AN ABSTRACT OF THE THESIS OF

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Abstract approved [redacted] (Major Professor)

Experiments conducted in the greenhouse showed that the leafhopper, Colladonus montanus reductus (Van Duzee), is a long-day insect. This conclusion is based on the production of diapausing eggs when the leafhoppers were kept under short days (ten hours) during the nymphal stage and the adult pre-oviposition period. Continuous development of generations occurred when insects were kept under long days (16 hours) during the nymphal stages and the adult pre-oviposition period. The effect of short days during the nymphal stages could be reversed if the nymphs were transferred to long days as they became adults; few diapausing eggs were produced under these conditions. The effect of long days during the nymphal stages was only slightly altered if the nymphs were transferred to short days as they became adults; very few, if any, diapausing eggs were produced. Embryos in diapause appeared to be in the anatrepsis stage of development; segmentation was taking place insofar as buds

of future legs and mouthparts could be seen.

Females deposited the majority of their eggs in the leaves of Trifolium subterraneum L., regardless of the combinations of photoperiods that they had experienced during their life cycles. Very few, if any, eggs were laid in the basal portions of the plants by adults that spent all of their life cycle under the 16-hour photoperiod. Females that spent part or all of their life cycle under the ten-hour photoperiod laid significantly greater percentages of their eggs in the basal portions of the plants than females that spent all of their life cycle under the 16-hour photoperiod. I suggested that diapausing eggs laid in the basal portions of the plants would be better protected from injury and dessication during the winter.

In general, adult leafhoppers that were reared as nymphs under eight-hour or ten-hour photoperiods were somewhat lighter in color than adults reared under a 16-hour photoperiod. Males were usually black and females were usually brown, although the color variation was such that the darkest females were about as dark as the color of the lightest males. The sexual dichromatism was much more striking than the seasonal dichromatism.

Adults reared as nymphs under ten-hour and 16-hour photoperiods did not vary significantly in length when each sex was compared separately in one of the experiments. In another experiment, females reared under an eight-hour photoperiod were significantly

shorter than females reared under a 16-hour photoperiod. Similarly, males reared under the eight-hour photoperiods were significantly shorter. I suggested that the significant difference in the latter experiment was due to the poor vigor of the plants that the nymphs fed on under the eight-hour photoperiod, rather than to a direct influence of the photoperiod on the leafhoppers.

INDUCTION OF DIAPAUSE IN *COLLADONUS MONTANUS REDUCTUS* (VAN DUZEE)

by

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INDUCTION OF DIAPAUSE IN COLLADONUS MONTANUS REDUCTUS (VAN DUZEE)

INTRODUCTION

Colladonus montanus reductus (Van Duzee) has been used in cherry virus transmission studies at Oregon State University. Prior to 1962, transmission studies were limited to approximately two months during the summer when sufficient numbers of leafhoppers were produced by greenhouse colonies. No leafhoppers were produced during the winter months; therefore, each spring new colonies had to be established with leafhoppers collected in the field. It was necessary to find some method of rearing C. m. reductus continuously in the greenhouse so that transmission studies could be conducted throughout the year.

Decreasing day lengths in the fall have been associated with the induction of a type of overwintering condition, or diapause, in other species of insects (Lees, 1955, p. 18-19; de Wilde, 1962; Beck, 1963). Diapause is a physiologically controlled arrest of development which is usually inititated, and may be terminated, by the environment (Andrewartha, 1952; Lees, 1955, p. 1-4; Beck, 1962). Environmental factors known to initiate diapause directly include photoperiod, temperature, and humidity; those known to promote diapause indirectly include temperature, "maternal physiology", and food

(Andrewartha, 1952; Lees, 1955). Of these environmental factors, photoperiod is by far the most common. Many species enter a state of diapause in the laboratory when subjected to 12 hours of light or less per 24-hour day, but continue to develop without interruption when reared under 16 hours of light or more (Andrewartha, 1952; Lees, 1955, p. 13-15; de Wilde, 1962).

During the summer of 1962, an experiment was conducted with C. m. reductus to determine the effect of rearing first instar nymphs to the adult stage under eight and 16 hours of light per 24-hour day. Ten pairs of leafhoppers which had been reared under eight hours of light laid a total of 312 eggs. Only 24 eggs, or 7.7 percent, hatched. Ten pairs of leafhoppers that had been reared under 16 hours of light per day laid a total of 325 eggs. All of these eggs hatched (Swenson, 1962).

The purpose of this paper is to corroborate the results of this experiment and to study further the effects of photoperiod on the induction of diapause in Colladonus montanus reductus.

REVIEW OF LITERATURE

Diapause Stages

Diapause occurs during the egg stage in some species of insects and occurs during the larval, pupal, or adult stage in other species. Diapause almost always occurs at only one characteristic stage in each insect species in which diapause is known to occur. Among species of Cicadellidae, diapause is known to occur in two different stages of the life cycle. In Colladonus clitellarius (Say) (George & Davidson, 1959), Scaphytopius acutus (Say) (Palmiter, Coxeter, & Adams, 1960), Euscelis plebejus Fall., and E. lineolatus Brullé (Müller, 1961), diapause may take place in the egg stage. Nephotettix bipunctatus cinctipes Uhler has a diapause during the fourth and fifth nymphal instars (Kisimoto, 1959). In one species of Delphacidae, Stenocranus minutus Fabr., diapause occurs in adult females that appear in late summer (Müller, 1957, 1958).

Environmental Factors Effecting Diapause

Photoperiod

Several studies have been made to determine the length of photoperiods which induce and suppress diapause. The majority of the insects studied have been shown to be long-day insects; those insects subjected to photoperiods of 16 hours or more per 24-hour day

continue to develop without diapause, while those which have been subjected to 12 hours of light or less per 24-hour day enter diapause. The relationship between the numbers of hours of light per 24-hour day and the incidence of diapause can be graphed to form a "response curve" (Lees, 1955, p. 15; de Wilde, 1962). Response curves of four long-day insect and mite species are presented in Figure 1.

Two features of the response curves of long-day insects have been noted by Lees (1955). First, the response curves show that the incidence of diapause drops abruptly between 12 and 16 hours of light per 24-hour day. The photoperiod at which this transition occurs has been termed the "critical photoperiod". Second, Lees pointed out that there is a decrease in the incidence of diapause at very short photoperiods, particularly those which are shorter than the shortest natural day length in the area in which the insects occur.

Temperature

Temperature modifies the effect of photoperiod in most insect species in which photoperiod is the primary initiator of diapause. The literature on this subject has been reviewed by Lees (1955) and de Wilde (1962).

In some insect species, both high and low temperatures eliminate the diapause-inducing effect of short photoperiods, while moderate temperatures do not change the diapause-inducing effect. The larvae

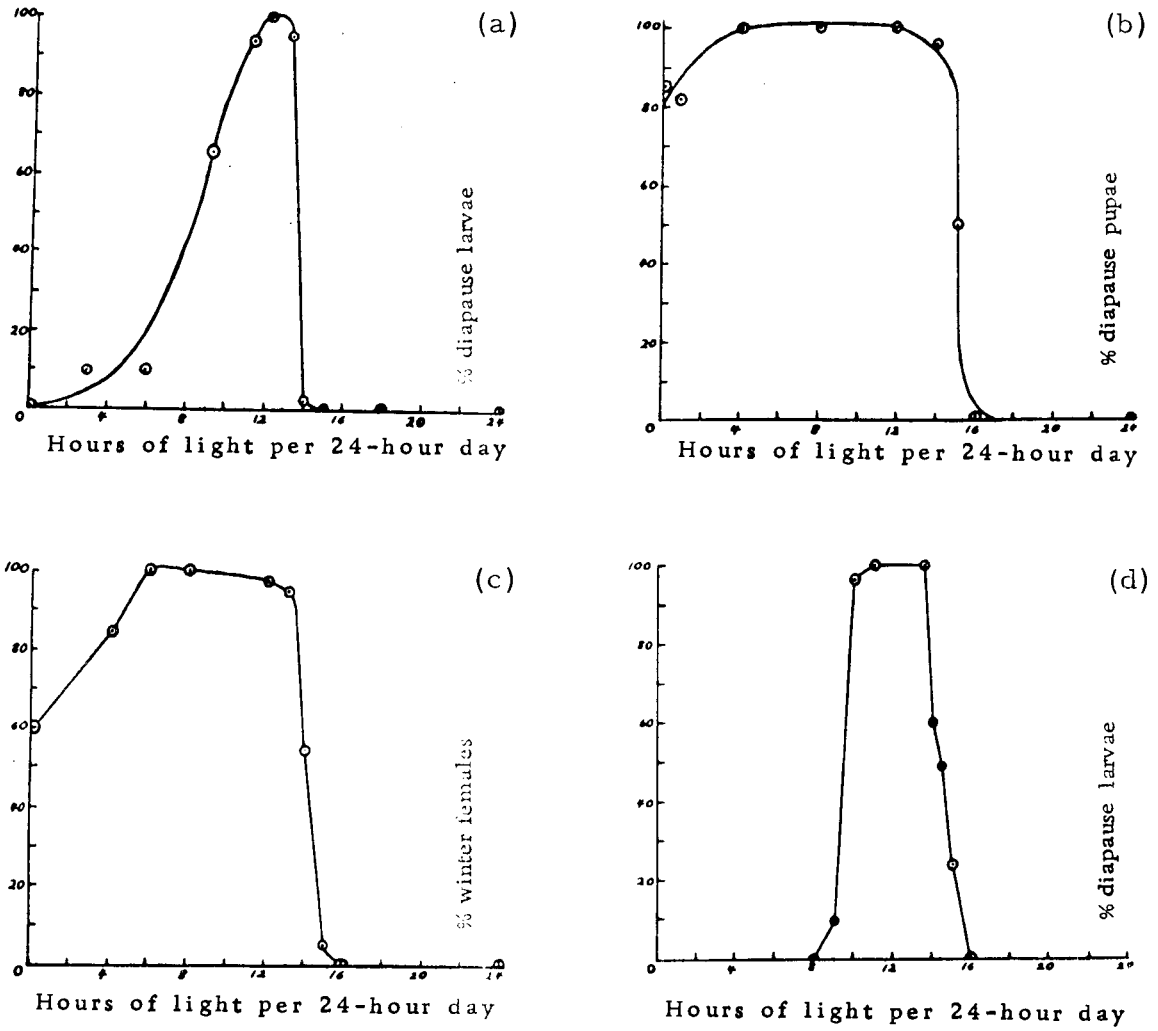

Figure 1. Response curves.

(a) Grapholitha molesta (24° C) (Dickson, 1949)

(b) Diataraxia oleracea (24° C) (Way & Hopkins, 1950)

(c) Metatetranychus ulmi (15° C) (Lees, 1953)

(d) Ostrinia nubilalis (30° C) (Beck, 1962)

of the oriental fruit moth, Grapholitha (=Laspeyresia) molesta (Busch.), entered diapause when reared under 12 hours of light per 24-hour day, at temperatures of 21, 24, and 26° C, but continued to develop without interruption when reared under the same photoperiod at 12 or 30° C (Dickson, 1949). Similarly, the highest percentage of diapausing pupae of the cotton moth, Chloridea obsoleta F., was obtained by rearing larvae at 23° C under ten hours of light per 24-hour day. Temperatures of 19, 25, and 30° C under ten hours of light resulted in smaller percentages of pupae in diapause (Komarova, 1959).

In most insect species only high temperatures eliminate the diapause-inducing effect of the short photoperiods; low and moderate temperatures have no effect on diapause. The tomato moth, Diataraxia oleracea L., exhibited a pupal diapause when the larvae were reared at 24° C under 12 hours of light per 24-hour day (Way & Hopkins, 1950). At temperatures of 30 and 34° C with the same photoperiod, diapause did not occur. However, low temperatures (12, 15, and 18° C), combined with photoperiods which would not otherwise induce diapause (16 hours of light per 24-hour day), resulted in diapause. A small percentage of mature larvae of the European corn borer, Ostrinia nubilalis (Hűbn.), entered diapause at a moderate rearing temperature (22° C) when the photoperiod was 16 hours per 24-hour day (Beck & Hanec, 1960). A greater percentage of larvae entered diapause when the rearing temperature was

lower (18° C) under the same photoperiod, but no larvae entered diapause when the rearing temperature was high (24 to 29° C).

De Wilde, Duintjer, and Mook (1959) concluded that photoperiod was the primary factor and that temperature played a minor role in effecting diapause in the adult Colorado potato beetle, Leptinotarsa decemlineata Say. Low temperature (17° C) had little effect in inducing diapause when the photoperiod was 20 hours per 24-hour day; under moderate temperatures this photoperiod did not induce diapause. High temperature (29° C) did not affect the percentage of adults entering diapause when the photoperiod was ten hours per 24-hour day; at 25° C, this photoperiod induced diapause.

Effect of Female's Age on Diapause Progeny

Although environmental factors, such as photoperiod and temperature, initiate the diapause response, the percentage of diapausing eggs, or insects, may increase with the age of the parent female (Andrewartha, 1952; Lees, 1955, p. 36). Matthée (1951) found that older females of Locustana pardalina (Walker) laid egg pods that contained higher percentages of diapausing eggs than did the egg pods laid by younger females. Similarly, Simmonds (1944, 1948) found that older females of Spalangia drosophilae Ashmead laid eggs that had a greater tendency to produce larvae which entered diapause. Bonnemaison (1961) and Prinsloo (1961) have also noted an increase

in diapausing progeny with an increase in the age of the parent female.

Saunders (1962) reported a statistically significant trend for older females of Nasonia vitripennis (Walker) to produce a greater percentage of diapausing larvae. Most females, however, produced eggs which developed into non-diapausing larvae both before and after the same females produced eggs which developed into diapausing larvae. Schneiderman and Horwitz (1958) also studied N. vitripennis but they did not find that the age of the female had any effect on the percentage of diapausing progeny. Saunders suggested that the difference between his results and those of Schneiderman and Horwitz may have been due to differences between the strains studied; Saunders worked with a strain from London, while Schneiderman and Horwitz studied a strain from Wood's Hole, Massachusetts.

In Lucilia sericata (Mg.) (Cragg & Cole, 1952) and Tritneptis klugii (Ratzeburg) (Schneiderman & Horwitz, 1958), the age of the female had no effect on the percentage of diapausing progeny.

Sensitive Period

The diapause response is determined by the environment during some stage or stages of development prior to the actual diapause stage. The stage when the insect is responsive to environmental stimuli has been called the "sensitive period" (Lees, 1955, p. 32-36;

de Wilde, 1962). The sensitive period, like the diapause stage (above), can occur at any period of the life cycle and only occurs during one characteristic period of the life cycle of each species.

The variation in the length of time between the sensitive period and the diapause stage of the life cycle can be seen in the following examples in which diapause occurs during the egg stage. This same variation occurs in insects and mites in which diapause takes place during stages of their life cycle other than the egg stage (de Wilde, 1962).

Kogure (1933) found that either diapausing or non-diapausing eggs were laid by Bombyx mori L. females, depending on the light and temperature that the females experienced during their embryonic development (cited by Andrewartha, 1952). In addition, it was possible to modify the proportions of diapausing and non-diapausing eggs by varying the light and temperature conditions during the larval stage, especially the first and second instars, of the parent females. Diapause in the egg stage of Orgyia antiqua (L.) was initiated by short photoperiods (six hours of light per 24-hour day) during the larval stage of the parent generation (Doskočil, 1957; de Wilde, 1962).

In the fruit tree red spider mite, Metatetranychus ulmi Koch, the deutonymph (the stage preceding the adult stage) was the sensitive stage, during which the length of the photoperiod, either eight- or 16-hours per 24-hour day, determined whether the eggs would be

diapausing or non-diapausing (Lees, 1953). Some mites remained sensitive until they had become adults and laid one to three eggs. During the sensitive period the females could be "switched" to laying the other type of egg (from laying diapausing eggs to non-diapausing eggs, or vice versa) by changing the photoperiod (from eight- to 16-hours, or vice versa).

Changing the environmental conditions can switch the response from diapause to non-diapause, or vice versa, during the sensitive period in other species of insects. De Wilde (1962) pointed out that it is much easier to reduce or eliminate the effect of a diapause-inducing photoperiod with a non-diapause-inducing photoperiod than to obtain the reverse change during the sensitive period.

MATERIALS AND METHODS

Definitions

Although Beck (1962) reported that the length of the dark period was more critical than the length of the photoperiod in the induction of diapause, the experiments in this paper will be described in terms of photoperiod, which is the number of hours of light per 24-hour day. Photoperiod refers to the time during which fluorescent lamps were used, either alone or supplementing daylight, and were controlled by a time clock or by covering and uncovering cages. Since all the references to photoperiod in this paper are given in the number of hours of light per 24-hour day, the length of the dark period can be determined by subtracting the number of hours in the photoperiod from 24.

Materials

Source of the Leafhoppers

Stock colonies of Colladonus montanus reductus were established with adults collected from clover at Metolius, Oregon, on July 6, 1962. The colonies were maintained in the greenhouse on Malva sylvestris L. and alsike clover, Trifolium hybridum L. The colonies were kept under photoperiods of 16 hours by supplementing daylight with Sylvania Gro-Lux fluorescent lamps from 7:00 AM to

11:00 PM. The leafhoppers in these colonies have increased in numbers since they were established in the greenhouse. Additional adults were collected from alfalfa near Madras, Oregon, on September 5, 1963. The colonies that were established from these adults were added to the other colonies after several months.

Rearing Cages

Screen cages described by Adlerz (1958) were placed over T. hybridum or M. sylvestris plants growing in six-inch clay pots and were used for the stock colonies in the greenhouse. For the experiments, the leafhoppers were reared either directly in the chambers (described above) or on plants in four-inch clay pots that were covered with lamp chimneys (Figure 2). The tops of the lamp chimneys were covered with several layers of cheesecloth which were fastened with a rubber band.

Chambers

Experiments One and Three were carried out in a series of eight chambers. Each chamber had a glass top, a removable front panel with cloth sleeves for access to the chamber, and a screened area on the rear panel which adjoined a central duct equipped with an exhaust fan for ventilation (Figure 3). Photoperiods were controlled by covering the glass top with a fiberboard panel and by

Figure 2. Lamp chimney cage.

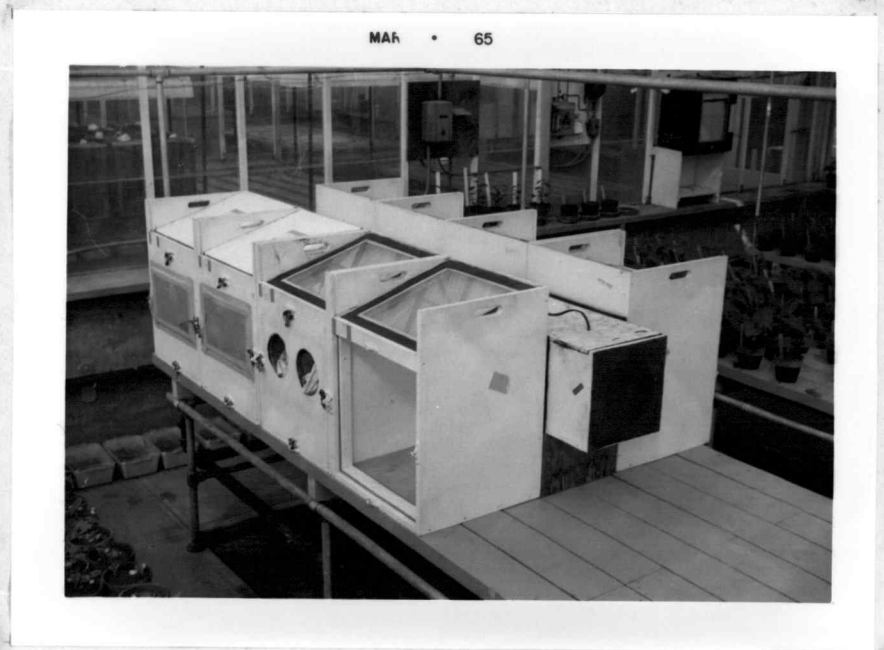

Figure 3. Series of eight chambers used for some experiments. The overhead fluorescent lamps have been removed.

taping a cardboard cover over the sleeves on the front panel. There were light baffles in the central duct. Sylvania Gro-Lux fluorescent lamps were hung one foot above the glass tops of the chambers. The temperature in the greenhouse where the chambers were located was controlled by a thermostatically regulated air conditioning unit and steam heating system; the temperature in the chambers fluctuated with the temperature in the greenhouse. On sunny days, the temperature in the greenhouse and in the chambers rose several degrees above the temperature that could be regulated by the air conditioning unit.

Another rearing chamber, 18" wide, 24" long, and 18" high was constructed of plywood for the second experiment. The top of the chamber was covered with Warp's Flex-O-Pane plastic. One side panel was removable to provide access to the chamber. The chamber was also placed in the greenhouse. Light was provided by Sylvania Gro-Lux fluorescent lamps which hung above the chamber. Black shading cloths were suspended over the lights and away from the sides of the chamber to reduce the high mid-day temperatures. Ducts were constructed which carried cold air from the air conditioning unit in the greenhouse into the bottom of the chamber.

Experiments Four and Five were carried out in two controlled-environment chambers, Model PGC-78, manufactured by Percival Refrigeration and Manufacturing Company. Each chamber was

equipped with Sylvania Gro-Lux fluorescent lamps and 50-watt incandescent lights. Light and dark periods were controlled by 24-hour cycle time clocks. The temperature in the chambers could be controlled by thermostats within 2^{o} F of the desired temperature.

Aspirators

Two types of aspirators were used to transfer leafhoppers from one cage to another. The aspirator used to transfer large nymphs and adults consisted of a 25 ml pipette which had the tip of the delivery stem cut off and a three-foot length of rubber tubing attached at one end. A piece of fine mesh plastic screen was inserted between the rubber tubing and the pipette so that the leafhoppers remained in the pipette. Smaller nymphs were transferred with an aspirator which consisted of a piece of four mm glass tubing several inches long, attached to a length of rubber tubing; a screen mesh was inserted between the glass and rubber tubing.

Methods

Experimental Procedure

Each experiment was started by transferring first- and second-instar nymphs from the stock colonies to the chambers or cages being used in the experiment. The transfers were made over a one-

to two-week period, because there were relatively few nymphs available in the stock colonies at any one time. Every time a transfer was made, the nymphs being transferred were divided equally among the different photoperiods under which the other nymphs were being reared.

The first teneral adults appeared approximately three weeks after the first nymphs were introduced to the experiment. The majority of the adults appeared over a one and one-half to two-week period following the appearance of the first adults. As the adults appeared, they were transferred daily, in pairs, to plants in chimney cages to mate and lay eggs.

The eggs were counted by examining the plants with a 10X hand lens. The majority of the eggs were inserted into the leaves, particularly the leaf margins. The females took from four days to three weeks to lay their eggs after they had been paired with the males. During this period of time the plants were examined every two or three days until from 25 to 40 eggs had been laid. If more than 40 eggs were laid, the additional eggs were removed. The adults were then killed and mounted. The eggs that hatched did so within two and one-half weeks after the adults were removed. The eggs that did not hatch were kept for an additional one to two weeks, after which they were assumed to be in diapause. Each experiment took from ten to 12 weeks to complete.

Examination of Diapause Eggs

In the fourth experiment, unhatched eggs were fixed and cleared so that they could be examined for embryos in diapause. The method for fixing and clearing eggs was taken from Hogan (1959). All of the eggs which appeared viable, i. e., fully inflated and opaque-white when examined under the microscope at 30X, were dissected from the plants and put in water for about 30 minutes. They were then put in a mixture of two parts glacial acetic acid, two parts chloroform, and one part absolute alcohol, at 34° C for 15 to 30 minutes. Then they were transferred to a mixture of one part glycerol and one part 70-percent alcohol, where they remained until the yolks became transparent and it became evident that the embryos, if present, were opaque-white. The majority of the eggs cleared within a few days. All were examined under a binocular microscope at 40X. Uncleared eggs were punctured and their contents removed to see if any embryos were present.

Many eggs apparently did not contain embryos, either in diapause or in any other stage of development. It is possible that embryos were present but not visible because they did not become fixed before the eggs were cleared. All eggs laid by each female were treated as a group during this procedure and were stored in 70-percent alcohol after examination.

Measurement of Adult Leafhoppers

In the third and fifth experiments, the length of the adult leafhoppers (from the anterior tip of the vertex to the posterior tip of the folded wings) was measured to determine if there was a difference between leafhoppers reared under short photoperiods and those reared under long photoperiods. The measurements were made on the mounted specimens after they had had several weeks to dry. A binocular microscope containing an ocular grid was used for the measurements. The measurements were made to the nearest 0.06 mm at 30X.

Statistical Methods

All of the eggs laid by the females from the same photoperiod were statistically treated as a single "group" of eggs in each experiment. The total number of eggs that were in diapause, that were viable, or that had hatched was tallied for each group of eggs. Each group of eggs was treated as a binomial sample since they could be counted as being in diapause or not in diapause, viable or not viable, and hatched or not hatched. The ratio of the number of eggs in a group in any condition (whether in diapause, viable or hatched) to the total number of eggs in the group was used as a sample mean. It was assumed that the population of sample means

followed a normal distribution. Two groups of eggs from different photoperiods were compared in regard to condition, using a "difference of means" test (Li, 1957, p. 407-410). To test the hypothesis that the samples means of two groups of eggs were equal, the statistic used was:

$$u = \frac{\bar{y}_1 - \bar{y}_2}{\sqrt{\bar{\bar{y}}(1-\bar{\bar{y}})(\frac{1}{n_1} + \frac{1}{n_2})}}$$

where " $\bar{\bar{y}}$ " equals the general mean of the two samples being compared. If "u" is greater than 1.96 or 2.57, the sample means are significantly different at the 5% or 1% level, respectively.

The lengths of adult leafhoppers were compared statistically by the Student's t-test. To test the hypothesis that the sample means of lengths of leafhoppers reared under short and long photoperiods were equal, the following statistic was used:

$$t = \bar{x}\sqrt{\frac{n(n-1)}{Sx^2}}$$

where " $\bar{x}$ " is the difference between the two sample means and "Sx^2" is the pooled sum of squares (Snedecor, 1950, p. 75-79).

DESCRIPTION OF EXPERIMENTS AND RESULTS

Experiment One

This experiment was designed to determine the photoperiods at which the majority of the eggs would change from the diapausing to the non-diapausing type. The series of eight chambers described under Materials was used in this experiment. Photoperiods of 9, 10, 11, 12, 13, 14, 15, and 16 hours were obtained by uncovering all the chambers at 7:00 PM and covering them at 4:00 PM, 5:00 PM, 6:00 PM, . . . , and 11:00 PM. The thermostat in the greenhouse was set at 20° C; the temperature in the chambers fluctuated with the greenhouse temperature, and was never lower than 19° C (at 7:00 AM) nor higher than 31° C (at 4:00 PM).

First- and second-instar nymphs were transferred from the stock colonies to M. sylvestris plants in the eight chambers. Adults were removed from the chambers as they appeared and put, in pairs, on young M. sylvestris plants in chimney cages under a 16-hour photoperiod in the greenhouse. In this manner, as many as 25 pairs of adults were obtained from each of the eight photoperiods. However, there were not enough adults to set up 25 pairs from some of the photoperiods.

The results of this experiment are summarized in Table I. The numbers of eggs that hatched probably would have been higher

TABLE I. Effect of the photoperiods under which adults were reared on the condition of the eggs which they produced (Experiment One).

	Photoperiod															
	9-Hour		10-Hour		11-Hour		12-Hour		13-Hour		14-Hour		15-Hour		16-Hour	
Replicates	T	H	T	H	T	H	T	H	T	H	T	H	T	H	T	H
1	28	11	41	0	34	14	41	19	25	11	17	0	41	27	38	30
2	37	19	36	16	28	11	25	17	31	3	40	3	41	4	28	24
3	35	7	31	15	41	31	39	39	32	26	35	25	28	15	42	42
4	27	19	25	18	25	8	34	12	31	10	34	15	40	1	39	39
5	17	17	44	44	28	4	33	15	33	25	--	--	44	37	39	12
6	29	7	35	14	33	33	24	0	--	--	37	11	38	29	30	19
7	40	26	28	17	--	--	29	0	44	44	40	24	31	17	36	36
8	31	31	29	11	26	0	26	15	27	2	26	0	39	0	43	28
9	13	4	41	11	40	13	48	22	27	8	29	6	34	9	29	12
10	40	22	33	15	38	24	44	32	33	13	42	42	34	22	43	20
11	27	0	40	40	28	28	--	--	24	9	25	19	44	16	26	26
12	31	31	--	--	33	27	32	32	36	31	24	23	33	33	18	6
13	32	20	--	--	38	0	38	26	34	11	26	16	25	16	40	14
14	--	--	--	--	34	0	27	10	25	2	35	19	46	40	28	11
15	--	--	27	0	40	25	35	9	53	53	27	5	31	27	41	26

(Continued on following page)

TABLE I. Continued.

Replicates	Photoperiod															
	9-Hour		10-Hour		11-Hour		12-Hour		13-Hour		14-Hour		15-Hour		16-Hour	
	T	H	T	H	T	H	T	H	T	H	T	H	T	H	T	H
16	20	10	22	0	--	--	34	9	25	24	10	0	32	32	57	57
17	30	4	40	27	--	--	26	17	19	6	38	10	29	22	30	22
18	--	--	31	11	35	20	30	16	31	9	27	19	27	13	25	18
19	--	--	29	29	15	1	29	26	29	13	31	16	34	7	25	25
20	--	--	--	--	25	0	--	--	25	10	32	8	26	0	39	22
21	--	--	38	4	37	25	34	0	31	22	30	3	27	14	29	29
22	--	--	28	4	25	2	39	0	--	--	33	2	24	24	30	29
23	--	--	28	7	--	--	38	25	26	13	--	--	38	25	17	1
24	--	--	25	2	25	24	--	--	36	36	--	--	27	14	--	--
25	--	--	29	0	28	0	16	7	23	8	36	0	32	20	25	6
Total	437	228	680	285	656	290	721	348	700	389	674	266	845	464	797	554
% hatched eggs:	52.2%		41.9%		44.2%		48.3%		55.6%		39.5%		54.9%		69.5%	

T = Total eggs laid
H = Number of hatched eggs

if many of the leaves containing eggs had not died and rotted before the eggs could hatch. The percentage of eggs that hatched in the eight photoperiod groups ranged from 39.5%, when the parent adults were reared as nymphs under a 14-hour photoperiod, to 69.5%, when the parent adults were reared as nymphs under a 16-hour photoperiod. All other photoperiods under which nymphs were reared had intermediate values. There was no obvious difference in the percentage of eggs that hatched when the parent adults were reared under short photoperiods (12 hours of light or less) when compared to the percentage of eggs that hatched when the parent adults were reared under long photoperiods (14 to 16 hours of light). The percentages of eggs that hatched when the parent adults were reared under short photoperiods ranged between 41.9% and 52.2% in this experiment; whereas in the experiment performed during the summer of 1962, only 7.7% of the eggs hatched when the parent adults were reared under and eight-hour photoperiod (see Introduction).

Experiment Two

This experiment was performed to determine why the results of the first experiment were not comparable to the results obtained in the experiment performed in 1962. An effort was made to control the high temperatures that occurred on sunny days in the greenhouse. Only eight- and 16-hour photoperiods were used.

The nymphs were reared in chimney cages in the chamber built for this experiment, as described under Materials. The temperature in the chimney cages fluctuated between 17 and 22° C with a few exceptions of only several hours duration. Eight-hour photoperiods were obtained by placing black cloth hoods over the chimney cages. The hoods were removed at 8:00 AM and put on at 5:00 PM. During the dark period which complemented the 16-hour photoperiod, the chamber was covered with a black shading cloth and the overhead fluorescent lamps were turned off. The 16-hour photoperiod began at 7:00 AM and ended at 11:00 PM.

Nymphs were reared in chimney cages containing T. hybridum plants. As the adults appeared in the rearing cages, they were transferred, in pairs, to young T. hybridum plants in chimney cages. The chimney cages containing the paired adults were placed on a bench in the greenhouse under Gro-Lux fluorescent lamps that were controlled by a time clock set for a 16-hour photoperiod. Eggs and nymphs were counted in the manner described under Methods. The results of this experiment are summarized in Table II. Only seven pairs of adults were set up in the 16-hour group because of the insufficient number of nymphs that were successfully reared to the adult stage. The percentage of eggs that hatched in the eight-hour group did not vary considerably from those that hatched in the 16-hour group. Some of the leaves containing eggs dried, which

TABLE II. Effect of the photoperiods under which adults were reared on the condition of the eggs which they produced (Experiment Two).

	Photoperiod			
	8-Hour		16-Hour	
Replicates	T	H	T	H
1	32	28	40	22
2	40	11	30	2
3	32	13	36	4
4	33	8	28	16
5	25	20	38	34
6	26	0	35	4
7	25	19	40	31
8	25	17	--	--
9	18	3	--	--
10	37	1	--	--
Total	293	120	247	113
% hatched eggs:	41.0%		45.7%	

T = Total eggs laid
H = Number of hatched eggs

probably decreased the number of eggs that hatched. When the parent adults were reared as nymphs under an eight-hour photoperiod, the percentage of eggs that hatched was 41.0%, in contrast to the experiment conducted in 1962 in which 7.7% of the eggs hatched when the parent adults were reared as nymphs under an eight-hour photoperiod.

Experiment Three

The purpose of this experiment was to find out if nymphs reared under short photoperiods (i.e., less than 12 hours) have to remain under short photoperiods after they become adults in order to insure that diapausing eggs would be laid. In Experiments One and Two, each day, as adults had appeared, they had been transferred in pairs to a 16-hour photoperiod to mate and lay eggs. Therefore, the adults that had been reared under a short photoperiod had remained there for a maximum period of 24 hours. In the experiment conducted during the summer of 1962, the paired adults were placed under natural daylight in the greenhouse beginning on August 16 (approximately 14 hours of light). Tables I and II show that the percentages of eggs that hatched that had been laid by adults from short photoperiods in the first two experiments were much greater than the percentage of eggs that hatched that had been laid by adults from the eight-hour photoperiod in the experiment conducted in the

summer of 1962.

The series of eight chambers used in Experiment One was also used in this experiment. Four of the chambers were used for photoperiods of eight hours and the other four chambers for 16-hour photoperiods; the former will be referred to as "eight-hour chambers" and the latter as 16-hour chambers". All of the chambers were uncovered at 7:00 AM; the eight-hour chambers were covered at 3:00 PM, and the 16-hour chambers were covered at 11:00 PM. The temperature in the chambers rose during sunny days above the temperature set on the thermostat; however, temperatures in the chambers were never lower than 18° C (at 7:00 PM) and never higher than 29° C (at 3:00 PM).

Nymphs were placed in three of the eight-hour and three of the 16-hour chambers. The nymphs fed on two large M. sylvestris plants (12 to 18" tall) in each chamber. The adults were removed daily, after they began to appear, and were put in pairs on Trifolium subterraneum L. plants covered with chimney cages. T. subterraneum plants were used because of their slow growth in the chimney cages and because leafhopper eggs were easily counted in the leaves and petioles. The adults in chimney cages were kept in the eight- and 16-hour chambers. As additional chamber space was needed, the nymphs were combined in the remaining chambers that were under the same photoperiod. This was possible because as more

adults appeared there were fewer nymphs, and therefore fewer chambers were needed for rearing nymphs.

As in every experiment, both sexes of each pair of adults in chimney cages had been reared as nymphs under the same photoperiod (either 8- or 16-hour). Pairs of adults from each photoperiod were alternately placed in other eight- and 16-hour chambers where they remained until the desired number of eggs was obtained. As a result, there were four combinations of the two photoperiods; nymphs that were reared in the eight-hour chambers and were left in the eight-hour chambers as adults (eight-eight-hour group), nymphs that were reared in the eight-hour chambers but were transferred to the 16-hour chambers as adults (8-16-hour group), nymphs that were reared in the 16-hour chambers and were left on the 16-hour chambers as adults (16-16-hour group), and nymphs that were reared in the 16-hour chambers but were transferred to the eight-hour chambers as adults (16-8-hour group). It was not possible to set up ten pairs of adults under each of the four combinations of photoperiods, as planned, because there were not enough adults to replace those that died before the necessary number of eggs were laid. The eggs and nymphs were counted in the same manner as in the other experiments.

The percentages of eggs that hatched under each of the four photoperiod combinations are given in Table III. The results were

inconclusive because of the lack of adults and, furthermore, approximately half of the plants in which eggs had been laid rotted or dried up, causing many of the eggs to be dessicated or spoiled. However, in Table III it can be seen that only 3. 1% of the eggs hatched in the eight-eight-hour group, which is comparable to the percentage of eggs that hatched in the 1962 experiment (7. 7%). The percentages of eggs that hatched in the 8-16-hour and 16- 8-hour groups (18. 4% and 9. 7%) were lower than the percentages of eggs that hatched in the 16-16-hour group (56. 7%).

During the process of counting the eggs, it was noted that some eggs were laid in the petioles of the T. subterraneum plants. The location of the eggs in the plants (whether laid in the leaves or petioles) is given in Table IV. Although the data are incomplete, the results show that the greatest percentage of eggs was laid in the petioles by females in the eight-eight-hour group (57. 2%). A larger percentage of eggs were laid in the petioles in the 8-16-hour and 16-8-hour groups (20. 2% and 25. 4%) than in the 16-16-hour group (5. 2%).

The lengths of the adult leafhoppers were measured to determine if there was a difference between females (and males) reared as nymphs under the 16-hour photoperiod, and females (and males) reared as nymphs under the eight-hour photoperiod (see Methods). The results are given in Table V. The mean lengths of both sexes

TABLE III. Effect of the photoperiods under which nymphs were reared and adults were kept on the condition of the eggs which they produced (Experiment Three).

	Photoperiod Combination							
	8-8-Hour*		8-16-Hour		16-8-Hour		16-16-Hour	
Replicates	T	H	T	H	T	H	T	H
1	27	0	35	0	14	0	37	37
2	30	0	34	2	42	7	36	23
3	29	0	25	11	26	6	15	12
4	27	0	37	26	--	-	27	27
5	--	-	32	12	24	0	29	0
6	--	-	25	0	28	0	25	17
7	13	4	36	0	--	-	25	0
8	10	0	27	0	--	-	28	17
9	25	1	--	-	--	-	27	7
10	--	-	26	0	--	-	28	17
Total	161	5	277	51	134	13	277	157
% hatched eggs:	3.1%		18.4%		9.7%		56.7%	

* = Eggs laid by adults that were reared as nymphs under an 8-hour photoperiod and remained under the 8-hour photoperiod during the adult and succeeding egg stages.

T = Total eggs laid
H = Number of hatched eggs

TABLE IV. Effect of the photoperiods under which nymphs were reared and adults were kept on the oviposition sites on Trifolium subterraneum L. (Experiment Three).

	Photoperiod Combination							
	8-8-Hour*		8-16-Hour		16-8-Hour		16-16-Hour	
Replicates	L	P	L	P	L	P	L	P
1	3	24	33	2	14	0	37	0
2	7	23	34	0	42	0	36	0
3	0	29	4	21	11	15	15	0
4	24	3	35	30	--	--	27	0
5	--	--	32	7	24	0	29	0
6	--	--	25	0	9	19	25	0
7	13	0	36	0	--	--	12	13
8	2	8	27	0	--	--	28	0
9	20	5	--	--	--	--	--	--
10	--	--	23	3	--	--	28	0
Total	69	92	249	63	100	34	237	13
% eggs in leaves:	42.8%		79.8%		74.6%		94.8%	
% eggs in petioles:	57.2%		20.2%		25.4%		5.2%	

* = Eggs laid by adults that were reared as nymphs under an 8-hour photoperiod and remained under the 8-hour photoperiod during the adult and succeeding egg stages.

L = Eggs laid in leaves.
P = Eggs laid in petioles.

TABLE V. Effect of the photoperiods under which nymphs were reared on the length of adults (Experiment Three).

Replicates	Males		Females	
	8-Hour	16-Hour	8-Hour	16-Hour
1	16.50	17.25	18.50	19.75
2	16.75	16.75	19.75	21.50
3	16.50	17.00	19.25	20.00
4	16.00	17.50	19.00	19.25
5	16.25	17.00	19.00	20.25
6	16.75	16.75	19.00	19.75
7	15.50	17.25	20.00	18.00
8	17.75	17.00	18.75	19.75
9	16.75	17.00	18.25	20.25
10	15.75	17.00	18.00	19.75
11	16.25	16.50	19.50	19.25
12	17.00	16.00	18.50	19.50
13	15.75	17.75	19.00	20.75
Total	213.50	220.75	246.50	257.75
Mean	16.42	16.98	18.96	19.83

1 unit = 0.26 mm

(taken separately) was significantly longer when the adults were reared as nymphs under the 16-hour photoperiod, than when the adults were reared as nymphs under an eight-hour photoperiod. The difference between the mean length of females reared under the 16-hour photoperiod and the eight-hour photoperiod was 0.23 mm, which was significant at the 0.5% level using the t-test (t = 3.12, 24 degrees of freedom) described under Methods. The difference between the mean length of males reared under the same photoperiods was 0.15 mm, which was significant at the 2% level (t = 2.73, 24 degrees of freedom).

Experiment Four

The purpose of this experiment, as of Experiment Three, was to determine if nymphs reared to adults under short photoperiods (12 hours or less) had to be left under short photoperiods as adults for more than one day in order to ensure oviposition of diapausing eggs. A ten-hour photoperiod was used, instead of the eight-hour photoperiod used in Experiments Two and Three, so that the plants would retain their vigor.

Controlled-environment chambers were used in this experiment; one chamber was set for a ten-hour photoperiod and the other chamber for a 16-hour photoperiod. The thermostat was set at 24° C during the photoperiods and at 18° C during the dark periods in both

chambers. Later in the experiment, after the eggs had been laid and the adults had been removed, the chimney cages containing the plants and eggs were transferred from the chambers to a greenhouse bench under a 16-hour photoperiod. The temperature in the chimney cages was set for 24° C on the thermostat in the greenhouse.

Adults from each photoperiod were collected daily from the chimney cages, and those of each sex were divided equally into two groups. Each group (consisting of males and females from the same photoperiod) was put on a T. subterraneum plant in a chimney cage. One group from each photoperiod was put in the ten-hour chamber, while the other group from each photoperiod was put in the 16-hour chamber. After seven days each group was separated into pairs of adults and placed in new chimney cages with T. subterraneum plants. In this manner, 15 pairs of adults were set up under each of the four combinations of short and long photoperiods that were described in Experiment Three.

Eggs and nymphs were counted as described under Methods. After the eggs laid by each pair of adults were given 30 days to hatch, the eggs that appeared viable were dissected from the plants and fixed and cleared (see Methods). Eggs laid by adults that had been reared as nymphs under the ten-hour photoperiod and were left under the ten-hour photoperiod as adults were examined first. All of the embryos visible were at the same stage of development (Figure 4);

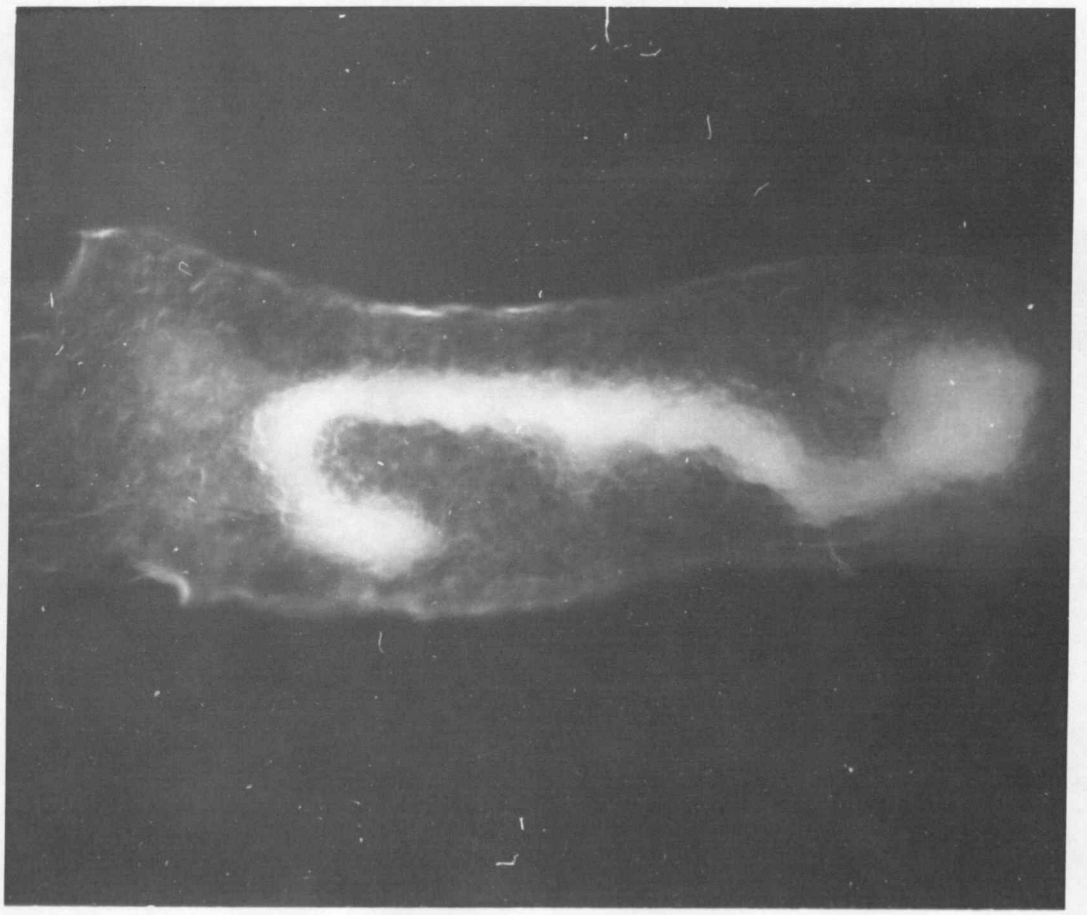

Figure 4. Embryo in diapause, lateral view (chorion removed).

therefore, it was assumed that this was the diapause stage. Following the examination of eggs from the ten-ten-hour group, the viable eggs from the other groups were examined using this technique.

The results of the experiment are given in Table VI. The data are incomplete because several days after the cages containing the plants and eggs were removed from the chambers and placed on a bench in the greenhouse, the rate of egg-hatching decreased abruptly, although the temperature and light conditions were similar to those in the chambers. However, Table VI shows that none of the eggs hatched and 20.0% contained diapausing embryos in the ten-ten-hour group (nymphs reared under a ten-hour photoperiod and left under the ten-hour photoperiod during the adult and subsequent egg stages). No diapausing embryos were observed in the 16-16-hour group, and 93.8% of the eggs hatched.

Large percentages of the eggs hatched in the 10-16- and 16-10-hour groups (72.4% and 42.3%), and only a small percentage contained diapausing embryos (2.3% and 3.7%). The percentages of eggs in diapause in the 10-16-hour and 16-10-hour groups were not significantly different, based on the difference of means test described under Methods ($u = 1.21$). The percentage of eggs in diapause in the ten-ten-hour group (20.0%) was significantly greater than the percentage of diapausing eggs in the 16-10-hour group (3.7%) ($u = 7.44$, $p < 0.01$), and therefore significantly greater than the percentage of

eggs in diapause in the 10-16-hour group (2.3%).

The location of the eggs in the plants was noted as the eggs were counted. The data are summarized in Table VII. The eggs included under the heading "petioles" includes eggs laid in the stipules (appendages at the base of the petiole) and in the outer layer of the root, directly below the base of the petioles. However, eggs in the petioles themselves accounted for at least two-thirds of the eggs laid in the basal portions of the plants in each of the four photoperiod groups.

The largest percentage of eggs in the basal portions of the plants were laid by females in the 10-10-hour and 10-16-hour groups (15.6% and 16.4%). A significantly smaller percentage of eggs were laid in the basal portions of the plants by females in the 16-10-hour group (4.9%) when statistically compared to the 10-10-hour group ($u = 5.83$, $p < 0.01$). The smallest percentage of eggs in the basal portions of the plants was laid by females in the 16-16-hour group (2.1%), when statistically compared to the 16-10-hour group ($u = 2.73$, $p < 0.01$).

TABLE VI. Effect of the photoperiods under which nymphs were reared and adults were kept on the condition of the eggs which they produced (Experiment Four).

Replicates	Photoperiod Combination											
	10-10-Hour*			10-16-Hour			16-10-Hour			16-16-Hour		
	H	D	S	H	D	S	H	D	S	H	D	S
1	0	5	25	34	0	0	7	0	18	22	0	8
2	0	0	24	23	3	0	20	0	10	29	0	0
3	0	4	21	25	1	0	12	0	17	29	0	0
4	0	16	26	30	0	0	29	0	3	33	0	0
5	0	10	25	34	0	0	23	0	2	30	0	0
6	0	3	25	27	0	0	0	0	31	26	0	0
7	0	6	21	0	0	30	27	0	2	36	0	1
8	0	1	36	14	3	16	17	0	15	19	0	8
9	0	11	14	22	0	10	1	0	28	30	0	0
10	0	0	27	31	0	0	21	0	8	40	0	0
11	0	1	26	14	1	18	0	0	32	25	0	4
12	0	4	31	20	1	5	0	5	18	30	0	5

(Continued on following page)

TABLE VI. Continued.

Replicates	Photoperiod Combination											
	10-10-Hour*			10-16-Hour			16-10-Hour			16-16-Hour		
	H	D	S	H	D	S	H	D	S	H	D	S
13	0	0	29	--	-	--	5	8	17	36	0	4
14	0	23	12	12	1	19	1	3	21	33	0	0
15	0	8	27	22	0	10	18	0	9	35	0	0
Total	0	92	369	310	10	108	181	16	231	453	0	30
% hatched eggs:	0.0%			72.4%			42.3%			93.8%		
% diapause eggs:	20.0%			2.3%			3.7%			0.0%		
% spoiled eggs:	80.0%			25.2%			54.0%			6.2%		

* = Eggs laid by adults that were reared as nymphs under a 10-hour photoperiod and remained under the 10-hour photoperiod during the adult and succeeding egg stages.

H = Number of hatched eggs.
D = Number of eggs containing diapause embryos
S = Number of spoiled eggs

TABLE VII. Effect of the photoperiods under which nymphs were reared and adults were kept on the oviposition sites on Trifolium subterraneum L. (Experiment Four).

	Photoperiod Combination							
	10-10-Hour*		10-16-Hour		16-10-Hour		16-16-Hour	
Replicates	L	P	L	P	L	P	L	P
1	49	3	35	6	25	0	47	0
2	19	5	38	8	31	0	48	0
3	25	0	40	0	23	6	29	0
4	38	4	32	2	35	0	52	3
5	44	0	0	35	25	0	44	2
6	19	8	38	0	31	0	39	3
7	27	0	30	0	43	0	52	0
8	33	4	37	7	58	0	61	5
9	35	19	39	5	46	3	54	0
10	41	4	43	5	29	0	53	2
11	41	16	23	14	32	0	38	0
12	28	7	44	3	31	7	35	0
13	29	0	--	-	39	4	55	0
14	38	9	27	7	20	5	43	0
15	42	15	42	0	32	1	45	0
Total	508	94	468	92	500	26	695	15
% eggs in leaves:	84.4%		83.6%		95.1%		97.9%	
% eggs in petioles:	15.6%		16.4%		4.9%		2.1%	

* = Eggs laid by adults that were reared as nymphs under a 10-hour photoperiod and remained under the 10-hour photoperiod during the adult and succeeding egg stages.

L = Eggs laid in leaves
P = Eggs laid in petioles

Experiment Five

This experiment was designed to determine if the adults would lay diapausing eggs when they had been reared under a short photoperiod and remained there as adults for one week before being transferred to a long photoperiod. The experiment was performed in the two controlled-environment chambers, as was Experiment Four. One chamber was set for photoperiods of ten hours and the other chamber was set for photoperiods of 16-hours. The temperature in both chambers was set at 21° C for both the light and dark phases of the 24-hour cycle.

Nymphs were reared on T. subterraneum and T. hybridum in chimney cages. Adults under each photoperiod were collected daily from the chimney cages and placed as a group in chimney cages on other T. subterraneum plants. After one week each group was separated into pairs of adults and put on new T. subterraneum plants in chimney cages. Pairs of adults from each photoperiod were alternately placed in the ten-hour and 16-hour chambers. Thus the four combinations of the two photoperiods described in Experiment Three were also made in this experiment. The difference between the four combinations in Experiment Three and Four and in this experiment was that in this experiment the adults remained for one week under the same photoperiod that they had been reared under as nymphs,

before being paired and separated into the four combinations of the two photoperiods.

All of the eggs that had not hatched from five to eight weeks after they had been laid were examined in situ with a 10X hand lens and the "viable" eggs (eggs that were fully inflated and opaque-white) were counted. This method for determination of diapause eggs has been used by Matthée (1951), Prinsloo (1961), and others. It is probable that only part of the eggs which were counted as viable were actually in diapause, the remainder being inviable or at least not containing visible embryos. This was found to be the case when the eggs were fixed and cleared in the fourth experiment.

The results of this experiment are given in Table VIII. None of the eggs hatched in the 10-10-hour group or the 10-16-hour group. A significantly greater percentage of eggs were in diapause (viable) in the 10-10-hour group (69.0%) than in the 10-16-hour group (48.3%) using the difference of means test described under Methods ($u = 6.25$, $p < 0.01$). Some eggs were counted as viable in the 16-16-hour and 16-10-hour groups (10.2% and 1.0%), but it is doubtful that few, if any, of these eggs were in diapause. There was no significant difference between the percentage of eggs that hatched in the 16-16-hour group (57.0%) and the percentage of eggs that hatched in the 16-10-hour group (63.3%) ($u = 1.55$).

The location of the eggs in the plants was noted when the eggs were counted (after the female oviposition period). The data are given in Table IX. The percentage of eggs laid in the petioles in the 10-10-hour group (20. 9%) was significantly greater than in the 10-16-hour group (1. 8%) ($u = 10.27$, $p < 0.01$), and therefore significantly greater than the percentage of eggs laid in the petioles in the 16-10-hour group (1. 4%). There was no significant difference between the percentage of eggs laid in the petioles between the 10-16-hour group (1. 8%) and the 16-10-hour group (1. 4%) ($u = 0.549$).

The length of the adult leafhoppers was measured as described under Methods. The data is presented in Table X. The mean lengths of both sexes (taken separately) were not significantly different when the adults had been reared as nymphs under a ten-hour photoperiod than when the adults had been reared as nymphs under a 16-hour photoperiod. The difference between the mean lengths of females reared under the 16-hour photoperiod and that of the females reared under the ten-hour photoperiod was 0. 04 mm, and the difference between the mean total lengths of similar groups of males was 0. 02 mm.

TABLE VIII. Effect of the photoperiods under which nymphs were reared and adults were kept on the condition of the eggs which they produced (Experiment Five).

	Photoperiod Combination											
	10-10-Hour*			10-16-Hour			16-10-Hour			16-16-Hour		
Replicates	H	V	S	H	V	S	H	V	S	H	V	S
1	0	20	14	0	20	15	12	3	12	0	1	23
2	0	28	0	0	23	13	21	0	13	29	0	0
3	0	18	14	0	0	34	27	0	0	0	18	10
4	0	30	0	0	0	28	24	0	9	20	2	3
5	0	30	0	0	3	26	20	0	8	42	0	0
6	0	0	24	0	57	2	22	0	3	29	0	0
7	0	19	6	0	0	19	26	0	8	0	9	19
8	0	34	0	0	30	2	11	0	20	8	0	17
9	0	31	0	0	0	29	0	0	27	0	0	16
10	0	16	8	0	31	1	20	0	3	8	0	8
11	0	22	3	0	25	3	--	-	--	31	0	0

(Continued on following page)

TABLE VIII Continued.

Replicates	Photoperiod Combination											
	10-10-Hour*			10-16-Hour			16-10-Hour			16-16-Hour		
	H	V	S	H	V	S	H	V	S	H	V	S
12	0	22	4	0	23	6	--	-	--	--	-	--
13	0	6	28	0	3	24	--	-	--	--	-	--
14	0	26	7	0	1	29	--	-	--	--	-	--
15	0	1	28	-	--	--	--	-	--	--	-	--
Total	0	303	136	0	216	231	183	3	103	167	30	96
% hatched eggs:	0.0%			0.0%			63.3%			57.0%		
% "viable" eggs:	69.0%			48.3%			1.0%			10.2%		
% spoiled eggs:	31.0%			51.7%			35.6%			32.8%		

* = Nymphs were reared as adults under a 10-hour photoperiod and remained under the 10-hour photoperiod during the adult and succeeding egg stages.

H = Number of hatched eggs
V = Number of "viable" eggs
S = Number of spoiled eggs

TABLE IX. Effect of the photoperiods under which nymphs were reared and adults were kept on the oviposition sites on Trifolium subterraneum L. (Experiment Five).

	Photoperiod Combination							
	10-10-Hour*		10-16-Hour		16-10-Hour		16-16-Hour	
Replicates	L	P	L	P	L	P	L	P
1	58	21	43	3	139	0	28	0
2	56	12	40	1	68	0	49	0
3	27	44	36	0	48	0	56	0
4	57	8	52	3	44	0	25	0
5	48	17	45	0	44	5	42	0
6	24	0	25	0	87	0	93	0
7	25	0	19	0	48	0	50	0
8	35	9	31	3	62	0	66	0
9	65	4	29	0	36	0	16	0
10	21	3	32	0	40	4	16	0
11	40	0	80	0	--	-	44	0
12	38	25	55	0	--	-	--	-
13	33	0	33	0	--	-	--	-
14	19	8	31	0	--	-	--	-
15	27	0	--	-	--	-	--	-
Total	573	151	551	10	616	9	485	0
% eggs in leaves:	79.1%		98.2%		98.6%		100%	
% eggs in petioles:	20.9%		1.8%		1.4%		0.0%	

* = Eggs laid by adults that were reared as nymphs under a 10-hour photoperiod and remained under the 10-hour photoperiod during the adult and succeeding egg stages.

L = Eggs laid in leaves
P = Eggs laid in petioles

TABLE X. Effect of the photoperiods under which nymphs were reared on the length of the adults (Experiment Five).

Replicates	Males		Females	
	10-Hour	16-Hour	10-Hour	16-Hour
1	17.25	18.00	20.75	20.50
2	17.00	16.50	20.50	20.75
3	17.50	17.00	20.50	19.50
4	17.25	16.50	20.00	18.50
5	17.00	15.50	18.50	19.00
6	16.75	16.00	18.25	20.50
7	16.00	17.00	20.00	19.50
8	17.00	16.25	18.50	19.00
9	16.00	16.00	19.00	19.00
10	16.00	16.00	18.50	20.00
11	17.00	18.00	18.25	18.25
12	17.00	17.00	19.00	18.50
13	17.50	16.50	18.50	19.00
14	17.00	17.75	19.50	18.75
15	16.75	16.25	17.50	19.50
16	16.25	15.75	20.50	18.00
17	16.75	17.00	20.25	18.50
Total	286.00	283.00	328.00	326.75
Mean	16.82	16.65	19.29	19.22

1 unit = 0.26 mm

Adult Coloration

A visual comparison was made of the color variations of the pinned adults from the five experiments. A "typical" male and female from the short photoperiod group and a similar pair from the long photoperiod group of Experiment Three were photographed (Figure 5). As a rule, the females were lighter in color than the males, varying from light to dark brown, in both the long and short photoperiod groups. The males varied in color from dark brown to black in both the long and short photoperiod groups. It was noted that the males and females from the long photoperiods were somewhat lighter in color than males and females from the short photoperiods. This is in agreement with the color variations reported by Swenson (1962).

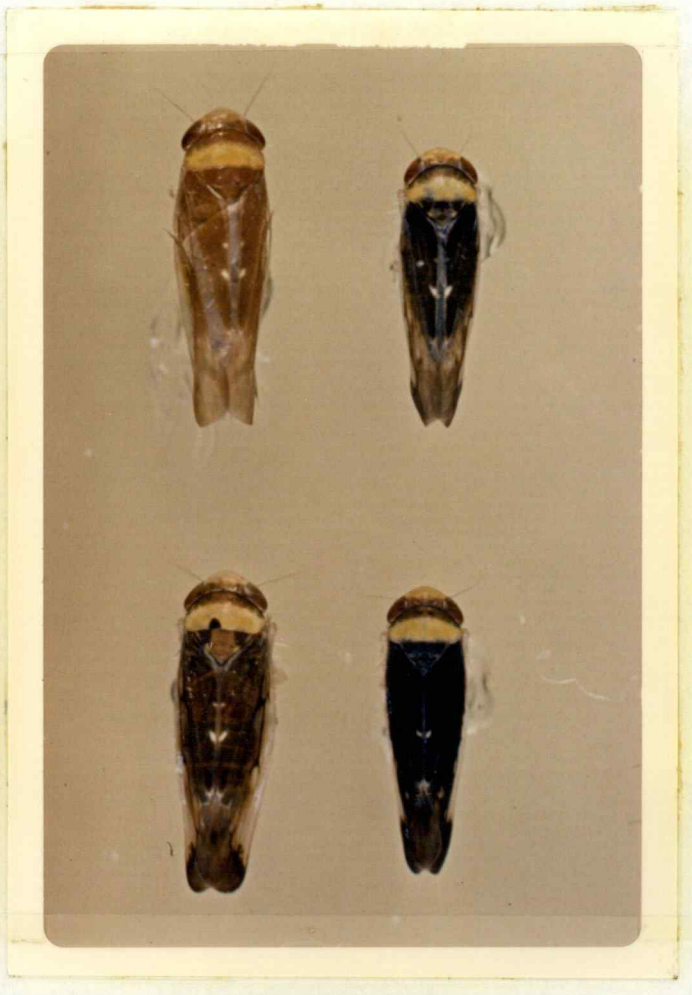

Figure 5. Colladonus montanus reductus (Van Duzee) from Experiment Three.

Upper left - 16-hr female.
Upper right - 16-hr male.
Lower left - 8-hr female.
Lower right - 8-hr male.

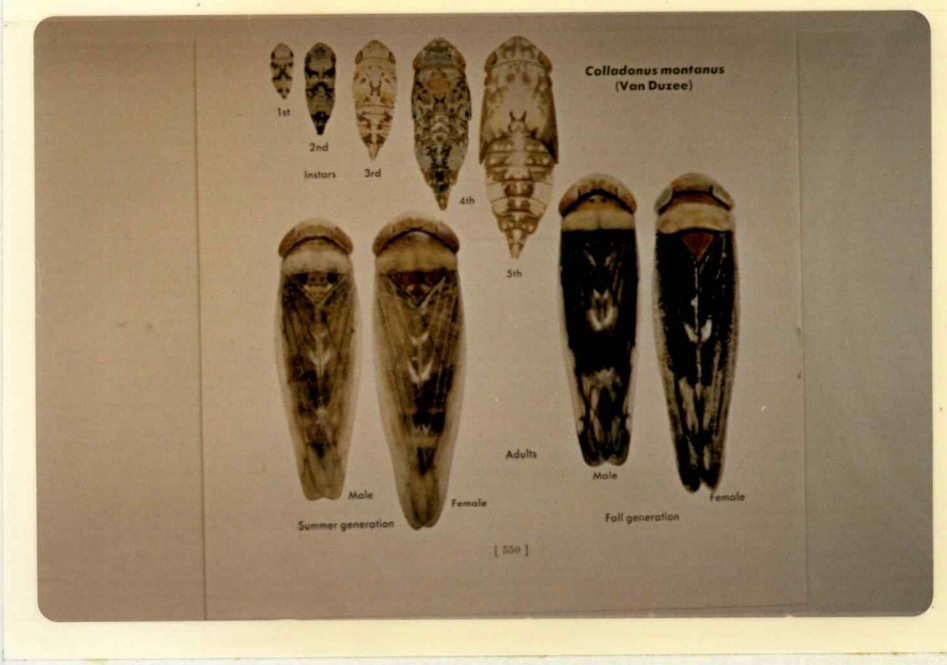

Figure 6. Colladonus montanus (Van Duzee) (reproduced from Severin & Klostermeyer, 1950).

DISCUSSION AND CONCLUSIONS

In the fourth experiment, 20.0% of the eggs laid under the 10-hour photoperiod by adults that had been reared as nymphs under the 10-hour photoperiod (10-10-hour group in Table VI) were in diapause, and none of these eggs hatched. When the nymphs were reared under the 10-hour photoperiod but were transferred to the 16-hour photoperiod as they became adults (10-16-hour group in Table VI), the adults laid only a few diapausing eggs and 72.4% of the eggs hatched. The 10-16-hour group of Experiment Four corresponds with the short photoperiod groups (less than 12-hours) of Experiments One and Two, because the nymphs reared under short photoperiods were also transferred as they became adults to 16-hour photoperiods in the earlier experiments. Large percentages of eggs hatched in the short photoperiod groups of Experiments One and Two (Tables I and II), as in the 10-16-hour group of Experiment Four (Table VI). In the fifth experiment, when the nymphs were reared under the 10-hour photoperiod and remained under the 10-hour photoperiod for one week as adults before being transferred to the 16-hour photoperiod (10-16-hour group in Table VIII), 48.3% of the eggs were viable (assumed to be in diapause) and none of the eggs hatched. These results show that nymphs reared under a short photoperiod (diapause-inducing) must remain under the short photoperiod as adults for one week

(perhaps less, but longer than one day) in order to insure that diapausing eggs will be laid. During the first several days of the adult stage the diapause response that has been induced by rearing the parent generation under short photoperiods can be reversed or "switched" as has been shown in other insect and mite species (see Review of Literature).

De Wilde brought attention to the fact that it is much easier to reduce or eliminate the effect of a diapause-inducing photoperiod with a non-diapause-inducing photoperiod than it is to obtain the reverse change during the sensitive period. In the fourth experiment, the four combinations of the two photoperiods were made as soon as the adults appeared. It was shown above that the adults are still sensitive to changes in the photoperiod at this time. The differences between the percentages of eggs that hatched in the 10-16-hour group and the 10-10-hour group was 72.4% and a significantly smaller percentage of the eggs were in diapause in the 10-16-hour group than in the 10-10-hour group (2.3% and 20.0%). However, only 3.7% of the eggs were in diapause in the 16-10-hour group compared to the lack of diapausing eggs in the 16-16-hour group and the percentage of eggs that hatched in the 16-10-hour group was 43.3% compared to the 16-16-hour group in which 93.8% of the eggs hatched. The differences in the percentages of hatched and diapausing eggs when the 10-16-hour and 10-10-hour groups are compared are much greater than

when the percentages of hatched and diapausing eggs in the 16-10-hour and 16-16-hour groups are compared.

It was not possible to consider the effect of temperatures in the first three experiments, because the diapause response was not clearly initiated and the data were incomplete. The temperatures in the chambers in the fourth experiment were 24° C during the photoperiods and 18° C during the dark periods. The temperature in the chambers during the fifth experiment was 21° C during both light and dark phases of the 24-hour cycle. The temperatures in these two experiments were moderate in terms of the temperatures which have been used in diapause induction experiments by other workers (see Review of Literature). These workers have found that moderate temperatures do not affect the response of other species to photoperiod.

A large number of the eggs in each experiment either did not hatch (Tables I, II, and III) or were spoiled (Tables VI and VIII). Slifer and King (1961) reported large percentages of spoiled eggs in studies of inheritance of diapause in Melanoplus differentialis (Thomas), which has a diapause in the egg stage. They cite similar reports by other workers for other species of Acrididae.

In the fourth and fifth experiments, the females laid an undetermined number of eggs before they were paired to lay the 25 to 40 eggs that were counted in the experiments. In the first three

experiments, the first 25 to 40 eggs laid were counted because the adults were paired as soon as they appeared. Severin and Klostermeyer (1950) found that three females of C. m. reductus laid 289, 324, and 348 eggs that hatched. Thus the number of eggs that were included in the experiments was only a small percentage of the potential number of eggs that any female can lay. From the data of the five experiments, it is not possible to determine the effect of the female's age on the percentage of diapause eggs or the effect of changing the photoperiod during the adult stage on the number of diapausing and non-diapausing eggs laid.

The location of the eggs in the plants was noted in the third, fourth, and fifth experiments. In each of these three experiments the females deposited their eggs in T. subterraneum plants. The majority of the eggs were inserted in the leaves, regardless of the photoperiod under which the leafhoppers had been reared. The rest of the eggs were inserted in the petioles, particularly near the base of the petioles; in the stipules; and in the exposed portion of the root directly below the base of the petioles. The percentages of eggs laid in the basal portions of the plants by females from the different photoperiod groups in the third, fourth, and fifth experiments are taken from Tables IV, VII, and IX and presented below:

	Exp. 3		Exp. 4	Exp. 5
8-8-hour	57.2%	10-10-hour	15.6%	20.9%
8-16-hour	20.2%	10-16-hour	16.4%	1.8%
16-8-hour	25.4%	16-10-hour	4.9%	1.4%
16-16-hour	5.2%	16-16-hour	2.1%	0.0%

A larger number of eggs were laid in the basal portions of the plants when the parents females spent a greater amount of their life cycle under short photoperiods.

The only published reports of a difference in oviposition site for diapausing eggs deal with the spider mite family, Tetranychidae (Cagle, 1946; Lees, 1950; Miller, 1950). The females of two species of spider mites that occur on apple trees deposit almost all of their winter (diapausing) eggs on the bark of the trees, while summer (non-diapausing) eggs are deposited on the leaves. Lees (1953) found that "winter females" are more active than "summer females". Before oviposition, the winter female leaves the leaf to lay its egg on the bark, "often under the petiole or in crevices near dormant buds". Diapausing eggs deposited on the bark would provide easy access to new tree foliage for the mite larvae during the following spring. Although the female C. m. reductus reared under short photoperiods deposit the majority of their eggs in the leaves, it seems likely that the diapausing eggs deposited in the petioles and other basal portions

of the plant would be better protected against dessication and injury over the winter.

In the fourth experiment, the eggs that did not hatch, but were viable 30 days after they had been laid, were fixed and cleared so that the diapausing embryos could be seen. These embryos were in an S-shaped position, when viewed laterally, and were located in the center of the yolk. Segmentation was visible insofar as mouthparts and legs could be seen as small buds. By comparing the diapausing embryos of C. m. reductus with illustrations and descriptions of the embryonic development of other insect species (Johannsen & Butt, 1941, p. 60-64; Lees, 1955, p. 8-11; Hogan, 1960), it seemed that the leafhopper embryos were in anatrepsis, the stage during which the embryo is moving caudally through the yolk. Lees (1955, p. 10) states that diapause occurs at the end of anatrepsis in many species of insects. Dr. F. H. Butt (personal communication) suggested that embryos are insulated by the surrounding yolk at this stage of development, making this stage particularly suitable for diapause to take place.

In the third experiment the average length of each sex of adults reared under the eight-hour photoperiod was significantly less than the average length of the same sex of adults reared under the 16-hour photoperiod. The significantly shorter males and females from the eight-hour photoperiod may have been the result of poor nutrition

during the nymphal stage because of the weak condition of the plants. In the fifth experiment the average length of adults reared under the ten-hour photoperiod was not significantly less than the average length of adults reared under the 16-hour photoperiod. In contrast, Müller (1957, 1958) found that short-day (eight-hour photoperiod) diapausing females were 18.2% shorter than the long-day (16-hour photoperiod) non-diapausing females of Stenocranus minutus. Similarly, the males were 13.5% shorter when reared under the eight-hour than under the 16-hour photoperiods.

It was noted that the males and females from the long photoperiods were generally lighter in color than the males and females from the short photoperiods, but the difference in color between males and females, regardless of the photoperiod under which they had been reared as nymphs, was much more striking. Severin (1934) and Severin and Klostermeyer (1950) reported the life history of Colladonus montanus and included a color plate of "summer" and "autumn" generations in each publication (reproduced in Figure 6). Severin (1934) did not consider Thamnotettix (= Colladonus) reductus Van Duzee to be a valid species separate from T. montanus because they could only be separated by color; he reported that DeLong could find no distinctive genital characteristics to separate the two species. Severin found that summer adults collected in four California valleys were dark brown, while specimens collected in the autumn were

usually black "with intermediates between the two color patterns". Nielson (1957) concluded that Severin and Klostermeyer (1950) had studied C. montanus, subspecies reductus. The difference between their populations and mine can be seen in Figures 5 and 6. The populations of C. m. reductus that they studied were seasonally dichromatic, whereas our laboratory cultures show some seasonal dichromatism, but a more striking sexual dichromatism. Nielson (1957) reported geographical variations in color for other species of Colladonus.

SUMMARY

When first- and second-instar nymphs were reared to the adult stage under ten hours of light per 24-hour day and were left under the ten-hour photoperiod for the first week (or more) of the adults stage, they produced diapause eggs. Adults reared as nymphs under the ten-hour photoperiod, but changed to a 16-hour photoperiod within one day after reaching the adult stage, produced only a few eggs that were in diapause and many eggs that hatched. Similarly, adults that had been reared as nymphs under a 16-hour photoperiod but were transferred to a ten-hour photoperiod, either one day or one week after reaching the adult stage, produced only a few eggs that were in diapause and many eggs that hatched. When the adults were reared as nymphs under a 16-hour photoperiod and were left under the 16-hour photoperiod to mate and lay eggs, none of the eggs produced were in diapause and a majority of the eggs hatched. These results show that Colladonus montanus reductus is a long-day insect.

Eggs laid by adults that had been reared as nymphs under a ten-hour photoperiod and left under the ten-hour photoperiod as adults contained embryos that were all at the same stage of development, which was assumed to be the diapause stage. These embryos were in the anatrepsis stage of development; appendage buds were present, indicating that segmentation was in progress.

Significantly greater percentages of eggs were laid in the basal portions of the plants by leafhoppers that had spent part or all of their life cycle, beginning with the first and second nymphal instars, under a ten-hour photoperiod. In two of the three experiments in which the location of eggs in the plants was noted, the greatest percentage of eggs deposited in the basal portions of the plants was laid by adults that had been reared as nymphs under the ten-hour photoperiod and had been left under the ten-hour photoperiod as adults. Few, if any, eggs were deposited in the basal portions of the plants when the adults were reared as nymphs under the 16-hour photoperiod and remained under the 16-hour photoperiod as adults. It is likely that eggs deposited in basal portions of the plants would be better protected from dessication and injury over the winter.

A visual comparison was made of the color variations of the pinned adults from the five experiments. Females varied in color from light to dark brown, and males varied in color from dark brown to black. Males and females reared under short photoperiods were generally lighter in color than the males and females reared under long photoperiods, but the color difference between males and females, regardless of the photoperiod under which they had been reared, was much more striking.

The total lengths of mounted adult leafhoppers from two of the experiments were compared statistically in regard to the photoperiod

under which they had been reared as nymphs. In one experiment, both males and females (each sex taken separately) which had been reared under an eight-hour photoperiod were significantly shorter than males and females reared under the 16-hour photoperiod. However, the shorter adults from the eight-hour photoperiod were probably the result of poor nutrition caused by the weak condition of the plants on which the adults fed as nymphs, rather than a direct effect of the photoperiod on the nymphs. In the other experiment there was no significant difference when the above comparisons were made. The plants on which the leafhoppers were reared in this experiment remained vigorous throughout the nymphal rearing period.

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