesperus* is terminated in 50 per cent of the natural populations by mid-November, and by mid-December, less than 20 per cent of the population is still in diapause (fig. 2). These times precede favorable developmental conditions by 2 to 3 months. After diapause termination, the adults can be found in the field until mid-February, whereupon the vast majority die soon after depositing their eggs. These eggs require 4 to 6 weeks to hatch, for nymphs are not collected until shortly before April 1. Thus, in the Davis area, *L. hesperus* passes early winter (November) as diapausing adults, midwinter (December-February) as ovipositing adults, and late winter (February-March) as eggs.

In many long-day species, high temperatures applied during the critical photoperiod have the ability to nullify the diapause-inducing short photophase

(de Wilde, 1962). We were unable to demonstrate this effect clearly in *Lygus hesperus* (table 4). It is conceivable, however, that although high temperatures cannot completely overcome the effects of short photophase, such temperature could significantly influence the intensity of diapause. Observations by Stitt (1940) at Yuma, Arizona, however, tend to refute this hypothesis. Although it is not known whether populations of *L. hesperus* enter diapause at Yuma, Stitt observed that 50 per cent of bugs he collected from mid-November to early December were reproductive; no nonreproductive bugs were collected after January 25. These data correlate well with data from Davis (fig. 2). If higher temperatures significantly influenced diapause by decreasing its intensity, one would expect a population of *L. hesperus* to become reproductive sooner at Yuma, Arizona, for the temperatures at Yuma are 6° to 10°C warmer during the fall than are Davis' fall temperatures.

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