This is for mum and dad

with love and thanks.

THE PHOTOPERIODIC CONTROL OF OVARIAN DIAPAUSE IN THE CABBAGE WHITEFLY, *ALEYRODES PROLETELLA* L.

A Thesis submitted for the Degree of Doctor of Philosophy at the University of London, and the Diploma of Membership of Imperial College.

by

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ABSTRACT.

Overwintering female cabbage whiteflies, Aleyrodes proletella L., exhibit an ovarian diapause characterized by the absence of pre-emergence and retarded post-emergence ovarian development, accompanied by fat body hypertrophy. Diapause induction is the result of a long-day - short-day transition.

The ovaries are acrotrophic and six stages of oöcyte development have been described. An ovarian score may be calculated for individual females.

Peak photoperiodic sensitivity is located in the third instar, although some degree of sensitivity is present during most of larval development.

Non-diel photoperiodic cycles show that the night length (scotophase) is most important in determining whether a particular regime constitutes a long or short day. However, the duration of the photophase may affect the critical night length (CNL).

Field samples have been dissected to monitior ovarian development throughout the year. The critical photoperiod has been shown to be the same as that recorded in the laboratory. Civil twilight is detected.

Systematically interrupting a long scotophase with one hour light pulses reveals four "dark stages": Stages 1 and 3 are photosensitive, Stage 2 is photorefractory to some degree, and Stage 4 occurs after the CNL.

Action spectra for dawn and dusk extensions of a short photophase both exhibit peak sensitivity at approximately 420nm with similar 50% response thresholds. This is consistent with the presence of a caroteno-protein photopigment.

An extensive series of "resonance" experiments has failed to implicate the circadian system in photoperiodic time measurement. Instead, the results indicate that the photoperiodic clock in this species is based on the hour-glass principle.

Immediately after diapause induction, females are refractory to photoperiod and temperature. Subsequently, the rate of post diapause morphogenesis is largely temperature dependent. Chilling may "prime" the ovaries for accelerated development in warmer conditions. Exogenous juvenile hormone has little, if any, effect upon termination.

GENERAL INTRODUCTION.

Diapause is the term applied to a period of arrest of growth and development (LEES 1955; SAUNDERS 1982b). It was first introduced by Wheeler in 1893 and refers to a particular phase in the embryogenesis of the grasshopper Xiphidium ensiferum and was, subsequently, applied to arrested growth in general, by Henneguy in 1904 (both cited in LEES 1955).

In contrast to quiescence, which is an immediate and direct response to "unfavourable" environmental stimuli, the arrest of growth and development constituting diapause occurs in response to environmental stimuli which may not, in themselves, be unfavourable but act as reliable and predictive cues to the forthcoming onset of cold, lack of food, lack of water etc. and serve to allow the organism to prepare itself for those conditions (LEES 1955; DANILEVSKII 1965).

The most recent attempts to sub-divide diapause into categories are those of Muller (1970) and Mansingh (1971). Muller introduced the term parapause, to describe obligatory diapause, which is genetically "fixed" and is, essentially, independent of any environmental influence; faculative diapause, induced by one environmental variable and terminated by another is termed eudiapause, and oligopause is a faculative diapause induced and terminated by the same environmental factor (MULLER 1965, 1970; see THIELE 1973). Mansingh attempted to elaborate further and

introduced additional terminology in order to describe low and high intensities of diapause as well as identifying five phases of the phenomenon: preparatory; inductive; refractory; activation and termination (MANSINGH 1973). This classification was criticised for a number of reasons (see THIELE 1973) and, although it is likely to prove more valuable in the light of further work on biochemical aspects of diapause, the terminology of Muller is currently preferred because of its more suitable simplicity. Recent work suggests that the cabbage whitefly, Aleyrodes proletella L. overwinters in eudiapause (IHEAGWAM 1976, 1977).

Amongst the insects are a number of species that diapause as eggs, larvae, pupae or adults (see SAUNDERS 1982b Appendix Table 4 for a comprehensive list and reviews by ANDREWARTHA 1952; LEES 1955, 1968; DE WILDE 1962; DANILEVSKII 1965; DANILEVSKII et al. 1970; TAUBER and TAUBER 1976; BECK 1980). This study will focus on adult reproductive diapause.

Adult diapause involves the arrest of growth and development of the gonads and is, characteristically, associated with fat body hypertrophy (LEES 1955). This is termed "gonotrophic dissociation" after the phenomenon first recorded in Anopheles maculipennis by Swellengrebel in 1929 (cited in SANBURG and LARSEN 1973).

There are a number of steps between the insect experiencing diapause inducing stimuli and the actual arrest of growth and development. In the case of a

photoperiodically induced ovarian diapause, photoreceptors respond to the photoperiod and some kind of "clock"measures/assesses the duration of the light period (photophase) or the dark period (scotophase) (see SAUNDERS 1982b). Inductive photoperiodic cycles are then "counted" until they exceed the "required day number" (RDN)(SAUNDERS 1981) at which point diapause is determined. However, diapause may not occur until much later in the life-cycle so some form of neuroendocrine "programming" must take place which stores the inductive information and results in a hormone balance which is unfavourable for ovarian development. The main reason for the retarded development has been shown to be a low titre of juvenile hormone (JH) (DE WILDE and DE BOER 1961; BOWERS and BLICKENSTAFF 1966; FERENZ 1977; SCHOONVELD et al. 1977; PORAS 1982). The role of JH in insect reproduction is well known in many species (eg: ENGELMANN 1970; ADAMS 1974; ABU-HAKIMA and DAVEY 1977; KOEPPE et al. 1981) and the low titre influences diapause by preventing vitellogenesis. In addition to JH, ecdysone is involved in the endocrinology of insect reproduction (RIDDIFORD 1980; FUCHS and KANG 1981; REDFERN 1982), but the role of this hormone in ovarian diapause is relatively little known.

It is the purpose of this study to establish the precise role of the photoperiod in the ovarian diapause of A. proletella. In particular, ovarian development under different conditions and the sensitive period will be determined and used to investigate the nature of the photoperiodic clock. The laboratory work will be used in

conjunction with field data to assess the physiology of the developmental arrest under natural conditions.

CHAPTER ONE.

REPRODUCTIVE BIOLOGY AND THE INDUCTION OF DIAPAUSE.

INTRODUCTION.

The cabbage whitefly, *Aleyrodes proletella* L. (=*brassicae* Walk.) is a minor pest of cruciferous crops in southern Britain (JONES and JONES 1975). Adult females lay their eggs on the underside of young leaves from late April until early September (BUTLER 1938a; IHEAGWAM 1976). The first instar larva is mobile for up to three days after hatching before it settles and the legs atrophy (DESHPANDE 1933; BUTLER 1938a; TREHAN 1940). Consequently, the larva is sessile for the remainder of its development. The fourth instar larva is sometimes referred to as a pseudo-pupa and the adult emerges through a T shaped split in the cuticle (BUTLER 1938a). During spring and summer, newly emerged adults tend to migrate to the younger leaves of the same, or a nearby, plant before oviposition commences (BUTLER 1938a) and, in the autumn and winter, migration to host varieties and species affording greater protection against the cold has been recorded (BUTLER 1938a; EL KHIDIR 1963). Senescing leaves and overcrowding may also cause adults to migrate (BUTLER 1938a,b). During the autumn, the proportion of males in the field falls from approximately 50% to zero (BUTLER 1938a; IHEAGWAM 1976). Since males are thought

to engage in mating activity very soon after emergence (EL KHIDIR 1963) and this reduces their cold- hardiness (BUTLER 1938a), the mortality, no doubt, occurs during early frosts.

According to Butler (1938a), overwintering A. proletella females are fully mature during the winter and cold merely arrests oviposition. However, subsequent work by El Khidir (1963) has shown that the ovarian development of these females is impaired. It was suggested that this was due to low temperatures and that the females did not mate (EL KHIDIR 1963). More recently, it has been demonstrated that the cessation of oviposition is a true reproductive diapause induced by photoperiod with a critical daylength of LD 15.75:8.25 at 15°C (IHEAGWAM 1976, 1977).

The reproductive system of female Aleyrodidae was first described by Deshpande (1933). He showed that the ovaries of A. proletella are acrotrophic (=telotrophic), each containing five ovarioles that, in turn, contain a number of oöcytes at different stages of development. Ovarian dissections of A. proletella have, since, shown that the proportion of non-gravid females increases markedly, from almost zero to nearly 100% during the course of September (EL KHIDIR 1963), but no further details of ovarian development are known at present.

The first description of discrete stages in the ovarian development of insects is that of Christophers (1911) for anopheline mosquitoes. Subsequently, that

system was modified by workers using other mosquitoes such as Culex pipiens pipiens (SANBURG and LARSEN 1973), Aedes aegypti (GWADZ and SPEILMAN 1973; CLEMENTS and BOOCOCK 1984) and Wyeomia smithii (O'MEARA and LOUNIBOS 1981). In addition, the ovarian development of a number of other insects has been divided into stages. These include the eye gnat, Hippelates collusor (ADAMS and MULLA 1967), Musca domestica (ADAMS 1974), the stable fly, Stomoxys calcitrans (VENKATESH and MORRISON 1980) the stick insect, Clitumnus extradentalis (MESNIER 1980) and Drosophila melanogaster (KING 1970). Each system provides a means of quantifying ovarian development and thus facilitates direct comparison with the results of other workers.

Much work on ovarian diapause has been based on measurements of pre- oviposition times (eg. OLDFIELD 1970; TAUBER et al. 1970a,b; HODEK 1971c; PENER and BROZA 1971; TADMOR and APPLEBAUM 1971; TAUBER and TAUBER 1969, 1973a,b,c, 1974, 1976b,c; IHEAGWAM 1976, 1977) or dissections to ascertain whether the ovaries were mature or immature (eg. NORRIS 1962; LEIGH 1966; MACLEOD 1967; STOFFOLANO and MATTHYSSE 1967; KAMM 1972; HERMAN 1973; JALANA et al. 1973; STORCH 1973; KAMBYSELLIS and HEED 1974; LUMME et al. 1974; ALI and EWIESS 1977; FERENZ 1977; HUDSON 1977; GOLDSON and EMBERSON 1980; JAMES 1982; PORAS 1982). They show that development does not proceed beyond the pre-vitellogenic stages. Such methods are useful in assessing the effects of photoperiod, temperature, nutrition and hormone application on diapause induction and/or termination but they fail to provide much

detailed information about the underlying reproductive physiology. Some workers have studied the condition of the ovaries in greater depth by dividing oöcyte development into stages, as above, (eg. KAMM and SWENSON 1972; SPEILMAN and WONG 1973; CASE et al. 1977; VANDERLIN and STREAMS 1977) and, in a few cases, a somewhat subjective assessment of fat body development has been made as well, thus providing a more complete account of the phenomenon (see: HARWOOD and HALFHILL 1964; DEPNER and HARWOOD 1966; HODEK 1971b; BEGON 1976; KONO 1982).

Behavioural and morphological changes may accompany gonotrophic dissociation as elements of the "diapause syndrome" (DE WILDE 1970) and these have been incorporated into some studies as indicators of diapause induction, intensity and termination. The Colorado beetle, Leptinotarsa decemlineata, exhibits distinctive pre-diapause behaviour by ceasing to feed and burrowing into the soil (DE WILDE 1954). Similarly, the Linden bug, Pyrrhocoris apterus, stops feeding and clusters in the leaf litter beneath lime trees (HODEK 1971c). Clusters of diapausing Monarch butterflies, Danaus plexippus are well known in California, following their famous migration down the west coast (WILLIAMS 1930, 1958; HERMAN 1973), and have also been recorded in Australia in the absence of any pronounced migratory behaviour (see JAMES 1982). The lacewings, Chrysopa carnea and C. mohave undergo similar colour changes from bright green (non-diapause) to pale yellow (diapause) (MACLEOD 1967; TAUBER and TAUBER 1969;

TAUBER et al. 1970a,b; TAUBER and TAUBER 1973a,b) and this is a good measure of diapause intensity in C. carnea (TAUBER and TAUBER 1973c). Amongst the Aleyrodidae, seasonally associated pigmentation changes are known in Aleyrodes proletella (EL KHIDIR 1963) and A. asari (BAHRMANN 1972) with the overwintering females being darker in both species.

Laboratory studies on diapause induction tend to focus on two features of the process: the Photoperiodic Response Curve (PhRC), which incorporates the critical photoperiod, and the sensitive stages.

The PhRC is obtained by subjecting the insect to one, "stationary", photoperiod throughout its development and observing the percentage of diapausing individuals resulting from different regimes. There is, typically, a very sharp switch from non-diapause to diapause and, sometimes, a change in photoperiod of as little as thirty minutes is sufficient to cause the change from continuous development to diapause which is indicative of the selective pressure that has moulded the response (LEES 1968). The critical photoperiod is reached when 50% of the insects enter diapause. In some cases, stationary photoperiods fail to elicit a complete diapause response and these insects need to experience a change in photoperiod during their development. The red locust, Nomadacris septemfasciata, will only enter an intense diapause if the hoppers experience a "long" day and the adults a "short" day (NORRIS 1962, 1965) whilst the carabid, Agonum assimile requires decreasing daylengths to

induce diapause (NEUDECKER and THIELE 1974).

The sensitive period is that part of the life-cycle which responds to the environmental conditions that determine whether development will be continuous or whether diapause will occur (LEES 1968). If diapause is determined, it usually ensues at a species specific stage (LEES 1968; SAUNDERS 1982b). Stages that are sensitive may be identified by transferring insects from diapause-averting to diapause-inducing conditions at different stages of development either permenantly, or for a few days (eg. the duration of one instar). All stages of the alfalfa weevil, Hypera brunneipennis, are said to be equally sensitive (MADUBUNYI 1978) however, normally, one phase of development is particularly sensitive such as the fifth instar larva of P. apterus (HODEK 1971b) and cocoon stage third instar plus the pupa of C. carnea (TAUBER et al. 1970b). Several insects are sensitive to photoperiod as adults, indeed larval treatment has little effect on diapause induction in L. decemlineata (DE WILDE et al. 1959) or Coccinella transversoguttata (STORCH 1973). Adult C. carnea are sensitive (TAUBER and TAUBER 1969) as are adult Aelia acuminata (HODEK 1971c) but, in both cases, peak sensitivity has been located in the immature stages where diapause induction is likely to be determined under natural conditions. It has been shown that photoperiodic sensitivity is confined to the immature stages of A. proletella and does not extend into the fourth instar (IHEAGWAM 1976, 1977).

Temperature and nutrition are known to be involved in the induction of diapause (LEES 1955, 1968). In nature, long days and high temperatures tend to occur together. However, in the laboratory, high temperatures may override the inductive effect of short days (WAY and HOPKINS 1950; LEES 1955, 1968; SAUNDERS 1982b) and temperature cycles, "thermoperiods", have been shown to mimic photoperiodic effects in constant darkness (SAUNDERS 1973a; see BECK 1983 for a review). Nutritional factors are very important in the diapause response of the spider mite, Metatetranychus (=Panonychus) ulmi (LEES 1953a) and L. decemlineata (DE WILDE and FERKET 1967) since, in both cases, feeding on senescing leaves may induce diapause even if the photoperiod and temperature are favourable for continuous development. C. mohave also has a food mediated diapause which ensues as prey becomes scarce towards the end of summer (TAUBER and TAUBER 1973b). Rearing A. proletella at 25°C averts diapause in all photoperiodic regimes. However, there is no significant effect of food quality on the response (IHEAGWAM 1976, 1977).

This chapter will be concerned with the ovarian development and photoperiodic sensitivity of A. proletella as well as with the role of stationary and changing photoperiods in the induction of diapause.

MATERIALS AND METHODS

Identification.

Aleyrodes brassicae Walk. is now considered to be a synonym of Aleyrodes proletella L. (TREHAN 1940; MOUND 1965; MOUND and HALSEY 1978) and the latter will be used throughout this study because of its historical precedence. Empty cases of 4th instar larvae were collected from brussels sprout leaves at Silwood Park and, subsequently, from laboratory cultures, and identified using the taxonomic features described by Mound (1965).

Culture.

Adult whitefly collected from the field in October and November 1981 formed the basis of the laboratory cultures, although these were occasionally supplemented by the addition of more field collected adults during the summer. The stock culture was maintained in LD16:8 in a 20±2 °C CT room. Once the eggs had been laid the leaves were kept in either a Fisons environmental cabinet (LD12:12 15±1 °C) or a Boro Labs. Labmark refrigerated incubator (LD16:8 15±1°C).

The eggs are characteristically laid in part or complete circles, and the mobility of the 1st instar larvae for 1-3 days after hatching ensures that developing

larvae in subsequent instars rarely overlap in spite of being almost totally sessile.

Attempts were made to transfer larvae from leaf to leaf in order to obtain localised populations of identical developmental age. However, the mortality level was unacceptably high so an alternative means was sought. Excised, young brussels sprout leaves had their petioles immersed in modified Hoagland-Snyder nutrient solution (ADAMS and VAN EMDEN 1972). These leaves take root, grow and remain healthy for the duration of the egg and larval stages even if kept in low light intensities or total darkness for ten days of the total developmental time. Leaves were placed in the culture cage where 200-350 eggs were normally deposited on them in the space of 1-3 days.

Recipe (for 1 litre stock solution):

$Ca(NO_3)$	...	0.82g;
KNO_3	...	0.50g;
KH_2PO_4	...	0.14g;
$MgSO_4$	...	0.12g;
Streptomycin sulphate	...	0.01g;
1.54% aq. solution Fe(EDTA)	...	1.0ml;
Distilled water to make	...	1.0l.

(To use: dilute 1 part stock: 3 parts distilled water).

Larval Development.

In view of the sessile nature of developing larvae, "leaf-maps" could be drawn and individual larvae numbered in order to follow their developmental history. The sexes are readily distinguishable upon emergence since the males possess a pair of claspers. In fact, as Table 1 shows, it is possible to ascertain the sex of an individual from the size of the 3rd and 4th instar larva.

Once the precise age of individual larvae is known, a leaf bearing larvae of a variety of ages may be transferred from one photoperiodic regime to another and the response of different developmental stages may be assessed by isolating the adults when they emerge. The age of a larva will be abbreviated throughout this study such that 3(2) signifies a larva that moulted to the third instar two days previously.

Diapause Criteria.

a) Pre-oviposition time. A female was adjudged to be in diapause if her pre-oviposition time exceeded 21 days (IHEAGWAM 1976, 1977). Non-diapausing females oviposit within 7 days of emerging whilst a pre-oviposition time of 7-21 days represents an intermediate response.

TABLE 1. Dimensions of 3rd and 4th Instar Larvae Destined to become Males and Females.

Instar.	Dimensions (mm) $\bar{x}$ ± SEM. ♂		♀	
	Length	Breadth	Length	Breadth
3rd.	0.70±0.05 (n=23)	0.49±0.02 (n=23)	0.70±0.03 (n=33)	0.54±0.02 (n=33)
4th.	0.99±0.06 (n=42)	0.72±0.04 (n=42)	1.17±0.05 (n=50)	0.88±0.05 (n=50)

b) Condition of the ovaries. Diapausing females do not contain any mature eggs (see below) when dissected 21 days after emerging. Non-diapausing females contain three or more mature eggs and intermediates contain one or two.

c) Presence of "relics" (see below). A relic indicates that an ovariole has released an egg prior to dissection and is a particularly useful criterion when field samples or females of unknown egg laying history are being dissected.

Ovarian Development.

A very precise assessment of the response to photoperiod can be achieved if post-emergence ovarian development is monitored. This allows the percentage of diapausing individuals resulting from each treatment to be ascertained (see b and c above) and also gives a dynamic picture of its effect on the target organ.

Females were briefly anaesthetised with chloroform, immersed in 90% ethanol to remove the abundant cuticular waxes and dissected in a dipteran saline (FINLAYSON and OSBORNE 1970) under a dissecting microscope using electrolytically "sharpened" tungsten needles. The ovaries were teased apart and the preparation viewed through a compound microscope. The developmental stage of the oöcyte in each ovariole (present studies show that approximately 25 ovarioles are present in each ovary and not 5 as stated by Deshpande (1933)) was assessed using

the scheme below, and the stages described are illustrated in Fig.1. :

Description of the developmental stages of oögenesis.

Stage 0: Little or no development of follicle cells or oöcyte. The ovariole consists solely of the germarium and terminal filament.

Stage 1: An oöcyte has been segregated from the germarium and the first follicle cells are present. The germarium begins to enlarge.

Stage 2: Cuboidal follicle cells have enveloped the oöcyte and nutritive cords (possibly only a single nutritive cord in A. proletella) are present. The germarium is still larger than the developing oöcyte and continues to grow. Towards the end of the stage some yolk deposition occurs giving the oöcyte a faint yellow colouration.

Stage 3: The oöcyte is now larger than the germarium and further yolk deposition gives it a distinct and uniform yellow colour. The follicle cells become more squamous than cuboidal indicating that they stretch, rather than multiply, as the oöcyte develops. The germarium begins to decrease in size.

Stage 4: Further yolk deposition gives the oöcyte a very deep yellow/brown colour which now obscures the nucleus. The germarium shrinks markedly until it is almost the same size as in Stage 1. The nutritive cord still connects the germarium to the oöcyte. Follicle cells begin to envelop a second oöcyte at the base of the germarium and the nutritive cord passes through this to retain contact with the primary oöcyte. By this stage the follicle cells surrounding the primary oöcyte are squamous. A deep yellow cap forms at the oviduct end of the oöcyte and this structure may harbour microorganisms.

Stage 5: Chorion formation takes place along the inner surface of the follicle cells and the connection with the germarium is lost. The pedicel forms at the oviduct end of the oöcyte and this will be inserted into the leaf as a kind of anchor when the egg is laid. At the time of oviposition the secondary oöcyte will have developed as far as late Stage 1 or, as occurs during the warm summer months, early Stage 2. After oviposition, the envelope of follicle cells is left as a loose sheath or "relic" and is readily identifiable in dissections indicating that a female has oviposited.

FIGURE 1.

Illustration of the stages of oöcyte development in *Aleyrodes proletella* L.

TF = Terminal Filament

F = Follicle Cell

G = Germarium

DO = Developing Oöcyte

N = Nucleus

NC = Nutritive Cord

Y = Yolk

P = Pedicel

RC = Red Cap

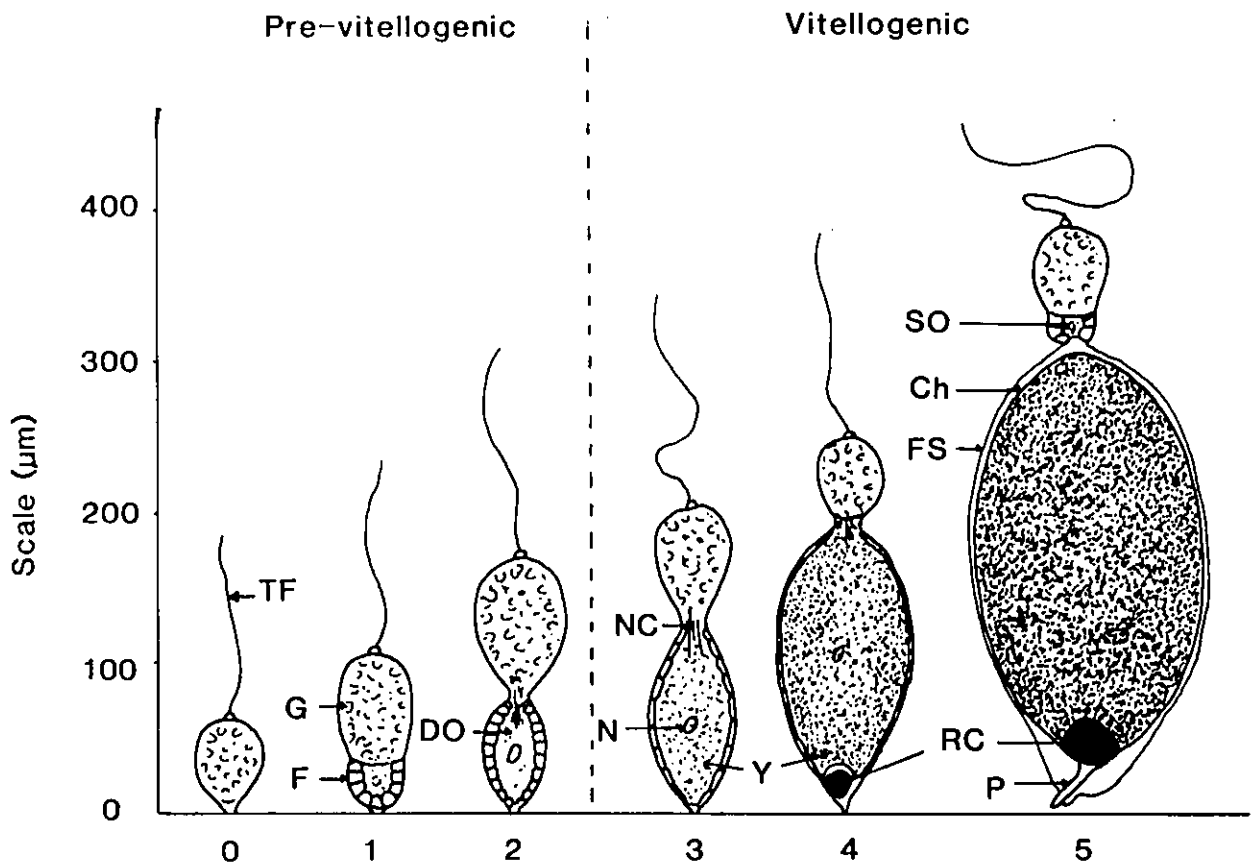
FS = Follicular Sheath

Ch = Chorion

SO = Secondary Oöcyte

(Further description in text.)

FIG. 1. Stages of Oogenesis



Ovarian Score.

An "ovarian score" may be obtained for any dissection using the following formula:

$$\text{Score} = \frac{ns1 + 2.ns2 + 3.ns3 + 4.ns4 + 5.ns5}{N}$$

where nsx = no. oöcytes of stage x

and N = total number of ovarioles observed.

Sensitive Period.

Leaves bearing larvae of various known ages were transferred from LD16:8 15 C to LD 12:12 15 C. When the adults emerged they were kept either as male-female pairs, or in small groups in which the females were the same developmental age when transferred. The pre-oviposition time criterion was used to determine whether the transfer induced diapause.

The range and degree of sensitivity was studied by transferring leaves from LD16:8 15°C to LD12:12 15°C for a few days and then back to LD16:8 15° C. Once again the pre-oviposition time criterion was used to assess the results.

Once the period of peak sensitivity to photoperiod had been established the effect of a wide range of photoperiods could be studied by subjecting the sensitive larvae to test conditions for a selected portion of their

total developmental period. Five 3 gallon honey tins, each fitted with a 12V 5W light bulb were used as the photoperiod chambers (see LEES 1973). The lighting regime in each tin was independently determined by a 5 channel punched tape enabling diel, non-diel and skeleton photoperiods of any description to be presented to the insects.

RESULTS.

Hourly observations of larvae whose position had previosly been mapped were made in order to ascertain whether egg hatch, larva-larva moults or adult emergence occurred on a rhythmic basis. Fig.2 a, b and c have no apparent rhythmic element. However, in all three instances there is a strong tendency to hatch/moult in the light. In Fig.2d it can be seen that approximately 45% of 3rd-4th instar moults occured during the three hours after "lights-on" (=dawn). As a result of these data, larvae were observed as late in the day as possible when their developmental age was being assessed.

Fig.3a shows that there was a distinct emergence rhythm with almost 60% of the adults emerging between one hour before and three hours after lights-on. The numbers of adults emerging in the hour before "dawn" was assessed by counting the number of insects with only partially expanded wings and very little surface wax when the leaf was observed at lights-on. Very few adults emerged during the rest of the night. Field observations have shown that peak emergence occurs during the three hours after dawn and that males tend to emerge before females (EL KHIDIR 1963). A similar trend is apparent in Fig.3b. Since newly emerged males have been observed mating with teneral females (EL KHIDIR 1963, 1972) it is possible that early emergence may facilitate insemination of all the females in the population.

FIGURE 2.

The hourly distribution of a) Egg Hatch; b) 1st to 2nd Instar moults; c) 2nd to 3rd Instar Moults and d) 3rd to 4th Instar moults

FIG. 2.

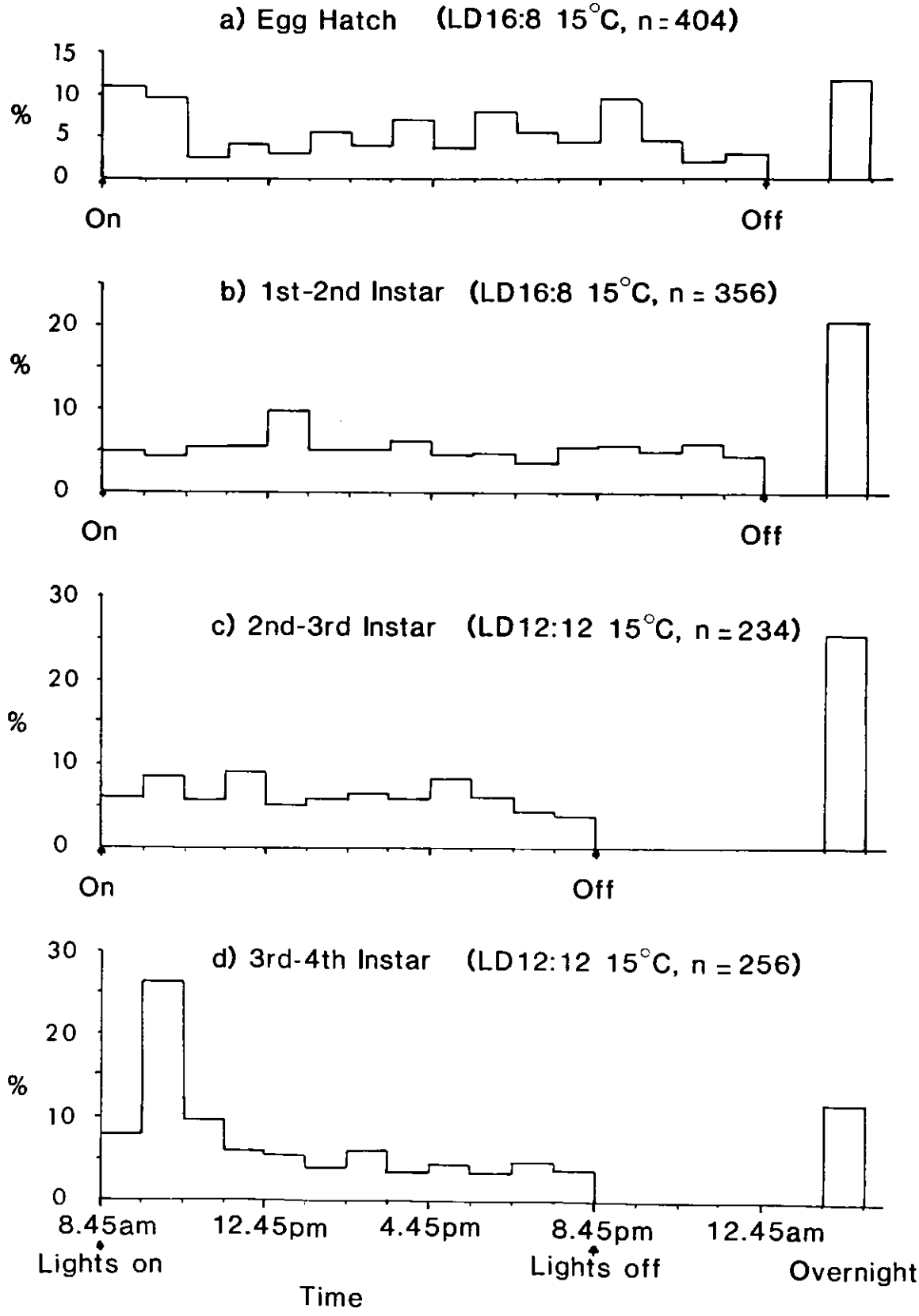
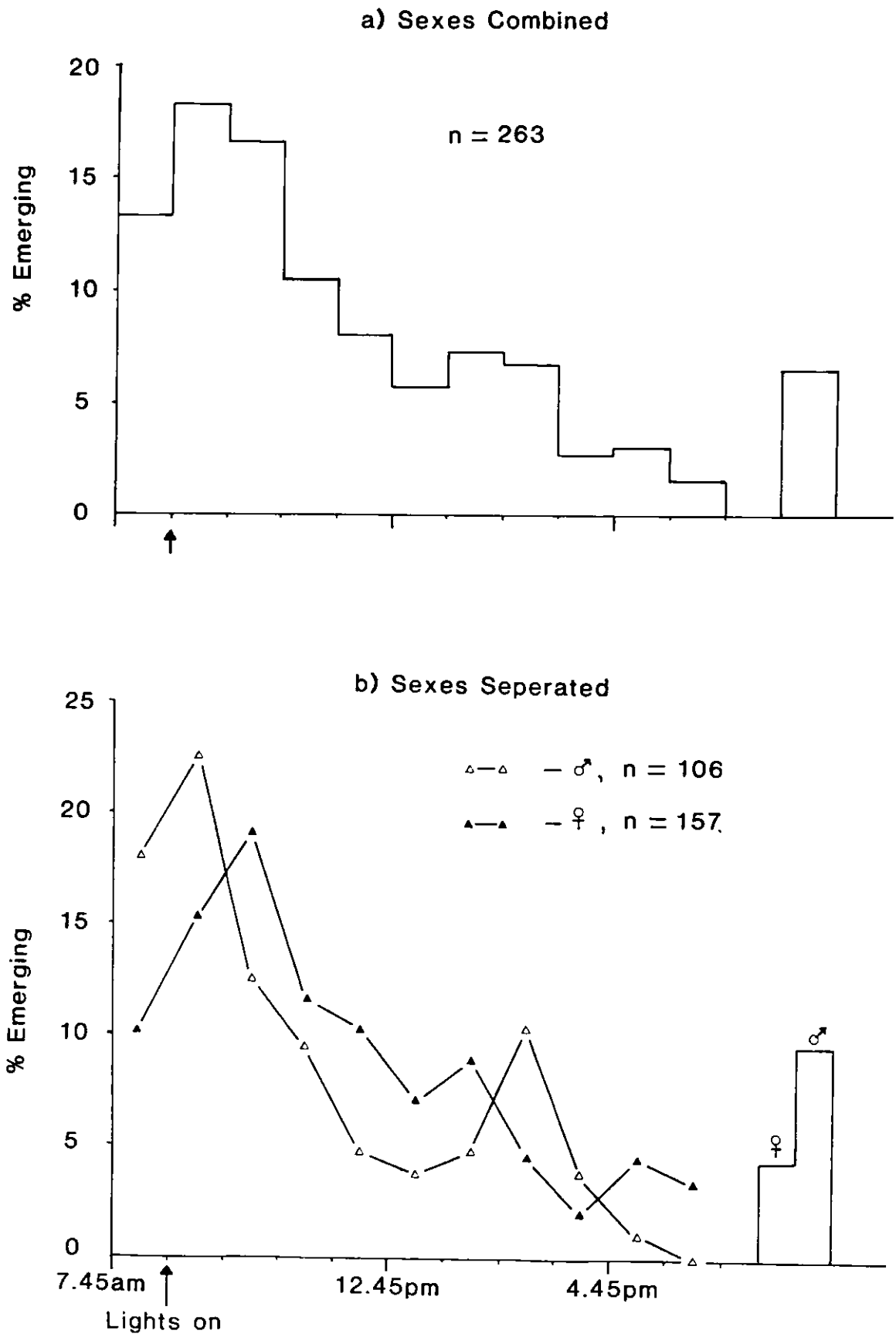

a) Egg Hatch (LD16:8 15°C, n = 404)
15
10
5
0
%
On
Off
b) 1st-2nd Instar (LD16:8 15°C, n = 356)
20
10
0
%
On
Off
c) 2nd-3rd Instar (LD12:12 15°C, n = 234)
30
20
10
0
%
On
Off
d) 3rd-4th Instar (LD12:12 15°C, n = 256)
30
20
10
0
%
8.45am
Lights on
12.45pm
4.45pm
8.45pm
Lights off
12.45am
Overnight
Time

FIGURE 3.

Adult Emergence Rhythm. The number of adults emerging each hour in LD16:8 15°C was recorded over a five day period. The data in a) has been divided into male and female distributions in b). Any adults with partly folded wings at lights-on were considered to have emerged in the previous hour.

FIG. 3.


a) Sexes Combined
n = 263
% Emerging
20
15
10
5
0
b) Sexes Seperated
— ♂, n = 106
— ♀, n = 157
25
20
15
10
5
0
7.45am
Lights on
12.45pm
4.45pm
♀
♂

Iheagwam (1977) has published a photoperiodic response curve for A. proletella with a critical photoperiod of LD15.75:8.25 at 15°C when the insects were reared in the experimental photoperiod throughout their development. The data presented in Fig.4 are in general agreement with the earlier findings, although the critical photoperiod appears to be closer to LD15.5:8.5. The pre-oviposition time criterion was used to assess the percentage of diapausing females in each photoperiod.

It is apparent from Fig.5 that ovarian development commences before emergence when whitefly are reared in LD16:8 15°C. Newly emerged females have an ovarian score of around 1.1 and contain a few vitellogenic oöcytes. By day 4 an equilibrium score of around 2.3 has been reached as the proportion of vitellogenic oöcytes increases. At this stage oviposition commences thereby accounting for the fall in stage 5 oöcytes. Since the secondary oöcyte starts to develop prior to the oviposition of the primary oöcyte, there is a progressive reduction in the proportion of stage 0 oöcytes until oögenesis has started in all the ovarioles. An equilibrium ovarian profile is reached by day 14 of adult life. These data were used as a non-diapause "standard".

When females were reared in LD12:12 15 C throughout development there was relatively little pre-emergence ovarian development (Fig.6) and the rate of ovarian maturation was clearly retarded compared with Fig.5. Almost 70% of the oöcytes were still pre-vitellogenic 21 days after emergence and, although an equilibrium score of

FIGURE 4.

Photoperiodic Response Curve of *Aleyrodes proletella*.

Eggs that had been laid in LD16:8 20°C the previous day were transferred to one of six stationary photoperiods at 15°C. The females were isolated at emergence and %Diapause was assessed using the pre-oviposition time criterion. The smaller figures indicate the number of females in each condition.

FIG. 4.

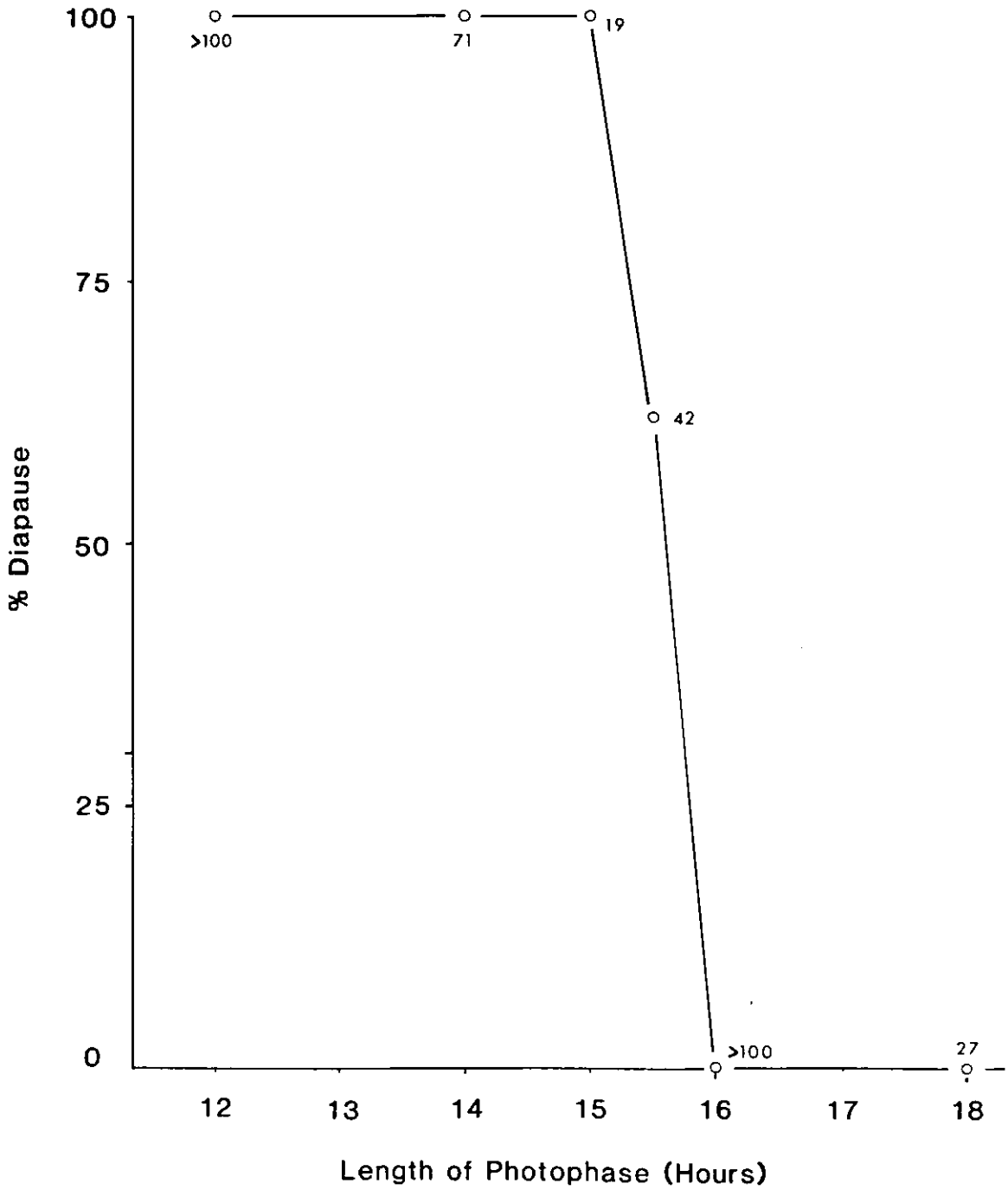

100
75
50
25
0
% Diapause
>100
71
19
42
>100
27
12
13
14
15
16
17
18
Length of Photophase (Hours)

FIGURE 5.

Long-Day Ovarian Development. Females that had been reared in LD16:8 15°C were collected within two hours of emergence and kept in daily groups, still in LD16:8 15° C, until dissection up to 21 days later. The ovaries were scored and the mean percentage of each ovarian developmental stage was also recorded for each sample.

FIG. 5.

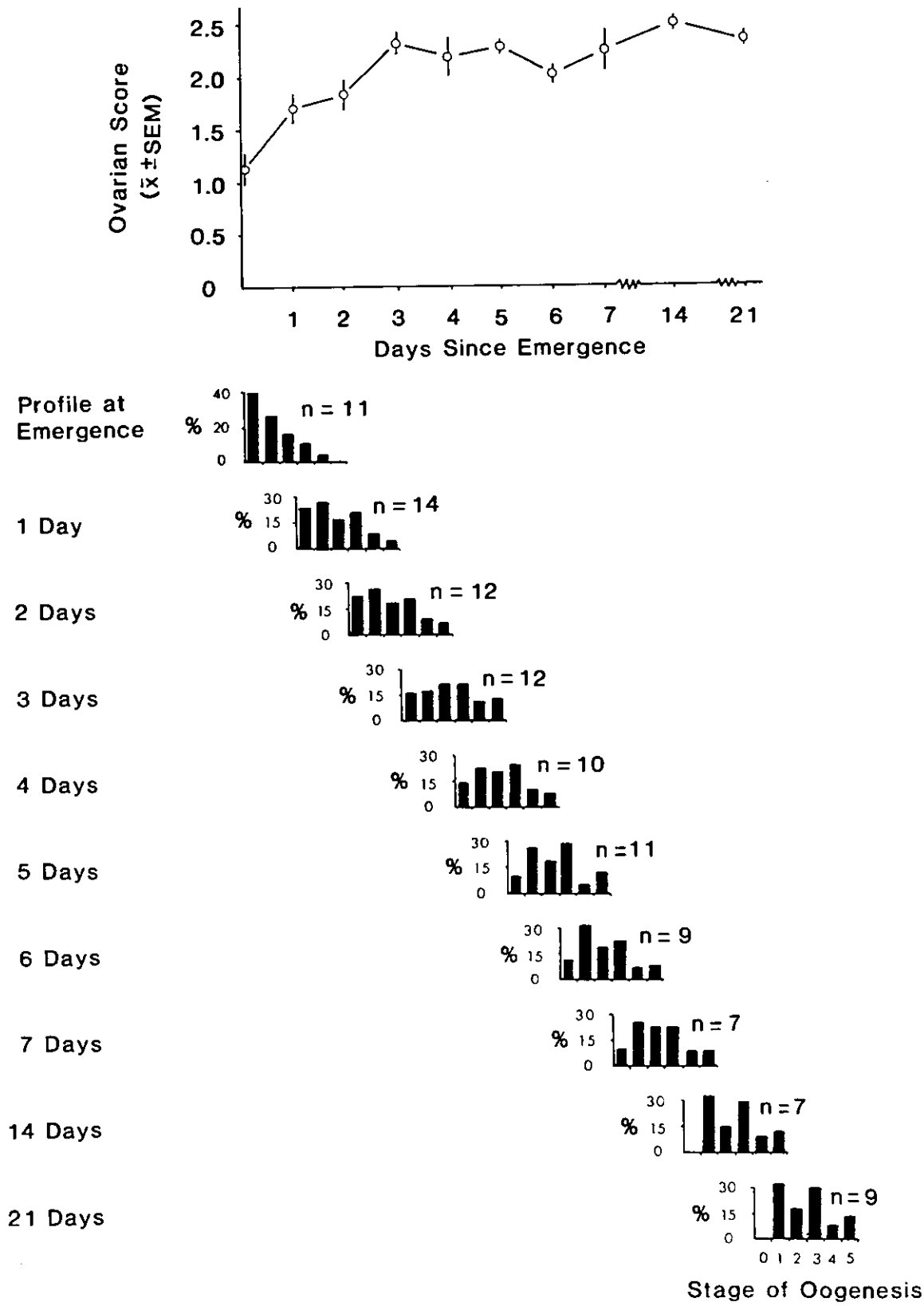
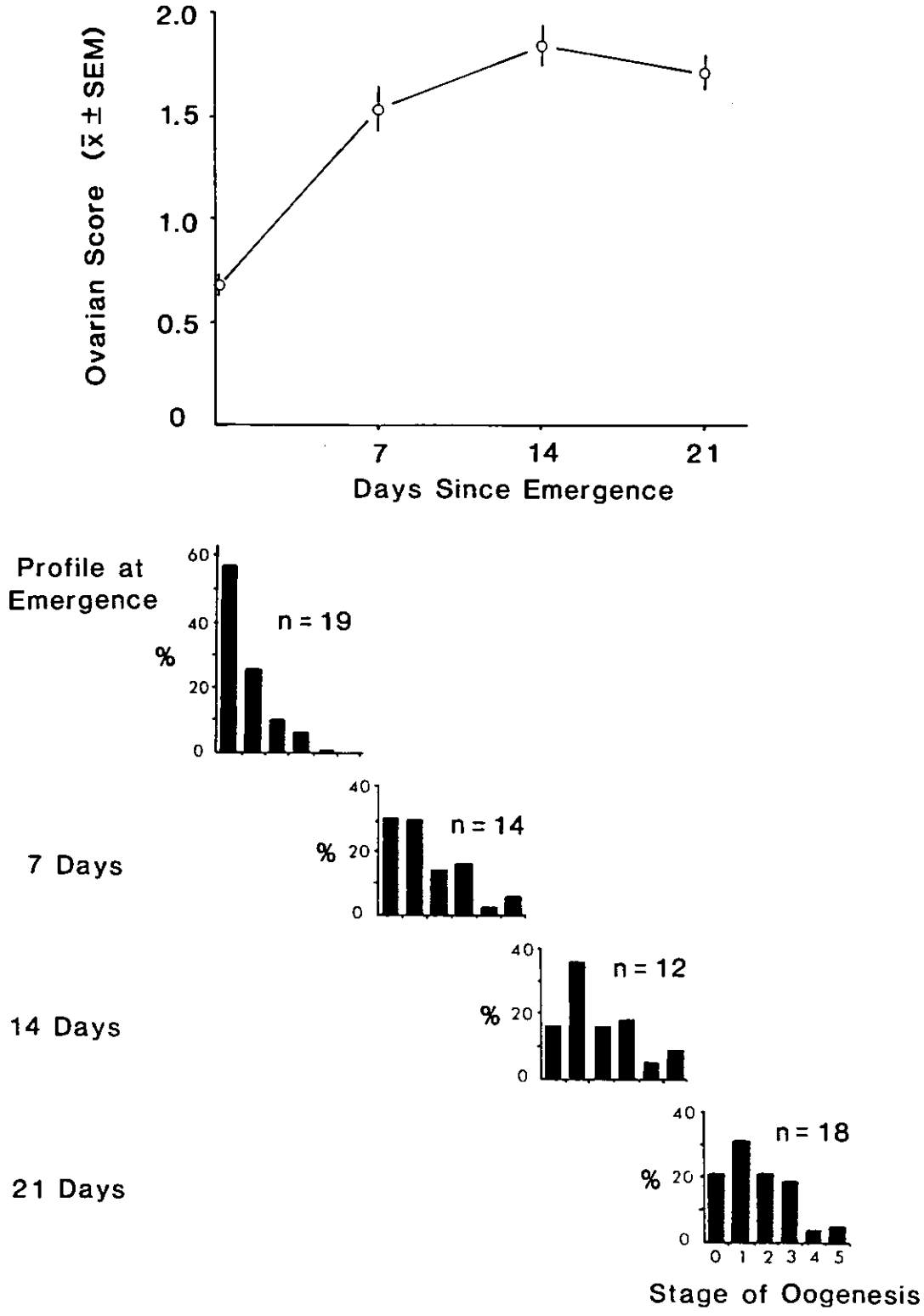

Ovarian Score
(x̄ ±SEM)
2.5
2.0
1.5
1.0
0.5
0
1 2 3 4 5 6 7 14 21
Days Since Emergence
Profile at Emergence
1 Day
2 Days
3 Days
4 Days
5 Days
6 Days
7 Days
14 Days
21 Days
n = 11
n = 14
n = 12
n = 12
n = 10
n = 11
n = 9
n = 7
n = 7
n = 9
%
0 1 2 3 4 5
Stage of Oogenesis

FIGURE 6.

Short-Day Ovarian Development. Females reared in LD12:12 15°C were collected daily as they emerged and kept in LD12:12 15°C until dissection, up to four weeks later. Mean ovarian score and mean percentage of each developmental stage were recorded for each sample.

FIG. 6.


Ovarian Score ($\bar{x} \pm$ SEM)
2.0
1.5
1.0
0.5
0
7
14
21
Days Since Emergence
Profile at Emergence
n = 19
7 Days
n = 14
14 Days
n = 12
21 Days
n = 18
%
Stage of Oogenesis

about 1.7 was reached by day 7, the ovarian profile was still changing two weeks later. These females were collected on the day of emergence and kept in groups until dissection and some of them oviposited before 21 days had elapsed (i.e. they would not have been classified as diapausing) which suggests that the isolated females in Fig.4 are in a low intensity diapause that is influenced, in some way, by the presence of other adults. However, this was not investigated.

Diapause could be induced by transferring larvae from LD16:8 to LD12:12 15°C. In Fig.7 it can be seen that such a transfer is only inductive if it occurs on or before day 2 of the 3rd instar (3(2)). Transfers later in development were less effective and no diapause was observed in females transferred after 4(5). These results were obtained using the pre-oviposition criterion for diapause assessment and are in agreement with those of Iheagwam (1977).

Daily batches of newly emerged adults were taken from leaves bearing larvae that had been in the 1st or 2nd instar when the leaf was transferred from LD16:8 to LD12:12 15°C. The adults were dissected and their ovaries scored at weekly intervals until 4 weeks after emergence. Table 2 shows that there were no significant differences in ovarian score during this post-emergence period. The grouped data from Table 2, combined with results from adults that had been either 1st or 2nd instar larvae on the same leaf when it was transferred, are shown in Fig.8. It is clear that pre-emergence ovarian development is

FIGURE 7.

Photoperiodic Sensitivity. Eggs and larvae of different developmental ages were transferred from LD16:8 15°C to LD12:12 15° C. The adult females were isolated at emergence and kept in LD12:12 15°C. Diapause was assessed using the pre-oviposition criterion. The small figures represent the number of insects studied at each stage.

FIG. 7.

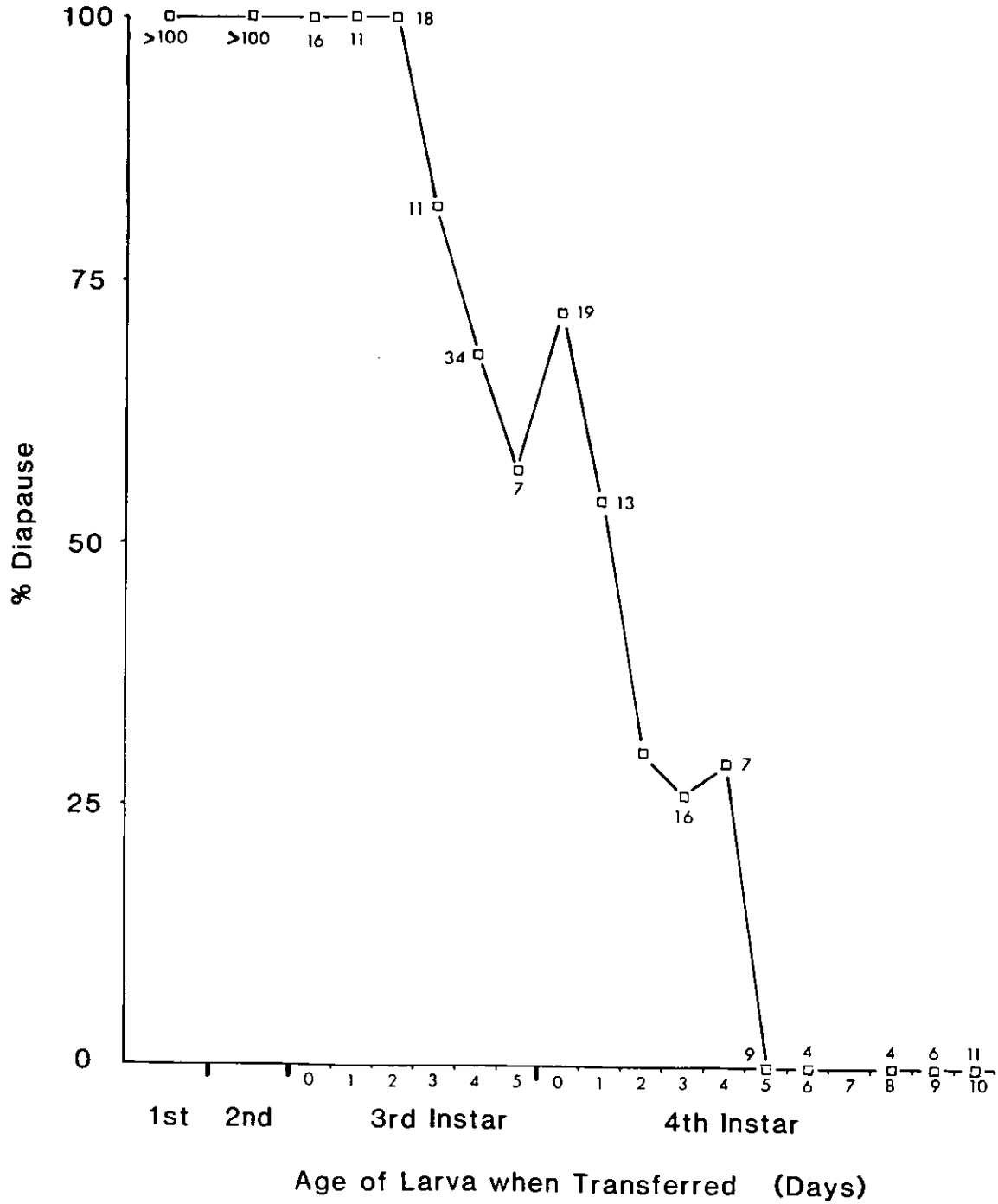

100
75
50
25
0
% Diapause
>100
>100
16
11
18
11
34
7
19
13
16
7
9
4
6
11
0 1 2 3 4 5 0 1 2 3 4 5 6 7 8 9 10
1st
2nd
3rd Instar
4th Instar
Age of Larva when Transferred (Days)

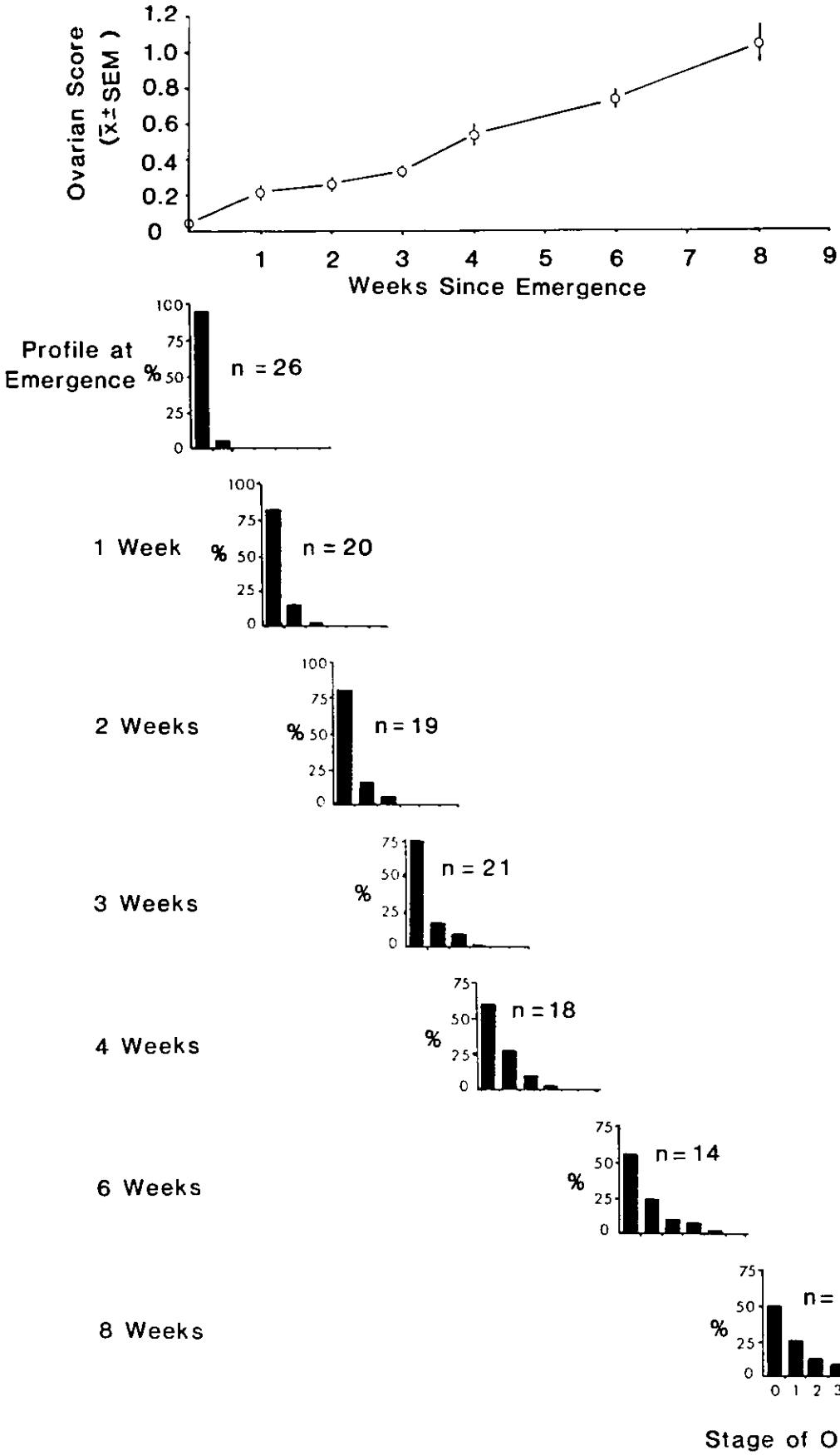
TABLE 2. Post Emergence Ovarian Development Following a Transfer from LD16:8 15°C to LD12:12 15°C during the 1st or 2nd Instar.

Time since Emergence	Ovarian Score $\bar{x}$ ± SEM.	
	1st Instar	2nd Instar
2 hours	0.08±0.02 (n=11)	0.03±0.01 (n=15)
1 week	0.19±0.02 (n=9)	0.24±0.03 (n=11)
2 weeks	0.29±0.03 (n=10)	0.23±0.05 (n=9)
3 weeks	0.31±0.03 (n=10)	0.34±0.06 (n=11)
4 weeks	0.53±0.07 (n=10)	0.54±0.12 (n=8)

FIGURE 8.

Ovarian Development of "Induced" Females. Whitefly were transferred from LD16:8 15°C to LD12:12°15 C during the first or second instar. The resulting adult females were collected daily as they emerged and kept in groups in LD12:12 15°C until dissection up to eight weeks later. Mean ovarian score and profile were recorded for each sample.

FIG. 8.


Ovarian Score
(X̄ ± SEM)
1.2
1.0
0.8
0.6
0.4
0.2
0
1 2 3 4 5 6 7 8 9
Weeks Since Emergence
Profile at Emergence
n = 26
1 Week
n = 20
2 Weeks
n = 19
3 Weeks
n = 21
4 Weeks
n = 18
6 Weeks
n = 14
8 Weeks
n = 15
%
100
75
50
25
0
0 1 2 3 4 5
Stage of Oogenesis

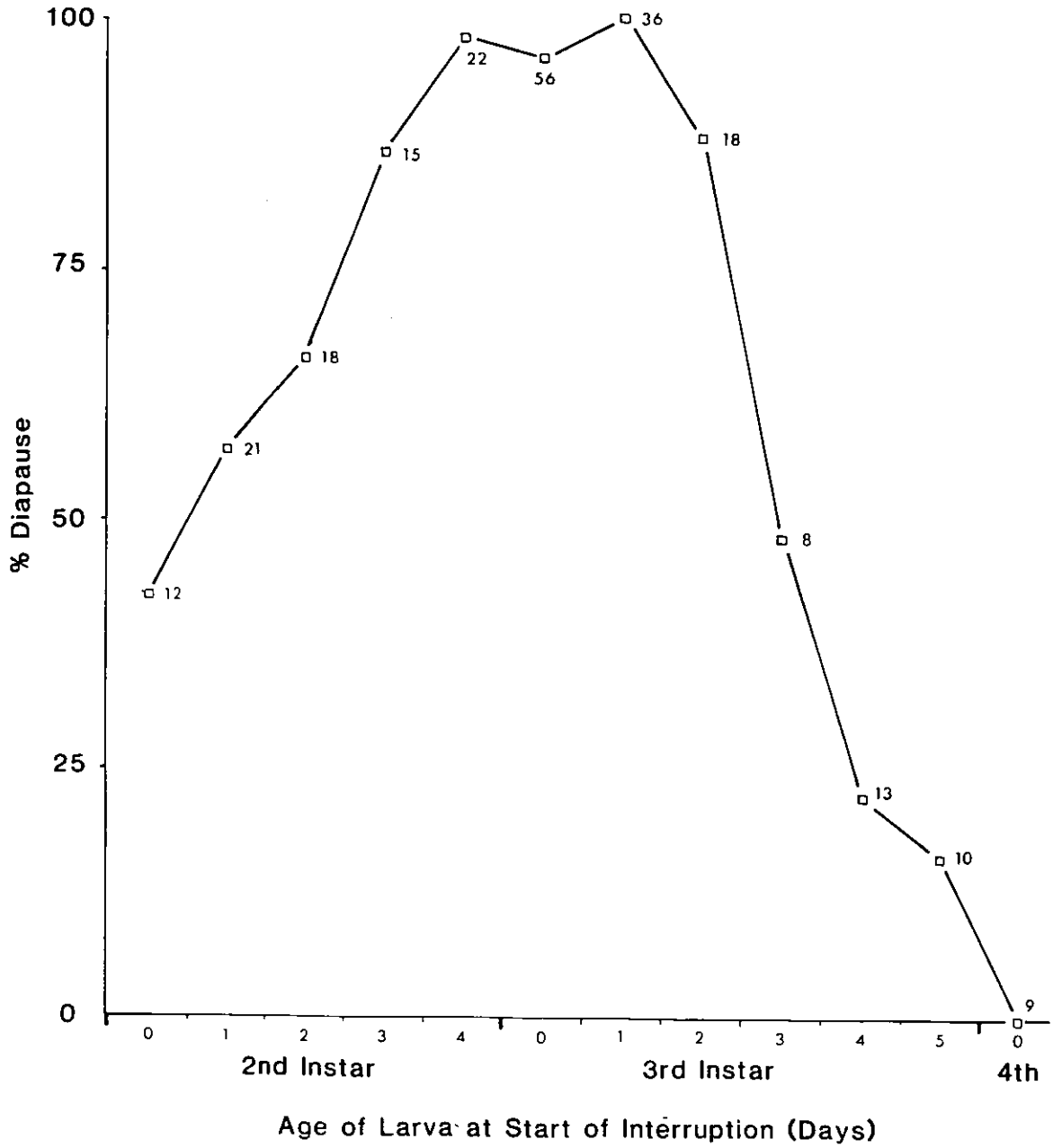
almost completely inhibited by a diapause inducing transfer and that post-emergence development progresses at a much lower rate than when the whitefly were kept in LD12:12 throughout development (Fig.6). If diapause determination was based on the number of short-day cycles experienced during the sensitive period then the intensity of the response in Figs.6 and 8 should have been the same. Thus, it is clear that the long-day to short-day transition is a very important feature of the inductive process.

In the next series of experiments, larvae of different developmental ages were transferred from LD16:8 to LD12:12 15°C for a period of 10 days before being returned to LD16:8. The results are illustrated in Fig.9 and pre-oviposition time was the diapause criterion. Over 50% of the females entered diapause if the 10 day period commenced between 2(1) and 3(2) with over 95% diapause resulting from a transfer on 2(4), 3(0) or 3(1). Some of the diapausing females were dissected and their ovaries scored 21 days after emergence. The scores obtained were usually between 0.3 and 0.5 suggesting that a similar diapause intensity was being induced to that occurring in the single transfer experiments (see Fig.8). By removing larvae that were developmentally young or old, it was possible to expose leaves bearing larvae between 2(4) and 3(1) to experimental photoperiods for the most sensitive part of their life cycle rather than throughout development. It was also possible to keep the adults emerging after experimental treatment in large groups

FIGURE 9.

Localised Photoperiodic Sensitivity. Larvae were transferred from LD16:8 15°C to LD12:12 15°C for a period of ten days commencing at different developmental ages. They were then returned to long day conditions and the females were isolated at emergence. Diapause was assessed using the pre-oviposition time criterion. The small figures represent the number of recordings for each developmental age.

FIG. 9.


100
75
50
25
0
% Diapause
12
21
18
15
22
56
36
18
8
13
10
9
0 1 2 3 4 0 1 2 3 4 5 0
2nd Instar
3rd Instar
4th
Age of Larva at Start of Interruption (Days)

without any significant effect on their ovarian development and dissect them 21 days after emergence to measure the proportion of diapausing individuals. Using this procedure, sensitive larvae were exposed to a range of diel photoperiods and the results, illustrated in Fig.10 showed that the critical photoperiod was almost identical to that in Fig.4.

Table 3 shows that the "inductive value" of short-day cycles is different. It has already been demonstrated that 10 LD12:12 cycles have different inductive effects depending on their position in the larval stage (Fig.9) and it is clear from Table 3 that the differences in sensitivity are quite substantial. As few as 7 short days are fully inductive if they start at 3(0) whilst as many as 17 cycles commencing at 1(3) are insufficient to cause 100% of the females to enter diapause.

The effectiveness of 7 LD16:8 cycles, interrupting a LD12:12 regime, in averting diapause, is shown in Fig.11. Diapause was assessed using the pre-oviposition time criterion. There were no indications of any developmental period where the long day treatment was effective which is a further indication that most of the larval developmental period exhibits some degree of sensitivity. Although the diapause value of a short day is highest during the 3rd instar, Fig.11 shows that the short day information that accumulates during the other days is sufficient to exceed the "threshold" and determine diapause. The results in Fig.11 also indicate that long days do not "destroy" the short day information that precedes them.

FIGURE 10.

Photoperiodic Response Curve. Larvae, reared in LD16:8 15 °C were transferred to the experimental diel photoperiod for a period of ten days commencing on or between the fourth day of the second instar, 2(4), and the first day of the third instar, 3(1). They were returned to LD16:8 15 °C and kept in a group until dissection, 21 days after emergence. Diapause was assessed using the ovary condition criterion.

FIG. 10.

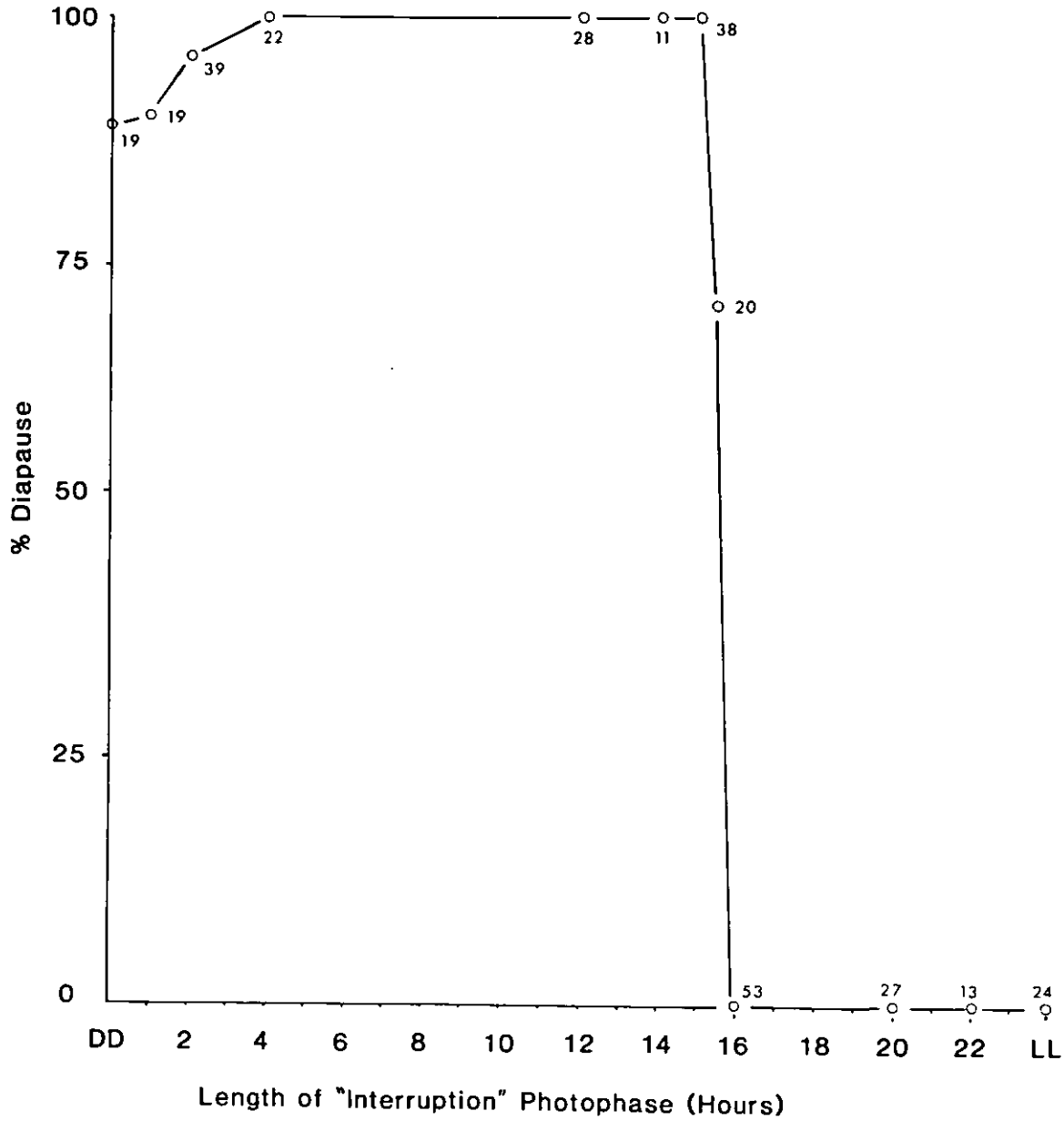

100
75
50
25
0
% Diapause
19
19
39
22
28
11
38
20
53
27
13
24
DD
2
4
6
8
10
12
14
16
18
20
22
LL
Length of "Interruption" Photophase (Hours)

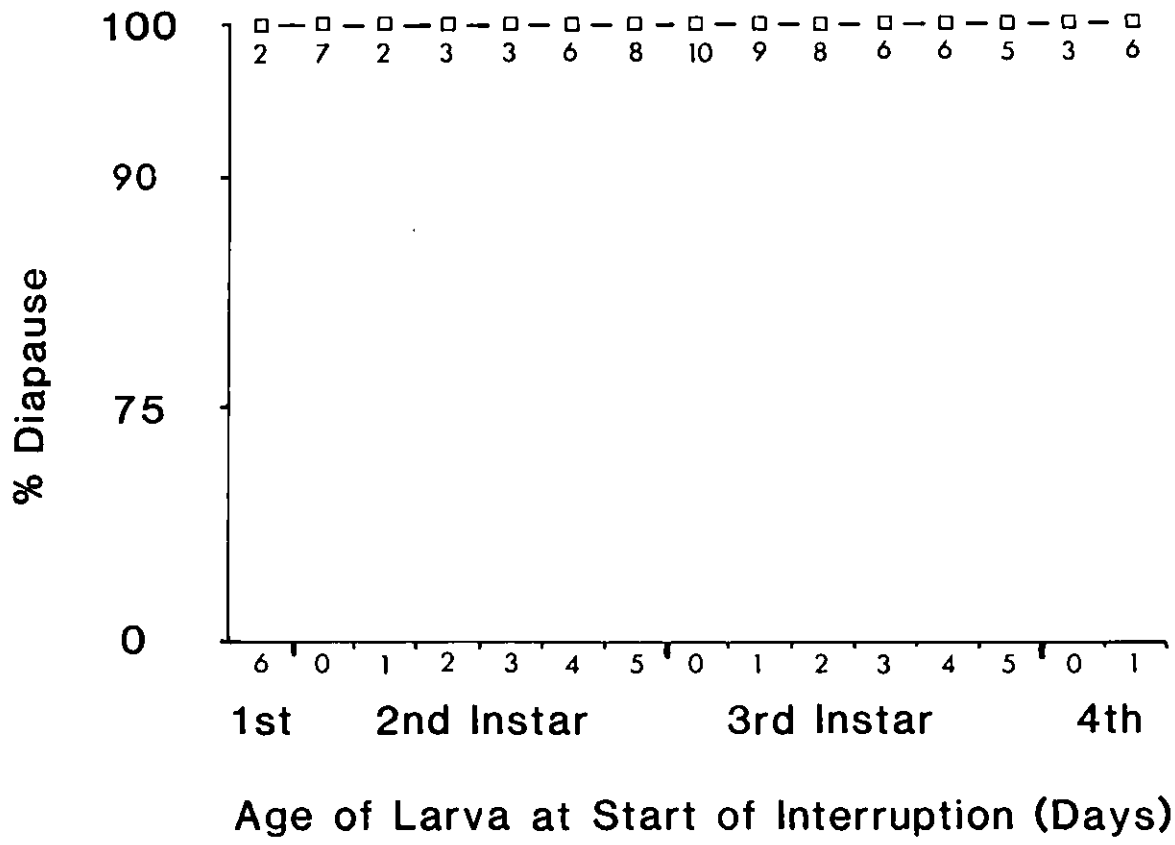
TABLE 3. The Incidence of Diapause when a Photoperiodic Regime of LD16:8 15°C is Interrupted by periods of LD12:12 15°C Cycles Commencing at two Stages of Development.

Age of Larva at Start of Interruption.	Number of Cycles.	Number of Larvae.	Diapause (%).
1(3)	12	11	45
1(3)	14	16	69
1(3)	17	15	82
3(0)	7	26	100
3(0)	8	10	100

FIGURE 11.

Diapause Averting Effect of Long Days. Larvae were reared in LD12:12 15°C. Transfers to long days were made at different developmental ages. The larvae spent 7 days in LD16:8 15° C before they were returned to short day conditions. Females were isolated at emergence and diapause was assessed using the pre-oviposition time criterion. The small figures represent the number of individuals tested at each developmental age.

FIG. 11.


100
90
75
0
% Diapause
2 7 2 3 3 6 8 10 9 8 6 6 5 3 6
6 0 1 2 3 4 5 0 1 2 3 4 5 0 1
1st
2nd Instar
3rd Instar
4th
Age of Larva at Start of Interruption (Days)

DISCUSSION

The oögenesis of A. proletella is of the acrotrophic (=telotrophic) type, typical of the hemiptera, with nutritive, cytoplasmic cords connecting the nurse cells to the developing oöcyte (ENGELMANN 1970; CHAPMAN 1971). A previous study of the structure of the whitefly reproductive system stated that each ovary was composed of five ovarioles which, in turn, contained a string of oöcytes (DESHPANDE 1933). Present studies clearly show that this is not the case. Instead, each ovary contains, approximately, 25 ovarioles in which the secondary oöcyte does not commence development until the primary oöcyte has reached stage 4 or 5 (Fig.1). Dissections of the greenhouse whitefly, Trialeurodes vaporariorum indicate that, like A. proletella, the secondary oöcyte does not commence development until the primary oöcyte has reached quite an advanced stage (stage 3 for this species) and does not develop beyond the pre-vitellogenic stage until the primary oöcyte has been oviposited (ADAMS unpublished observations). Within the ovariole of another hemipteran, Rhodnius prolixus, only one oöcyte undergoes vitellogenesis at any one time (PRATT and DAVEY 1972).

Usually the definition of stages of ovarian development has been based on a visual description of the morphology of the developing oöcyte (eg. CHRISTOPHERS 1911; SPEILMAN and WONG 1973; ADAMS 1974; VENKATESH and MORRISON 1980; O'MEARA and LOUNIBOS 1981) however, the

most recent classification of the stages of oogenesis in Aedes aegypti has incorporated vital staining to elaborate upon previous work (CLEMENTS and BOOCOCK 1984). This technique permitted the identification of early vitellogenic and resorbing follicles and provided a much more precise and dynamic account than any of its predecessors. Similar studies will, undoubtedly, prove to be invaluable in the study of so-called "developmental gates" in oögenesis where development ceases unless and until a particular stimulus is experienced (BARTON BROWNE et al. 1979) but it is unnecessarily time consuming if a measure of the ovarian development of a sample at any one time is sought as a comparitive measure. A common index for this is the length of a sample of terminal oöcytes (eg. GWADZ and SPEILMAN 1973; SANBURG and LARSEN 1973; ADAMS 1974; VENKATESH and MORRISON 1980) but this is best suited to species with a marked, synchronous gonotrophic cycle whilst whiteflies develop their ovaries continuously. Consequently, the scheme presented in Fig.1 is more appropriate for A. proletella.

Hourly recordings of egg hatch, larva-larva moults and adult emergence suggested that only the 3rd-4th instar moult and adult emergence had any rhythmic basis in A. proletella (Figs.2 and 3). In the absence of observations in prolonged dark periods however, the circadian nature and natural periodicity (τ) remain equivocal (SAUNDERS 1982b). Nevertheless, it may be possible to construct a Phase Response Curve (PRC) for this species using either or both of these rhythms and the potential value of this

in the study of the photoperiodic clock will be discussed later (see Chapter 3).

The Photoperiodic Response Curve (PhRC) of A. proletella reared in stationary photoperiods (Fig.4) is typical of a "long-day" insect (DANILEVSKII 1965; LEES 1968) with continuous development in photoperiods of 16 hours or more, and diapause when the photoperiod falls below 15 hours. An almost identical PhRC was recorded for this species by Iheagwam (1976, 1977). Comparable response curves have been observed in numerous other species (see LEES 1968; SAUNDERS 1982b). The critical photoperiod is important, ecologically, in determining that individuals do not invest energy either in egg production or in further larval development if subsequent cold susceptible developmental stages would be likely to experience unfavourable autumn and winter conditions. Instead, diapause is determined and the insect is able to overwinter in a specific, cold resistant stage and adjust its whole life cycle to seasonal changes. It is clear that the long-day response has evolved to enhance the chances of surviving cold conditions and yet the primary stimulus for diapause is the photoperiod. Photoperiod is a reliable "noise-free" (provided there is some temperature compensation) predictor of forthcoming conditions, that gives the insect time to prepare, physiologically, for unfavourable conditions rather than responding to them in a more direct and immediate manner as in quiescence (SAUNDERS 1982b). The selective pressure that moulded the PhRC, and the critical photoperiod in

particular, is likely to have been considerable since late entry into diapause would expose some individuals to unfavourable conditions, possibly resulting in a high degree of mortality, whilst an early entry might supply other species in a similar habitat with a competitive edge.

Female cabbage whiteflies, reared in LD16:8 15° C oviposit their first egg about three days after emergence, although higher temperatures substantially reduce the pre-oviposition time (IHEAGWAM 1976). Measurements of the post- emergence ovarian development of this insect show that an equilibrium ovarian score of around 2.3 was reached at the time of first oviposition although it was another week before oögenesis had commenced in every ovariole (Fig.5). It is clear that oögenesis commences some time prior to emergence under long day conditions.

When LD12:12 15° C was used as a stationary photoperiod, no oviposition was observed in females either isolated or kept in male-female pairs for twenty one days after emergence (Fig.4). This is a typical response to a short photoperiod and previous work has shown that the mean pre-oviposition time under these conditions is over two months (IHEAGWAM 1976, 1977). Protracted pre-oviposition times have also been demonstrated for other species kept in short day conditions in the laboratory (eg. NORRIS 1962, 1965; TAUBER et al. 1970a; HODEK 1971d) whilst even longer pre-oviposition times occur in the same species under field conditions which is probably due to a combination of low temperatures and

short photoperiods (see Chapter 4). In view of these considerations, the ovarian profiles presented in Fig.6 are most surprising. Although the ovarian score at emergence and the subsequent rate of development are much reduced when compared with Fig.5, the profile as little as seven days after emergence reveals that some stage 5 oöcytes are present so at least some of the females in the sample must have been either intermediates or non-diapausing. Overwintering A. proletella have been recorded as having very small ovaries, without any mature eggs (EL KHIDIR 1963), and this is also the case in numerous other species which diapause with pre-vitellogenic oöcytes such as the thrips, Anaphothrips obscurus (KAMM 1972), the coccinellid, Coccinella transversoguttata (STORCH 1973), the stink bug, Nezara viridula (ALI and EWIESS 1977) and the carabid Pterostichus nigrita (FERENZ 1977). Since it has been shown that A. proletella only enters an intense diapause in response to a transfer from long to short days (Fig.8), the main question raised by the results in Fig.6 concerns the differences when stationary short day females are isolated at emergence compared to when they are kept in mixed groups of about twenty adults. One possibility is that crowding is responsible for overriding the "weak" diapause inducing effect of a stationary photoperiod. In the black bean aphid, Aphis fabae, crowded short day aphids develop into alate gynoparae, whilst uncrowded short day aphids become apterous gynopara producers (LEES and HARDIE 1981; LEES 1983). This response to crowding has been shown to be the result of tactile stimulation

received by the first instar larvae (LEES and HARDIE 1981). However, it is unlikely that larval crowding is the causal factor in A. proletella since the larvae are sessile and larva-larva overlap was rare at the densities used in these experiments. When overlaps did occur, the larva "on top" usually died at the subsequent moult. Similarly, the adult densities were too low for any great degree of "jostling" to occur so it seems more likely that some chemical factor is responsible although no experiments have been performed to investigate this possibility.

Transferring insects of different developmental stages from a diapause averting to a diapause inducing photoperiod has been the method most commonly used in the search for the periods during which the insect is sensitive to the light regime. However, some of the conclusions drawn are based on experiments that lack detail and tend to treat a long period of development, such as all the larval instars, as one stage. In several species that exhibit an ovarian diapause, adult sensitivity to photoperiod has been demonstrated. The Colorado beetle, Leptinotarsa decemlineata, entered diapause when a transfer to short days occurred immediately after adult emergence and the adult is the major photosensitive stage (DE WILDE et al. 1959) although food quality also influences the induction process (DE WILDE and FERKET 1967). Other species in which photoperiodic sensitivity of the adult has been demonstrated include Coccinella septempunctata (HODEK and

CERKASOV 1961), Co. transversoguttata (STORCH 1973), Hypera postica (ROSENTHAL and KOEHLER 1968), Pyrrhocoris apterus (HODEK 1968 cited in HODEK 1971a), Chrysopa carnea (TAUBER and TAUBER 1969) and Ch. harisii (TAUBER and TAUBER 1974). However, close examination of the late larval stages of P. apterus revealed an additional phase of photoperiodic sensitivity since diapause could be averted in some individuals if the short day treatment did not commence prior to three days before larva-adult ecdysis (HODEK 1971a) whilst Ch. carnea exhibited maximum diapause intensity when the transfer to short days occurred during the cocoon stage third instar or the pupal stage (TAUBER and TAUBER 1970b). In Notonecta undulata, the fifth instar and adult appear to be the stages with greatest sensitivity (VANDERLIN and STREAMS 1977). Cumulative sensitivity of all developmental stages was inferred from experiments with Chilocorus bipustulatus (TADMOR and APPLEBAUM 1971) but since the entire larval stage was subjected to one combination of photoperiod and temperature, and the adult to another, it is impossible to judge whether this was true or whether the stage of maximum sensitivity had simply been masked. A simple cumulative theory was not supported by work with Culex pipiens although no truly critical sensitive stage was isolated either and sensitivity appeared to consist of a complex interaction during development (SANBURG and LARSEN 1973).

Of the hemiptera that enter an ovarian diapause, 100% diapause was induced when Nezara viridula was transferred to short days before the end of the third instar but the incidence of diapause fell as the transfer occurred later in development with some (31%) females diapausing when transferred as fifth instar larvae but none when the adult stage alone experienced the short photoperiod (ALI and EWIESS 1977). Similarly, previous work with A. proletella has shown that a reduction in diapause incidence occurs as the transfer to short days is delayed until late in the third instar, with zero diapause when "early" fourth instar larvae or teneral adults were transferred (IHEAGWAM 1976, 1977). The results presented in Fig.7 are largely in agreement with Iheagwam's data, although it is apparent that sensitivity extends into the fourth instar, something which may have been masked if Iheagwam's early fourths were comprised larvae five or more days into the instar.

Post-emergence ovarian scores and profiles observed when A. proletella larvae were transferred from LD16:8 15°C to LD12:12 15°C are shown in Table 2 and Fig.8. Clearly, the transfer has been effective at preventing pre-emergence ovarian development compared to the stationary short day results (Fig.6) and the intense diapause induced occurs amongst females kept in groups of twenty or more individuals. Thus, if a crowding effect of some kind is responsible for the relatively "labile" stationary short day effect, some other factor must be at work to cause the inhibition of pre-emergence oögenesis in

one case, whilst failing to prevent it in the other. This effect appears to be attributable to the change in photoperiod. Since the field photoperiod is shortening as the whiteflies enter diapause, it seems likely that the detection of a change in daylength has an important role in the induction of an intense diapause in nature. Stationary short day treatment may result in a long pre-oviposition period due to the lack of long day stimulation rather than short day inhibition of ovarian development.

A "long-day - short-day" photoperiodic effect was first demonstrated with the red locust, Nomadacris septemfasciata which only entered an intense reproductive diapause when the hoppers experienced a longer photoperiod than the adults (NORRIS 1962, 1965). A comparable effect was demonstrated with the mosquito, Culiseta inornata, which did not enter diapause if kept in LD12:12 10° C throughout development, but did so if a transfer from LD16:8 20°C to LD12:12 10°C had occurred, although the inductive effect was much less when the transfer took place after adult emergence (HUDSON 1977). Unfortunately, the relative effects of temperature and photoperiod changes were not clearly established, however there was little doubt that a change of some sort was necessary. Similarly, a short diapause (as determined by the pre-oviposition time) and "pale" waxy green/ yellow winter colouration resulted when Chrysopa carnea was reared in stationary short day conditions (TAUBER et al. 1970b) whilst a long day to short day transfer induced an intense

diapause accompanied by the typical pale yellow/red winter colour (TAUBER and TAUBER 1970b). Working with Leptinotarsa decemlineata, Hodek (1971a) subjected adults to a near critical photoperiod and larvae to either the same photoperiod or a long day. It was already known that the adult was the principal sensitive stage (DE WILDE et al 1959) however, Hodek recorded a higher incidence of diapause when the larvae were kept in long days followed by the intermediate photoperiod. Although a large amount of variation in the incidence of diapause would be expected from several replicates using a stationary critical photoperiod, these results demonstrated that L. decemlineata also exhibits a slight long day-short day effect. Therefore, it seems likely that closer investigation of diapause intensity resulting from stationary and transfer photoperiodic treatments will reveal a long day-short day effect of some sort in the full diapause response of some other species.

An alternative way of assessing the relative sensitivity of different developmental stages to photoperiod is to subject the insect to diapause inducing conditions for a pre-determined period whilst the remainder of developmental time is spent in diapause averting or neutral conditions. Unfortunately, few detailed studies have been performed with species entering an ovarian diapause. However, sequences of short days have been inserted into the developmental period of the red spider mite, Tetranychus urticae (VEERMAN 1977a). It was revealed that seven short day cycles could induce a

maximum of close to 50% diapause when they were experienced during the protonymphal and deutonymphal instars. Longer short day sequences were not investigated. Amongst species that diapause during developmental stages other than the adult, maximum photoperiodic sensitivity has been shown to lie in the Sarcophaga crassipalpis (DENLINGER 1971; GNAGEY and DENLINGER 1984) and another flesh fly, S. argyrostoma (SAUNDERS 1971). In the latter case, differential sensitivity was revealed which declined during the larval stages and was lost shortly before puparium formation (SAUNDERS 1971; SAUNDERS and BRADLEY 1984).

Interrupting the long day development of A. proletella with ten LD12:12 15°C cycles induced over 95% diapause when the transfer to short days occurred on or between 2(4) and 3(1) suggesting that maximum sensitivity is exhibited by the third and early fourth instars (Fig.9). In Fig.10 the PhRC recorded when the experimental photoperiod commenced during this developmental period is shown and the critical photoperiod is the same as before (Fig.4). Thus, an experimental photoperiod can be presented to the insect at this stage in the knowledge that previous and subsequent long day treatment will not override a short day effect. Extensive use of this feature will be made in subsequent work. The significance of the results in very short photoperiods and constant darkness will be discussed later (Chapter 3).

From Fig.9 and Table 3 it is apparent that seven (and possibly fewer) short day cycles experienced during the phase of peak sensitivity may induce 100% diapause whilst seventeen cycles commencing much earlier in development, but still covering that period, only result in 82% diapause. This suggests that the time at which the transfer to short days occurs influences the "inductive value" of the cycles experienced in the remainder of the sensitive period. It has already been shown that post-emergence ovarian developmental rate is essentially identical when a single transfer to short days takes place during the first or second instar (Table 2) which, at first sight, contradicts the inductive value hypothesis. However, the seventeen day interruption finshed before the end of the sensitive period and it is likely that a few more cycles would have induced a full response. It is possible that the long to short day transition that is experienced late in the sensitive period somehow enhances the inductive value of the remaining sensitive days to ensure that a full, intense diapause is induced in the first diapausing females that emerge in the field. This may be a means of preventing warm conditions in late August and early September from overriding the effect.

A reciprocal series of experiments, with seven long days interrupting a short day regime did not supply any indications that long days were as strong, or stronger at averting diapause than short days were at inducing it (Fig.11), although this is believed to be the case in S. argyrostoma (SAUNDERS 1971). However, it has been shown

that the transfer to short days is essential for an intense diapause (Table 2 and Fig.8) and most of the long day interruptions did not finish until well after 3(2) which is the latest stage in development when a single transfer to short days can induce 100% diapause whilst a few did not finish until after 4(4) which is the last stage at which a transfer is at all effective (Fig.7). Thus, it appears that the earlier accumulation of short day information is retained and a "late" transfer to short days is able to induce a full diapause response when post-transfer inductive cycles alone would have been ineffectual. Without the transfer the earlier short day cycles would probably have been ineffective or, at best, would have produced a very low intensity diapause (cf. Fig.6).

This Chapter has focussed on photoperiodic sensitivity with respect to diapause induction. The use of ovarian scores as a measure of diapause intensity has helped to show that A. proletella is a long day-short day insect. Photoperiodic sensitivity is maximal in the third and early fourth instars and seven short days at this time can induce 100% diapause even when the remainder of development is spent in diapause averting conditions. Since 100% diapause was also induced when this period was spent in long days and the remainder of development in short days it is clear that photoperiodic sensitivity is extensive and differential in the developmental stages of this insect.

CHAPTER TWO.

THE INDUCTION OF DIAPAUSE IN THE FIELD.

INTRODUCTION.

It has been said that:"...conclusions drawn from experiments in the laboratory should best be proved again in the field."(THIELE and FIELDER 1981), and yet very few workers have investigated, in any detail, ovarian development in the field during the induction of ovarian diapause.

Whilst many workers state the time of the year when diapausing adults appear in the field, such as towards the end of the rainy season for the red locust Nomadacris septemfasciata (NORRIS 1962), during September for the bug Lygus hesperus (LEIGH 1966), Drosophila littoralis (LUMME et al. 1974), Culex tarsalis (HARWOOD and HALFHILL 1964), the lacewing Chrysopa carnea (MACLEOD 1967) and Aleyrodes proletella (EL KHIDIR 1963) it is frequently unclear how closely the more detailed experiments with controlled conditions of photoperiod, temperature etc, relate to the field data. Field samples dissected to ascertain whether the ovaries are mature or immature have been taken several weeks apart (eg. LUMME et al. 1974; BEGON 1976), and the absence of data from newly emerged adults makes it difficult to assess exactly when diapausing adults appear and, by extrapolation, when the sensitive stages first

encounter inductive environmental conditions. Consequently, it is frequently impossible to ascertain what the natural inductive conditions comprise and so the relevance of the contolled experiments cannot be determined.

Measurements of effective light intensities, for a few species, indicate that the photoperiodic response thresholds of insects are below the intensities encountered during dawn and dusk twilight and above full moonlight (eg. LEES 1953b; DE WILDE and BONGA 1958; PARIS and JENNER 1959; DE WILDE 1962; LEES 1968; SAUNDERS 1982b). However, only a few studies have attempted to discover whether the effective field photoperiod, including or excluding civil twilight, resembles that determined in the laboratory. The importance of such parallel studies is highlighted by work with Chrysopa carnea in which the critical photoperiod in the laboratory was found to be LD13.75: 10.25 at 24° C (which occurs in the field in mid September) whilst the first diapausing adults emerged in late August when the field photoperiod, including civil twilight, was close to LD14.5:9.5 (TAUBER and TAUBER 1973c). At first sight it seems that C. carnea does not "see" twilight. However, the situation is complicated by the fact that this species has been shown to respond to a decrease in photoperiod above the critical photoperiod determined using stationary light cycles (TAUBER and TAUBER 1970a), although the intensity threshold is unknown. Since an increase in diapause intensity has been detected in a few species

subjected to a change in photoperiod compared to stationary "short" days (see Chapter 1) it is likely that a closer look at the field population will reveal key stages in the complete process of diapause induction.

In addition to recording the condition of the ovaries, the development of the fat body has been assessed from field samples of a few species (eg. HARWOOD and HALFHILL 1964; DEPNER and HARWOOD 1966; HODEK 1971b; BEGON 1976; KONO 1982). Since fat body hypertrophy is an integral part of the gonotrophic dissociation that characterises ovarian diapause, it is important to incorporate an estimate of fat body development in field studies in order to achieve a more complete picture of the physiological consequences of inductive environmental conditions.

The insemination status of diapausing females of several species has also been determined. Species that diapause as mated females include Culex tarsalis (HARWOOD and HALFHILL 1964) and Drosophila macroptera (KAMBYSELLIS and HEED 1974) whilst the females are unmated in D. obscura (BEGON 1976), D. littoralis (LUMME et al. 1974), Musca autumnalis (STOFFOLANO and MATTHYSSE 1967) and the blowfly Phormia regina (STOFFOLANO et al. 1974). Virgin females may overwinter with males that are also in diapause since the cessation of mating behaviour (HODEK 1971d; ORSHAN and PENER 1979), absence of spermatozoa production and empty accessory glands (NORRIS 1962) and fat body hypertrophy (STOFFOLANO and MATTHYSSE 1967; BEGON 1976) have been reported for the males of some

species. Under these circumstances the proportion of males in the overwintering population may remain at a similar level to the summer population, although there is no data to confirm or deny this in the studies mentioned above. However the sex ratio of overwintering Drosophila grisea is 16 :1 compared to 1:1 in July (KAMBYSELLIS and HEED 1974). This suggests that the males of this species have a lesser capacity to diapause, if they do so at all. However, the diapausing females are known to be mated (KAMBYSELLIS and HEED 1974), and an even sex ratio is restored in the first post-diapause generation.

Previous work with Aleyrodes proletella has shown that the sex ratio during the summer is 1:1 and the proportion of males in the population declines towards zero during the autumn, suggesting that males cannot overwinter (BUTLER 1938a). Subsequently, daily dissections of females collected from the field during September showed that the proportion of non- gravid (=diapausing) females increased from almost zero at the start of the month to nearly 100% by the end (EL KHIDIR 1963). The overwintering females were recorded as having darker pigmentation than the summer form and they did not appear to engage in mating activity (EL KHIDIR 1963).

Here, an attempt is made to relate the environmental conditions inducing diapause in A. proletella, in the field, to those known to be influential in the laboratory. The changes in the overall ovarian and fat body scores of the field populations are followed as diapausing females appear, and the date when the first overwintering adults

emerge is also recorded. An estimate of the earliest inductive conditions in the field will then be based on these data and the sensitive stage results given in Chapter 1.

MATERIALS AND METHODS.

Field Samples

Weekly field samples of adult whitefly were collected, with a pooter, from brussels sprouts at Silwood Park between November 1982 and February 1983, and from June 1983 until April 1984. Females were dissected and a mean ovarian score calculated for each sample (see Chapter 1). The 1982-3 collections commenced after the diapausing females had emerged and the data will be presented in Chapter 4 together with the latter part of the 1983-4 data.

Fat Body Development

Females scored for ovarian development were also scored for fat body development in accordance with the following scheme:-

0 = little or no development;

1 = some development;

2 = extensive development.

Spermatheca

In most dissections the spermatheca was clearly visible so mated and unmated females could be distinguished on the basis of the presence or absence of spermatozoa.

Sex Ratio

The sex ratio during the autumn of 1982 and 1983 was recorded from the samples collected for dissection. Males are readily distinguishable by their smaller size and claspers (BUTLER 1938a; TREHAN 1940).

Diapause Status of Emerging Adults.

Sprout leaves, from the field, bearing larvae of different developmental ages were excised and kept inside a 30cm square nylon mesh covered cage in the field with their petioles immersed in nutrient solution. Newly emerged adults were collected every one or two days and transferred to smaller cylindrical cages containing one young sprout leaf taking root in a 7.5x2.5cm specimen tube of nutrient solution. These cages were also kept in field conditions. Three weeks after emergence, the females were dissected and the percentage of diapausing females determined.

RESULTS.

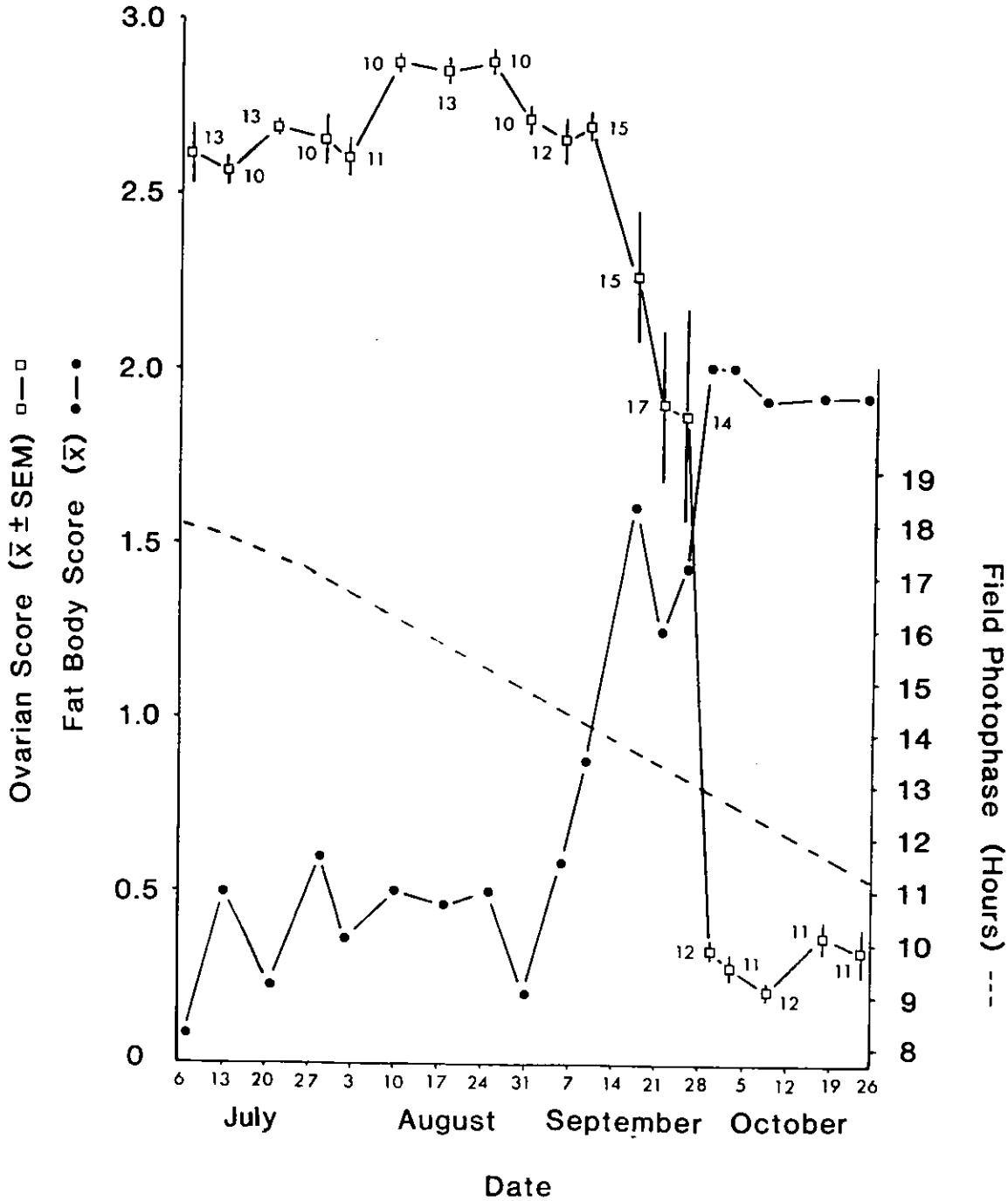
Fig.12 shows the ovarian and fat body development of females collected from the field between the beginning of July and the end of October 1983. The mean ovarian score fluctuated between 2.55 and 2.88 during July and August and the fat body score only exceeded 0.5 on one occasion. It can be seen that the situation changed dramatically during September when the ovarian score of the field population fell from 2.7 to 0.3 in the space of three weeks with a concurrent rise in fat body score. The large standard error values for the ovarian score during this period were due to the presence of two "classes" of female: non-diapausing summer individuals with scores of around 2.5 and diapausing females with scores around 0.2. The change in mean ovarian score from sample to sample reflects the proportions of the two classes, not a gradual reduction in the ovarian development of individuals since females that would have been classified as intermediates (see Chapter 1) were almost non-existent.

During the transition from summer to overwintering females, some morphological differences were readily apparent. Once the adults had been immersed in 90% ethanol to remove the white cuticular waxes, summer females were seen to be creamy yellow in colour with a few small areas of darkly pigmented cuticle and to have swollen abdomens due to the presence of 7-12 stage 5 oocytes in their ovaries. Diapausing winter females were

FIGURE 12.

Ovarian and Fat Body Development in the Field during the summer and early autumn of 1983. Field collections were taken at approximately weekly intervals. The females were dissected and a sample were scored for ovarian and fat body development. This sample reflected the proportions of diapausing/intermediate/non-diapausing females in the original collection. The number of females scored is indicated by the figure next to each point. The fat body and ovarian scores for each date were taken from the same females.

FIG. 12.


Ovarian Score (x̄ ± SEM) □—□
Fat Body Score (x̄) •—•
Field Photophase (Hours) ---
3.0
2.5
2.0
1.5
1.0
0.5
0
19
18
17
16
15
14
13
12
11
10
9
8
6 13 20 27 3 10 17 24 31 7 14 21 28 5 12 19 26
July
August
September
October
Date

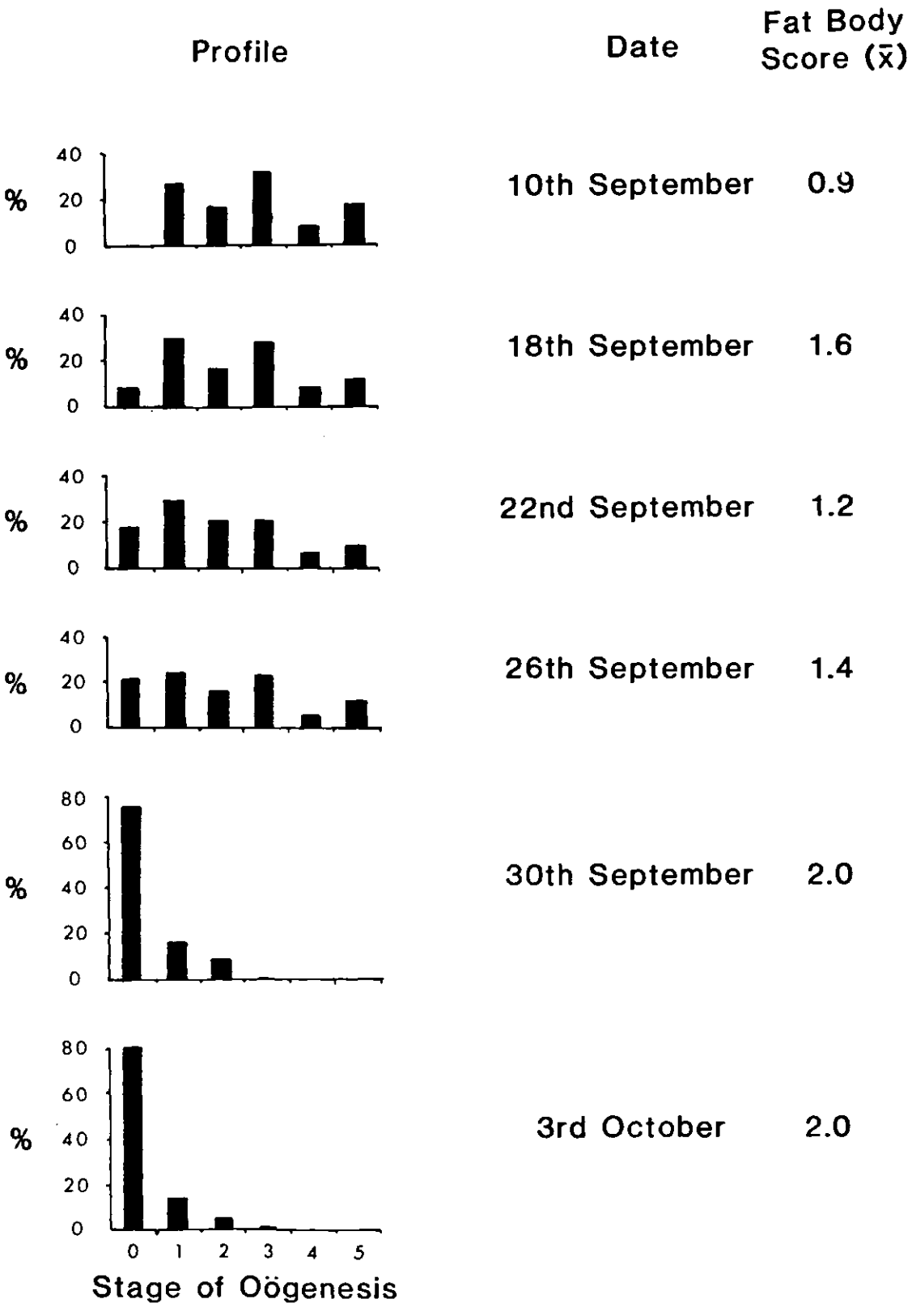
bright yellow with large areas of darkly pigmented cuticle and their abdomens were not swollen although the accumulation of reserves in the fat body kept the size of the abdomen large in comparison with the males. Close examination revealed that the same areas of the body were darkly pigmented in the summer and winter forms but the pigmentation was far darker in the latter.

Ovarian profiles for the non-diapause to diapause transition in the field are shown in Fig.13. On September 10th the profile was typical of that calculated throughout June, July and August and closely resembled the proportions of the six stages in females 14 or more days after emergence in LD16:8 15 C (see Fig.5) since there were 0% stage 0 oöcytes, around 30% stage 1 and 3 oöcytes, 15% stage 2 and 5 oöcytes, and 10% stage 4. In the course of the next three weeks, diapausing females constituted an increasing proportion of the field population and this was illustrated by the increasing percentage of stage 0 oöcytes. As the proportion of non- diapausing females fell the percentage of stage 3, 4 and 5 oöcytes decreased. By the end of September the field population consisted entirely of females in diapause and the profile was comparable to that of induced females three weeks after emergence (see Fig.8) with over 75% of the ovarioles without any signs of oögenesis, about 15% stage 1 and 10% stage 2. Vitellogenic oöcytes were almost completely absent.

FIGURE 13.

Ovarian Profiles from the Non-Diapause to Diapause Transition Period in the Field. The mean percentage of each ovarian developmental stage in the dissections scored in Fig.12 are presented for the last half of September.

FIG. 13.


Profile
Date
Fat Body Score (x̄)
%
40
20
0
10th September 0.9
18th September 1.6
22nd September 1.2
26th September 1.4
80
60
40
20
30th September 2.0
3rd October 2.0
0 1 2 3 4 5
Stage of Oögenesis

The lower panel of Fig.14 shows that mean daily temperatures in the field were around 18°C in August 1983 and 14°C during September. These temperatures are unlikely to have much influence on the induction of diapause apart from a slight effect on the critical photoperiod (IHEAGWAM 1976).

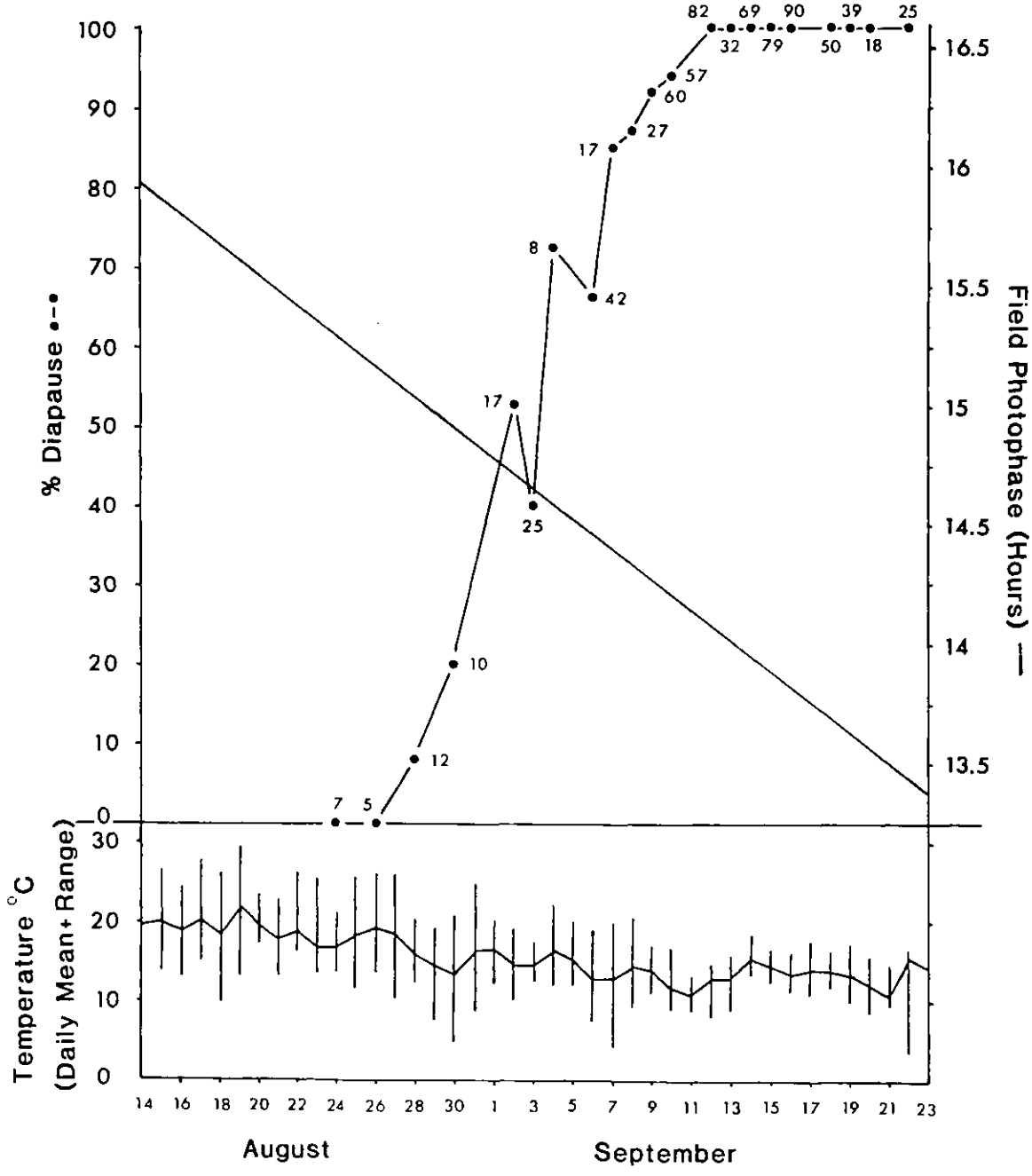
In Fig.12 the field photophase has been plotted for Ascot (51°28'N; 220 feet above mean sea level), including the periods of civil twilight. Since 50% diapause results in a mean ovarian score of approximately 1.25, the photophase when half the field population is in diapause is around 13 hours. However, diapause is determined by the photoperiod at the time of the moult to the 3rd instar, which is approximately 14 days before emergence at the prevailing field temperatures, so the critical photoperiod is apparently close to LD14:10. Clearly, this is far removed from the critical photoperiod obtained in stationary and interruption experiments in the laboratory where a 50% response was seen in LD15.5:8.5 at 15°C (Figs.4 and 10 respectively).

Ovarian scores from field samples may be representative of the ovarian development of the population at any one time, but they do not provide a direct means of assessing the diapause/non-diapause status of newly emerged females. The influence of environmental factors on the induction of diapause may only be ascertained with any degree of certainty if newly emerged adults are studied. In Fig.14 it is evident that the first diapausing females emerged at the end of August, and

FIGURE 14.

Diapause Status of Emerging Females During late summer 1983. Newly emerged adults were collected each day and kept in daily groups, in the field, until 21 days after emergence. Diapause was assessed using the ovary condition criterion. The numbers for each point represent the number of females that were collected on that day.

FIG. 14.


% Diapause •–•
Field Photophase (Hours) —
Temperature °C
(Daily Mean+Range)
August
September
82 69 90 39 25
32 79 50 18
57
60
27
17
8
42
17
25
10
12
7 5
100
90
80
70
60
50
40
30
20
10
0
16.5
16
15.5
15
14.5
14
13.5
30
20
10
0
14 16 18 20 22 24 26 28 30 1 3 5 7 9 11 13 15 17 19 21 23

100% of the emerging females were diapausing before the middle of September. However, diapausing individuals did not have a significant impact on the field sample dissections until late September (see Fig.12).

The larvae destined to become the first diapausing females, which emerge at the end of August, would have moulted to the 3rd instar in mid August when the field photophase, including civil twilight, was close to 15.75 hours (Fig.14). The last non-diapausing adults emerged on September 10th so these insects would have moulted to the 3rd instar towards the end of August in a field photophase of 15.25 hours. Both these findings are consistent with a critical photoperiod of LD15.5:8.5 found in laboratory studies (IHEAGWAM 1976, 1977; see also Figs.4 and 10).

Table 4 shows the changing sex ratio of A. proletella during the autumn of 1982. Clearly, there was a substantial change, in favour of females, over that period of time with very few males present in the field population by the beginning of December. This is in agreement with earlier observations on this species (BUTLER 1938a). During this period, 83% (n=113) of the field collected females were mated and subsequent studies over the same period in 1983 revealed that 96% (n=116) of the diapausing females had sperm in their spermathecae.

TABLE 4. Sex Ratio of A. proletella Collected from the Field during Autumn 1982.

Date	No. Collected	♀	♂	♀:♂
22 Sept.	81	58	23	2.5
29 Sept.	44	39	5	7.8
6 Oct.	46	37	9	4.1
13 Oct.	49	39	10	3.9
20 Oct.	55	49	6	8.2
27 Oct.	42	36	6	6.0
3 Nov.	77	74	3	24.7
10 Nov.	49	44	5	8.8
17 Nov.	53	50	3	16.7
24 Nov.	55	52	3	17.3
1 Dec.	50	50	0	-
8 Dec.	65	63	2	31.5
15 Dec.	48	48	0	-
21 Dec.	43	42	1	42.0

DISCUSSION

Earlier workers with A. proletella suggested that low autumn temperatures initiated the production of overwintering females (BUTLER 1938a; EL KHIDIR 1963). More recently, the role of photoperiod as the major inductive factor has been recognised (IHEAGWAM 1976,1977). However, the first studies on the appearance of overwintering females were based on samples of the entire field population (EL KHIDIR 1963). As a result, the impact of non-gravid females on the population was followed but the actual dates of emergence of diapausing females were not established. This information is necessary if a realistic appraisal of inductive field conditions is to be made since it is known that the difference between a "long" and a "short" day, for this species, is less than one hour (IHEAGWAM 1977; see also Figs.4 and 10) and the decrease from a field photoperiod, including civil twilight, of 16 hours to 15 hours occurs in just two weeks (Fig.12). If diapausing females are not adjudged to emerge until one or two weeks after they actually do, then the effect on the extrapolated inductive field photophase will clearly be substantial. Similarly the relative sensitivity of all the sensitive stages must be established in order for an estimate to be made as to when the first developing larvae encounter fully inductive conditions. For example, if short days are required throughout development, there will be a much greater temporal difference between the onset of inductive

conditions and emergence than if those conditions only have to be experienced from the middle of the 3rd instar.

A good estimate of developmental rate in the relevant field temperatures is necessary in order to calculate the time between the latest developmental stage when induction may commence, and emergence. Since developmental rate may be significantly different in a thermoperiodic regime with one temperature during the photophase and another throughout the scotophase, compared with a constant temperature that is the mean of the two (see SAUNDERS 1982b), such experiments will enable the developmental rate to be estimated with greater accuracy. However, a much better estimate can be made if the larval development of individual insects is monitored as inductive conditions approach and compared with the subsequent diapause status. To date, this has not been monitored in the field although whitefly with their sessile larval stages are ideal candidates for investigation. However, the effect of constant temperatures of up to 25°C upon the developmental rate has been established for A. proletella (IHEAGWAM 1976).

The first attempt to reconcile laboratory findings with field data using A. proletella was inaccurate because some of the factors mentioned above were either incompletely known or were misjudged. Iheagwam (1977) estimated that diapausing females would emerge about the third week in September. This estimate was based on a critical photoperiod of LD15.75:8.25 which occurs in the field in late July if sunrise and sunset times are taken.

This is one hour shorter than if civil twilight is included as part of the photophase. In addition, Iheagwam assumed that short days were required throughout the developmental period even though he had established that 100% diapause could be induced by a transfer from long to short days as late as the early 3rd instar (IHEAGWAM 1976, 1977). However, until the light intensity threshold for this species has been established an element of doubt as to whether civil twilight really is detected photoperiodically must remain.

Recent work with the fall webworm, Hyphantria cunea, has shown that there is a significant asymmetry between the dawn and dusk twilight intensity thresholds and it appears that the effective field photophase commences about 40 minutes before sunrise and ends 20 minutes after sunset (TAKEDA and MASAKI 1979). Different dawn and dusk responses suggest that there are differences in the underlying photoreceptive physiology at these two stages of the day and different spectral sensitivity thresholds, at similar wavelengths of peak sensitivity, for dawn and dusk have been found with Chaoborus americanus (BRADSHAW 1972, 1974) and Nasonia vitripennis (SAUNDERS 1975c). Unfortunately, many of the studies of threshold sensitivity have failed to isolate dawn and dusk. For example, a short photophase was extended by low light intensities which, if detected, would evoke a long day response in Metatetranychus ulmi (LEES 1953b) and Metriocnemus knabi (PARIS and JENNER 1959). In both cases, the threshold represented that of dusk and the dawn

response remains unknown.

Later in this study, it will be shown that the dawn and dusk light intensity thresholds for A. proletella are around 1.5uWcm^{-2} and 2.5uWcm^{-2}, respectively, at 411nm (Figs.21 and 22). Both are slightly above the intensity of full moonlight (0.65uWcm^{-2}) and below twilight intensities. Although experiments using dim white light have not been performed, these results indicate that this species exhibits a small asymmetry in its response at dawn and dusk, but its photoperiodic photoreceptor does respond to twilight. This will be discussed further in Chapter 3.

Studies incorporating field samples to look at the reproductive condition of Drosophilids during the year have been performed with D. littoralis (LUMME et al. 1974) and D. obscura and D. subobscura (BEGON 1976). Unfortunately, in both cases, the samples were taken at approximately monthly intervals and the overwintering population was either not located or not sampled. Consequently, whilst correlations between mated status and/or fat body development and ovarian development were possible, the switch from summer females with mature ovaries to overwintering females with immature ovaries appeared as a "step" rather than a gradual transition which made it impossible to estimate, with any degree of accuracy, when diapause was induced in the field. The ovarian scores (Fig.12) and corresponding profiles (Fig.13) of weekly field samples of A. proletella show that the transition from a non-diapausing population to a diapausing one is quite sudden and is due to the change in

proportions of summer and winter females during September and not to any marked ovarian regression in the non-diapausing individuals. Field samples of the entire whitefly population (Figs.12 and 13) do not reveal the presence of diapausing females until two or three weeks after these females actually emerge (Fig.14) due to the fact that the non-diapausing females are present in vast numbers and effectively dilute the impact of diapausing individuals. As the numbers of diapausing females increase and some of the summer females die, the changing proportions become apparent in field samples. The time lag between the date when the first diapausing females emerge and the date when they become apparent in field samples is, clearly, a very significant one since it represents a difference of almost an hour in the inductive field photoperiod. This highlights the importance of the information presented in Fig.14.

Weekly samples of Chrysopa carnea, that were then reared in field conditions, showed that 50% of the females that emerged in the first week of September were in diapause (TAUBER and TAUBER 1973c). Calculations made using a critical photoperiod of LD13.75:10.25, that had been determined using stationary photoperiods, had predicted that diapausing females would emerge some three weeks after they were actually observed. It had been shown that transferring C. carnea from "long" to "short" photoperiods increased the intensity of diapause, particularly when the transfer occurred during the cocoon stage third instar or the pupal stage (TAUBER and TAUBER

1970b). However, the adult is also sensitive (TAUBER and TAUBER 1969) so the response to inductive photoperiodic conditions may be almost immediate in the first diapausing adults that are observed in nature. In another experiment, 29% of C. carnea adults entered diapause in response to a decrease in photoperiod even though the "short" daylength represented a "long-day" as a stationary photoperiod (TAUBER and TAUBER 1970a). Clearly, this insect has a more complicated response than that suggested by the initial laboratory work and relative sensitivity of all developmental stages, combined with the naturally decreasing photoperiod are important factors. All these features of the induction process must be known before confident conclusions about the response in the natural habitat may be drawn.

Contrary to earlier suggestions (see EL KHIDIR 1963), diapausing whitefly are inseminated. In addition the changing sex ratio during the autumn (BUTLER 1938a; see also Table 4) suggests that males do not overwinter. It has been shown that mating activity reduces the cold hardiness of A. proletella males (BUTLER 1938a) and it seems likely that this is the main reason for the predominantly female population that is prevalent during late autumn and winter. Since the overwintering females contain sperm, the first post-diapause generation probably restores the even sex ratio which also appears to be the case in Drosophila grisea (KAMBYSELLIS and HEED 1974). Parthenogenesis has been demonstrated in A. proletella and the eggs of virgin females were always male (BUTLER

1938a). Thus, overwintering females need to mate before the frosts kill all the males in order for the 1:1 sex ratio to be restored towards the beginning of the following summer. Clearly, an all-male generation at the start of the summer would be extemely unfavourable for population increase.

In this study the date of emergence of diapausing A. proletella females has been used in conjunction with laboratory located sensitive period data to estimate the prevailing field conditions, particularly photoperiod, when diapause is induced in nature. The results obtained show that the inductive field photoperiod is the same as the inductive photoperiod found in the laboratory if, as seems likely, the light intensity threshold lies below the intensity of civil twilight. Thus, the induction process, in this species, appears to consist of detecting that the photoperiod is sub-critical. Whilst the length of the critical photoperiod may be slightly affected by temperature, the field photoperiod is undoubtedly the major diapause inducing factor. If the photoperiod falls below critical before the end of the third instar, some individuals will accumulate sufficient short day information during the remainder of the sensitive period for diapause to be determined. The fact that the critical photoperiod in the field is the same as that observed in laboratory studies suggests that a decrease in photoperiod is not inductive unless it crosses the critical value. However, the decrease in photoperiod induces an intense diapause, compared to a stationary, "short" photoperiod

(Chapter 1. Figs.6 and 8) so it probably has substantial ecological significance since the strong inhibition of oogenesis resulting from the detection of a long day-short day transition cannot even be overridden by warm autumnal conditions.

CHAPTER 3.

THE PHOTOPERIODIC CLOCK OF ALEYRODES PROLETELLA L.

INTRODUCTION.

The response of insects to photoperiod in the field and the laboratory is well documented for numerous species (LEES 1955; DANILEVSKII 1965; LEES 1968; SAUNDERS 1982b). In the majority of cases, however, the precise means by which a short-day (or long-night) is distinguished from a long-day is largely unknown.

Photoperiodic time measurement requires a photoreceptor, which incorporates some form of photopigment, a "clock" to measure the duration of light and/or dark, and a counter to accumulate the information from the light-dark cycles experienced during the sensitive period. The resulting "packet" of information can only program the insect for diapause if it exceeds a particular threshold value.

Some recent work with *Pieris rapae* suggests that a photoreceptor organelle is present in the giant glial cells of the brain (KONO *et al.* 1983). Areas of cuticle overlying the brain of two species have been locally illuminated to assess the sensitivity of the underlying structures (LEES 1964; WILLIAMS and ADKISSON 1964). In *Megoura viciae* the photoreceptor is believed to lie just

lateral to the group I neurosecretory cells (LEES 1964; STEEL and LEES 1977) and, in Antheraea pernyi it is located beneath the transparent patch of facial cuticle (WILLIAMS and ADKISSON 1964; WILLIAMS et al 1965). In other insects the precise location of the photoreceptor is unknown. However, extra-retinal photoreception for diapause and polymorphism has been demonstrated in several species (DE WILDE et al. 1959; LEES 1964; TRUMAN 1976; SHIMZU 1982), and the photoperiodic control of adult diapause in the eyeless mite, Amblyseius potentillae (VEERMAN et al. 1983), lends further support to the idea that the compound eyes and ocelli are not directly involved in the response. However, the compound eyes do appear to be the photoperiodic photoreceptors in male carabids, Pterostichus nigrita (FERENZ 1975) and female bean bugs, Riptortus clavatus (NUMATA and HIDAKA 1983).

The photopigment has not been located visually in M. viciae (LEES 1966) or by transmission studies in which the protocerebrum was scanned (HARDIE et al. 1981). However, orange pigmented areas have been observed associated with the membranous covering of A. pernyi brain which may be involved with photoreception (NORRIS et al. 1969). Ideally, whole animal action spectra would reflect the absorption characteristics of the pigment however several factors invalidate this assumption. In particular, the fact that the precise location of the pigment is not known makes it difficult to allow for the filtering action of the tissues and cuticle overlying the photoreceptor (LEES 1981), although these have been measured in M. viciae

(HARDIE et al. 1981) and the corrected action spectra have been published (LEES 1981). Nevertheless, energy compensated action spectra and, to a lesser extent, spectral sensitivity studies are of great value in elucidating the physiology of one link in the chain of events previously masked by the black box between the photoperiodic stimulus and the observable response of diapause or polymorphism. Action spectra for the control of polymorphism in M. viciae show blue sensitivity in the early part of the scotophase and a more general sensitivity in the late scotophase (LEES 1966, 1981). Blue sensitivity has also been demonstrated in the diapause termination action spectra of Laspeyresia pomonella and Antheraea pernyi (NORRIS et al. 1969) whilst the same process in the aquatic larva of Chaoborus americanus shows peak sensitivity in the yellow-green part of the spectrum (BRADSHAW 1972, 1974). Numerous, less comprehensive studies show that, in general, insects are most sensitive to blue wavelengths and are almost completely insensitive to red light (see LEES 1968; BRADSHAW 1974).

Convincing evidence that carotenoids or caroteno-proteins are necessary for the photoperiodic response may be obtained using artificial diets since insects are unable to synthesize carotenoids de novo (SHIMIZU and KATO 1984). To date, carotenoid based pigments have been implicated in three arthropod species: the spider mites Tetranychus urticae (VEERMAN and HELLE 1978; VEERMAN 1980) and Amblyseius potentillae (VAN ZON

et al. 1981; VEERMAN et al. 1983) and the silkmoth Bombyx mori (SHIMIZU and KATO 1984). Blue sensitive action spectra are consistent with these findings (LEES 1981).

The implication of early work on insect photoperiodism was that the clock was an "hour-glass" (LEES 1968; SAUNDERS 1982b). This explanation is perfectly acceptable in diel photoperiods within the ecological range of the species involved but a complete understanding of the process of time measurement can only be gained if the response to abnormal photoperiodic regimes is observed. By coupling a "long" scotophase with a photophase of 48 hours, or more, it has been shown that night-length is most important in the process of time measurement in many species including Antheraea pernyi (TANAKA 1950), Megoura viciae (LEES 1966) Adoxophyes orana (BONNEMAISON 1978) and Ostrinia nubilalis (BONNEMAISON 1978). These results are consistent with the idea that an hour-glass measures the duration of the dark period.

Following work on the daily leaf movements of Phaseolus, Bunning (1936) proposed that endogenous rhythms had a central role in photoperiodism. In his original model, each day consisted of a 12 hour "photophil" (light requiring) and a 12 hour "scotophil" and if the light period extended into the scotophil, a long-day response was elicited. Subsequently, this principle was used to account for the induction of pupal diapause in Pieris brassicae (BUNNING and JOERRENS 1960). Although this model is no longer tenable in its original form, the

principle of endogenous (circadian) rhythms having a central role in insect photoperiodism has been developed within the "external coincidence" model (PITTENDRIGH and MINIS 1964; PITTENDRIGH 1966). In this model, light has two functions: to entrain the rhythm and to induce the response. The position of a short photoinducible phase, øi, (which is equivelant to a truncated scotophil from Bunning's original model) is "set" by a particular point in the light/dark cycle (such as the end of the photophase). If oi then falls in the light, a long-day response will occur. Since the entrained rhythm cannot be observed, the behaviour of readily apparent rhythms, such as pupal eclosion or oviposition (SAUNDERS 1982b), is recorded on the assumption that these rhythms will be linked to the photoperiodic clock in the insect's multioscillator system (PITTENDRIGH 1972, 1981). The best example of an insect with this type of clock is the flesh-fly Sarcophaga argyrostoma (SAUNDERS 1973b, 1975b, 1978a, 1979, 1984).

A model of "internal coincidence" whereby two or more oscillators are entrained by the light cycle has been developed by Tyshchenko (1966, in DANILEVSKY et al 1970) and Pittendrigh (1972). If the light cycle is such that particular phases of the oscillators coincide then a particular developmental pathway will be followed but light itself plays no part in the actual inductive process. The dawn and dusk oscillators of Nasonia vitripennis (SAUNDERS 1974) provide the best evidence in support of this model. Several other two component

systems have been proposed with various depths of experimental support including a dusk commencing hour-glass combined with a dawn commencing rhythmic timer for Carpocapsa pomonella (HAMNER 1969), a double oscillator for Mamestra brassicae, Ostrinia nubilalis and Adoxophyes orana (BONNEMAISON 1978) and the "S" and "P" components of Beck's Dual System Theory for Ostrinia nubilalis (BECK 1974a,b, 1975, 1980).

In a comprehensive series of experiments the hour-glass principle has been developed for the aphid Megoura viciae (LEES 1966, 1968, 1973). There is no direct circadian role in this model of the clock, instead time measurement commences at dusk and proceeds through four stages of which stages 3 and 4 are the most ecologically significant since they lie either side of the critical nightlength (LEES 1973). By analogy, the role of light is to turn the hour-glass over in readiness for the start of the next night (LEES 1971). Convincing evidence for an hour-glass clock has also been obtained for Ostrinia nubilalis (SKOPIK and BOWEN 1976) whilst some degree of hour-glass behaviour has also been implicated in several other insects including Carpocapsa pomonella (HAMNER 1969), Pectinophora gossypiella (ADKISSON 1966) and Aedes atropalpus (BEACH and CRAIG 1977).

One of the methods most commonly used to obtain evidence for or against the involvement of the circadian system in photoperiodic time measurement is the so called "resonance" experiment based on the experimental protocol of Nanda and Hamner (1959). A "short" photophase is

combined with a series of scotophases of up to 72 hours in length. If a circadian element is present it will "free-run" in constant darkness (DD) with a periodicity (τ) close to 24 hours. Consequently, in a cycle of LD12:24 the second photophase will fall in the subjective night and illuminate oi to effect a long-day response and the series of light cycles will show rhythmic maxima and minima of long and short day responses. Resonance experiments have demonstrated a circadian influence in Sarcophaga argyrostoma (SAUNDERS 1973b, τ =24 hours), Nasonia vitripennis (SAUNDERS 1974, τ=24 hours) a central European stock of Pterostichus nigrita (THIELE 1977a, τ =24 hours), Pieris brassicae (CLARET et al. 1981, τ=22 hours) and the red spider mite, Tetranychus urticae (VEERMAN and VAZ NUNES 1980, τ=20 hours), whilst apparently negative results have been recorded with Pectinophora gossypiella (ADKISSON 1966), Carpocapsa pomonella (PETERSON and HAMNER 1968), Megoura viciae (LEES 1973), Ostrinia nubilalis (SKOPIK and BOWEN 1976; BONNEMAISON 1978), a Swedish stock of Pterostichus nigrita (THIELE 1977b) and Adoxophyes orana (BONNEMAISON 1978). Some additional, unpublished, examples are cited by Saunders (1981).

There are no known insects in which diapause may be determined by a single 24 hour photoperiodic cycle so, clearly, information from the clock must be accumulated by some form of "counter" mechanism (SAUNDERS 1981).

The number of cycles necessary to induce diapause in 50% of the population has been termed the "required day number" or RDN (SAUNDERS 1981). The RDN has been shown to be temperature compensated in Nasonia vitripennis (SAUNDERS 1965, 1966), Sarcophaga argyrostoma (SAUNDERS 1971) and in Acronycta rumicis, Mamestra brassicae and Dendrolimus pini (GORYSHIN and TYSHCHENKO 1970, 1972, 1974, see SAUNDERS 1981). Consequently, at high temperatures, the insect will develop through its sensitive period without experiencing enough cycles for diapause to be determined regardless of the "long" or "short" nature of the prevailing photoperiodic regime.

In natural conditions, inductive photoperiodic cycles will occur consecutively and it has been shown that intervening long days can nullify the effect of short days in Tetranychus urticae (VEERMAN 1977a). Conversely, the accumulation of long day information by Megoura viciae is unaffected by such treatment (LEES 1971). In addition the inductive "value" of a particular cycle is dependent on its position in the sensitive period (see Chapter 1). The interaction of these factors determines whether enough information accumulates to exceed the "induction sum" (VEERMAN and VAZ NUNES 1984) and program the diapause response.

In many experimental regimes it is very difficult to separate the effects of the clock and the counter. One recent suggestion, that the performance of the photoperiodic timing process should be optimal in light/dark cycles close to resonance with the periodicity

of the circadian system (PITTENDRIGH 1972), has been developed into an "hour-glass timer - oscillator counter" model for the adult diapause of Tetranychus urticae. In this model, the clock is a Megoura type hour-glass, but the output of the clock and its value to the counter, is influenced by the relationship between the experimental cycle and the circadian pacemaker (VAZ NUNES and VEERMAN 1982b; VEERMAN and VAZ NUNES 1984).

There have been no investigations into the mechanism of photoperiodic time measurement in the cabbage whitefly, Aleyrodes proletella L.. However, its small size, sessile larval stage, relatively short generation time and ease of handling in large numbers make it an ideal subject for such work.

In this chapter, the relative influence of the photophase and scotophase in photperiodic timing, the light sensitivity of the scotophase, action spectra for the light sensitive parts of the scotophase and the influence of the circadian system plus, to some extent, the role of the counter, upon the induction of ovarian diapause, will be investigated.

MATERIALS AND METHODS.

Culture, Diapause Criteria and Ovarian Development.

See Chapter 1.

Fat Body Development.

See Chapter 2.

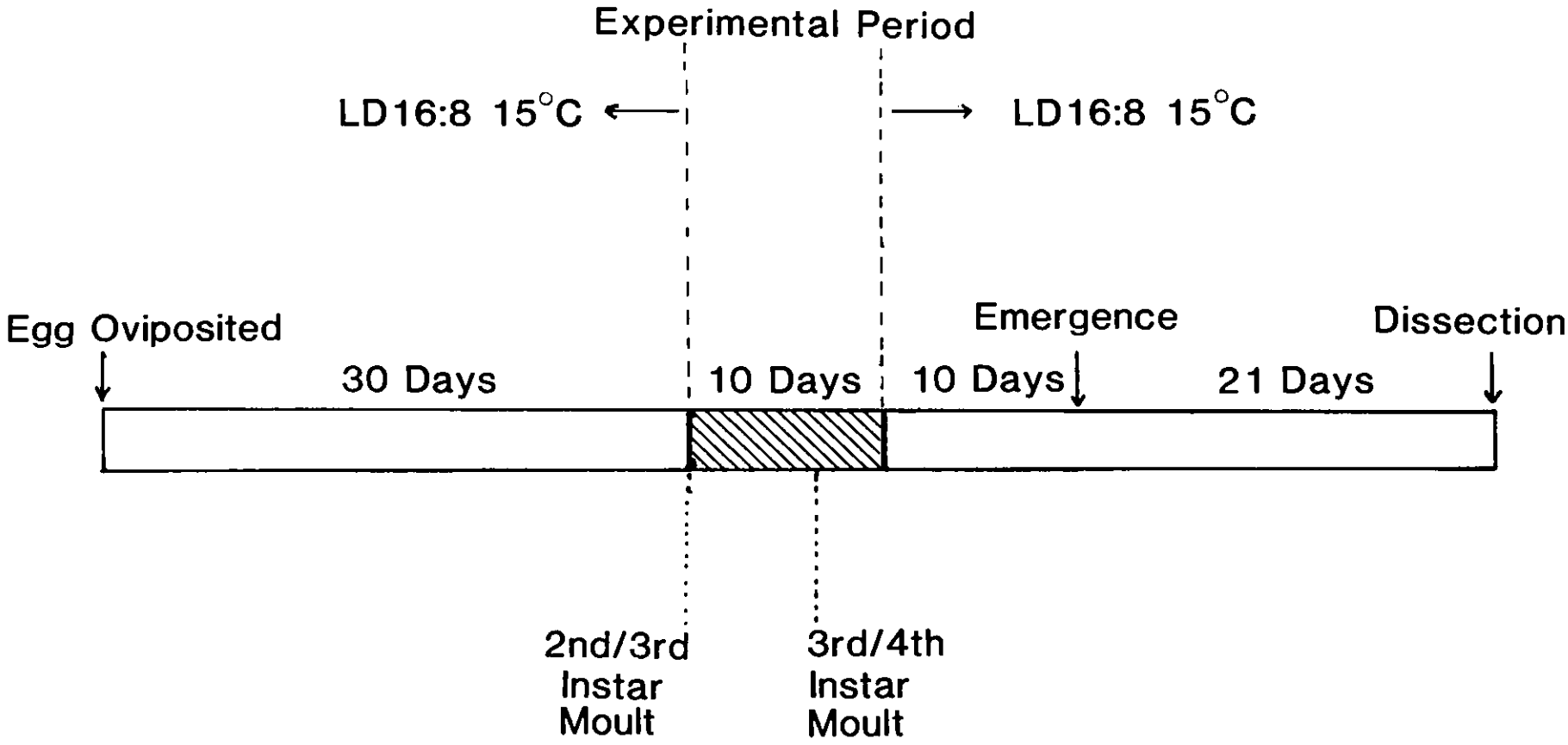
Sensitive Period Treatment.

The phase of development with peak sensitivity to diapause inducing conditions has already been identified (see Fig.9). In accordance with this, the experimental pattern shown in Fig.15 was adopted so that all the insects developed in LD16:8 15°C until the late 2nd (L2) instar (L2 = 2(3)-2(4)) or early 3rd (E3) instar (E3 = 3(0)-3(1)). Any larvae that were developmentally "young" or "old" were removed from the leaf immediately prior to the transfer to the experimental conditions. The sensitive larvae were then exposed to the photoperiodic regime under test for 10 days (± 1 day depending on the length of the cycle). At the end of this period any 3rd or very early 4th instar larvae were removed from the leaf as an additional control measure and the insects were then returned to LD16:8 15°C. Clearly, an insect in LD12:36 will experience 5 cycles in 10 days compared to 10 cycles in LD12:12 and this may, in some cases, have a significant effect upon the amount of information that reaches the

FIGURE 15.

Sensitive Period Experimental Procedure. Based on the results in Fig.9 the procedure shown here was adopted. Since diapause may be induced in all females if they experience 10 short day cycles commencing at around the time of the 2nd to 3rd instar moult, whitefly were kept in LD16:8 15°C until they had reached that stage before they were transferred to the experimental photoperiod. After the test period the insects were returned to long days until 21 days after emergence when they were dissected.

FIG. 15.


Experimental Period
LD16:8 15°C
LD16:8 15°C
Egg Oviposited
30 Days
10 Days
10 Days
Emergence
21 Days
Dissection
2nd/3rd Instar Moult
3rd/4th Instar Moult

counter. However, it was decided to standardize the period of presentation to experimental conditions, rather than the number of cycles, on the basis that any counter effects could be allowed for more easily than attempting to evaluate the diapause value of cycles experienced at different times during the entire sensitive period.

Most of the experiments were conducted at 15°C throughout. However, some resonance experiments were performed at 17°C and 20°C. At these higher temperatures the larval developmental rate increases (IHEAGWAM 1978) so that the duration of the peak sensitive period becomes shorter. In these instances, the test photoperiod was started a few days earlier in an attempt to ensure that all the cycles were experienced at a time when the insect was sensitive to some degree.

Action Spectra.

Several experimental protocols for investigating the spectral sensitivity of selected parts of the photoperiodic cycle have been described and discussed previously (BRADSHAW 1974; LEES 1981). The regimes used in this study are based on Bradshaw's Fig.4F and G (1974) for dawn and dusk extensions of a white light short day.

The apparatus used was, essentially, that described by Lees (1981). Since sensitive A. proletella larvae are sessile, leaf cages were not required and it was simply necessary to ensure that any larvae outside the circle of

leaf surface illuminated by the spectral light were removed prior to treatment. The sensitive larvae were then exposed to LD14:10 15°C with an additional 2 hours of monochromatic light replacing either the first or last 2 hours of the scotophase.

Two light sourses were used to vary the incident energy reaching the leaf surface: BMC 12V 48W bulbs (lower energies) and Phillips 6V 100W bulbs (higher energies) which both have tightly coiled tungsten filaments. Different intensities could be obtained by using neutral density filters or covering the rear half of the bulb with aluminium foil. All the lamps were placed at the focal length of a 10.5cm collecting lens. The light then passed through one of the nine selected narrow band Balzer interference filters (Filtraflex B-20 series, 6nm band width at half peak). A calibrated radiometric silicon diode (Oriel No.7190) was used to measure the energy reaching the leaf surface (irradiance) at the beginning and end of the experimental period and a mean value was calculated in uWcm . Whilst the filters only transmit a narrow band of visible light, some infra-red energy emitted by the light bulbs will also reach the larvae. In order to determine whether this had any influence on the response an interference filter was replaced by a Wratten 87c gelatine infra-red (i-r) filter. This filter transmits 95% peak at 1200nm and 1% at 800nm which is outside the range of the photocell so a Hilger-Schwarz thermopile with a silicon dioxide window and a spectral range of 180-3400nm was used, in

conjunction with a digital multimeter, to measure the infra-red energy. The i-r energy reaching the leaf was also measured when the 470nm Balzer interference filter was in place.

In Fig.16 the results of coupling a 10 hour scotophase with a series of photophases are shown. Diapause was assessed by dissecting the females 21 days after emergence. A representative sample of females from each experiment was also scored for ovarian and fat body development. The high incidence of diapause, even with a photophase of 44 hours reflects the relative importance of the dark and light periods in the process of time measurement. The trends are for decreasing percentage diapause, increasing ovarian score and decreasing fat body score with increasing duration of the photophase. It is possible that the CNL increases slightly in parallel with longer light periods. However, a closer look at the number of cycles experienced during the experimental period reveals that this may also be influential. The results suggest that 4 cycles including a 10 hour scotophase are sufficient to induce a 50% response.

Since 100% diapause resulted from cycles with a 10 hour scotophase and a photophase between 2 and 24 hours in duration (Fig.16), it is clear that the scotophase is the most important part of the photoperiodic cycle, with respect to diapause induction. Consequently, the sensitivity of the dark period to light was investigated in an attempt to determine the means of time measurement. The result of scanning the night of a LD14:10 15°C cycle with 1 hour light breaks is shown in Fig.17. When the

FIGURE 16.

The effect of coupling a 10 hour scotophase with a range of photophases up to 80 hours long upon a) the incidence of diapause; b) the mean ovarian score and c) the mean fat body score. The small figures represent the number of females in each condition. The same females were scored for ovarian and fat body development and they represent the proportions of non-diapause/ intermediate/diapause females in a).

FIG. 16.

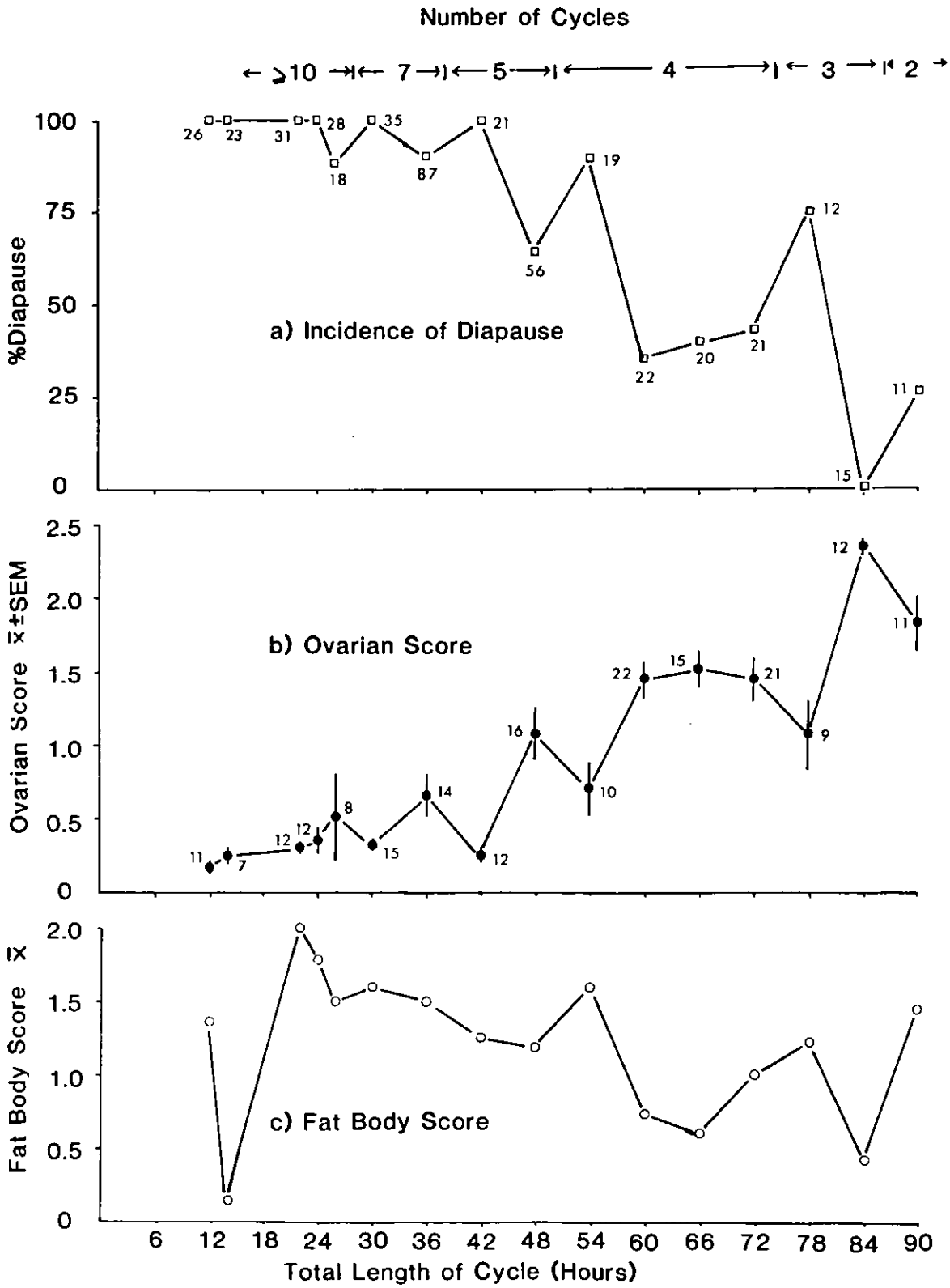
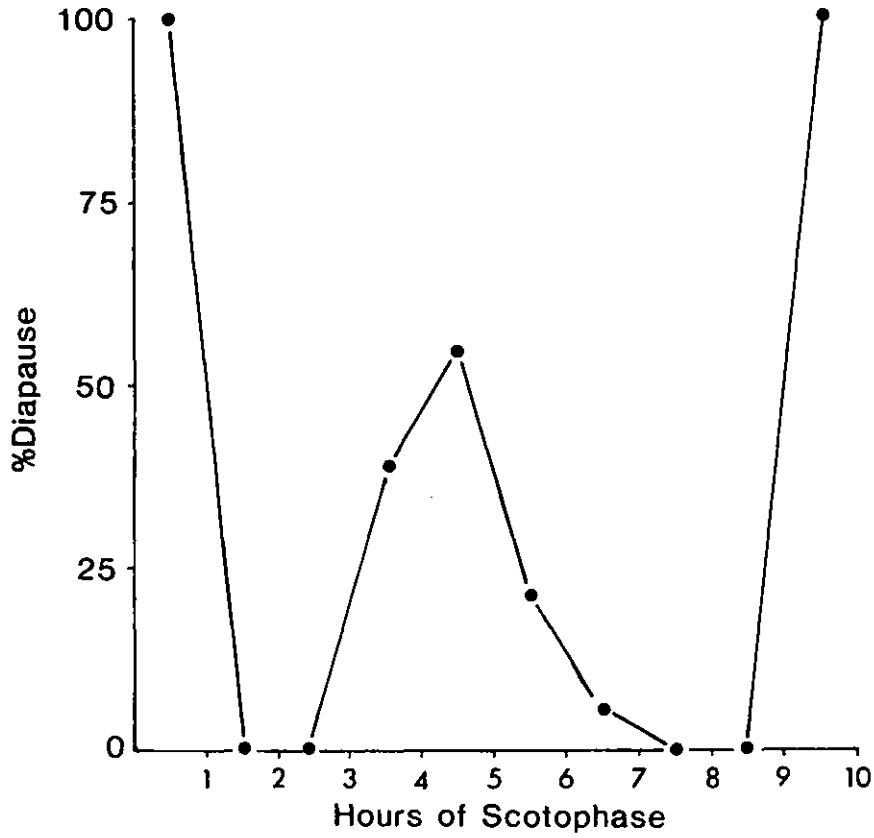
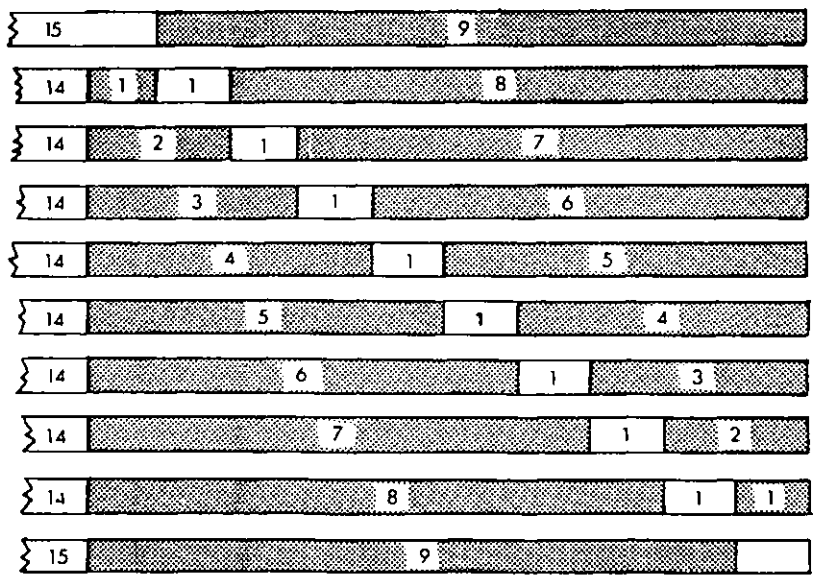

Number of Cycles
≥10
7
5
4
3
2
%Diapause
a) Incidence of Diapause
Ovarian Score x̄ ±SEM
b) Ovarian Score
Fat Body Score x̄
c) Fat Body Score
Total Length of Cycle (Hours)

FIGURE 17.

Night Interruption Experiments. The scotophase of a LD14:10 15°C regime was systematically interrupted by one hour periods of light. The effect of light breaks at different times during the night was assessed by recording the condition of the ovaries 21 days after emergence. Shaded areas signify periods of darkness and the numbers represent the number of hours of light or dark.

FIG. 17.





%Diapause	n
100	38
0	14
0	18
39	18
55	20
21	14
6	17
0	16
0	12
100	38

light break commenced 1-2 or 6-8 hours into the night, it completely reversed the long night effect and these two periods of high sensitivity were separated by a phase exhibiting some degree of photorefractoriness. The two photosensitive phases have been termed the "A" and "B" peaks (SAUNDERS 1968) and have been shown to occur in several species (see SAUNDERS 1982b).

An inspection of Fig.18 shows that the two high sensitivity phases have underlying differences. In this series of experiments, the light interruption was followed by a scotophase of 9 hours (>CNL). The diapause response was completely restored after an "early" light break but the long dark period was not as effective following interruptions 5-8 hours into the night. When the post-interruption dark period was further extended to 10 or 11 hours (Fig.19) the percentage of females entering diapause increased in most cases. However, the effect of a light break 8 hours into the night remained relatively "resistant" to the long scotophases.

In the series of experiments shown in Fig.20 the length of the light interruption was extended to 4 hours. It can be seen that this restored the capacity to respond to a 9 hour post-interruption dark period in all cases.

Energy compensated whole animal action spectra for dawn and dusk extensions of an LD14:10 regime are shown in Figs.21 and 22. If the spectral light was "seen" the cycle would be equivelant to LD16:8 and long day effects would result. In Fig.21 it can be seen that the whitefly

FIGURE 18.

Post-Interruption Night Extension Experiments. The length of the scotophase following a one hour light break was extended to 9 hours (>CNL) in all cases and the effect upon the incidence of diapause was assessed by dissection 21 days after emergence.

FIG. 18.

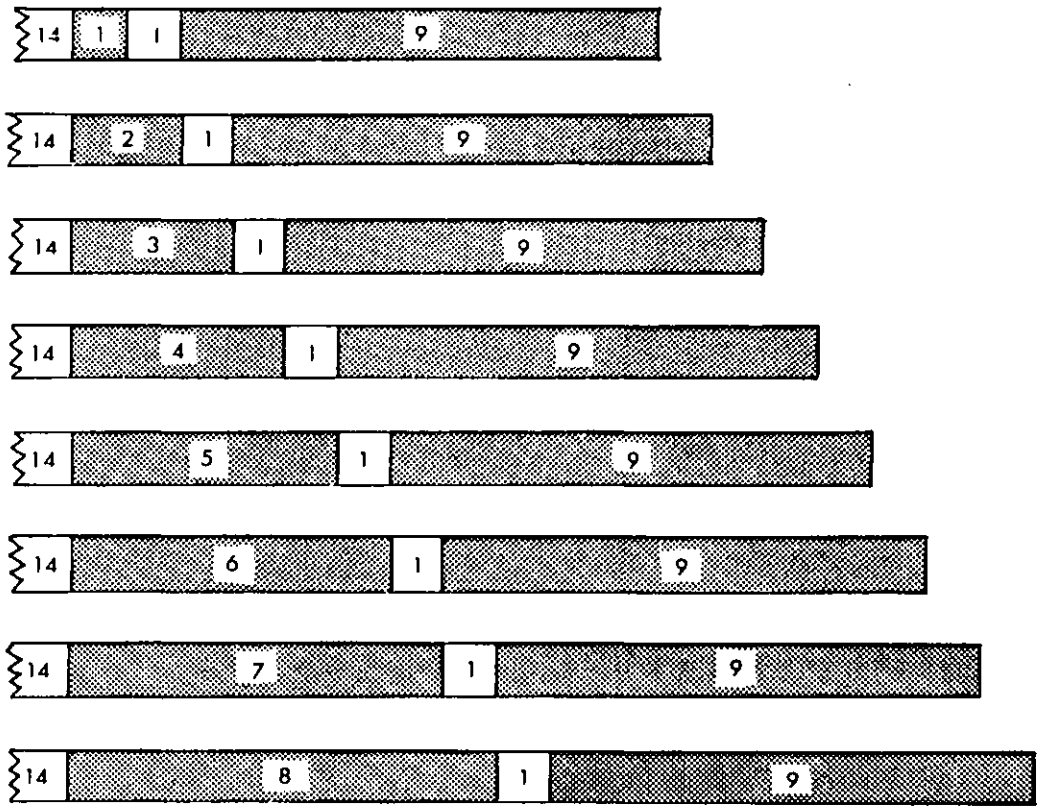

%Diapause
n
14 1 1 9
100
30
14 2 1 9
100
10
14 3 1 9
100
17
14 4 1 9
100
22
14 5 1 9
88
24
14 6 1 9
75
172
14 7 1 9
78
41
14 8 1 9
38
26

FIGURE 19.

Post-Interruption Night Extension. One hour light breaks commencing 4 to 8 hours into the scotophase were followed by a 10 or 11 hour dark period. The effect was assessed by dissecting the females 21 days after emergence.

FIG. 19.

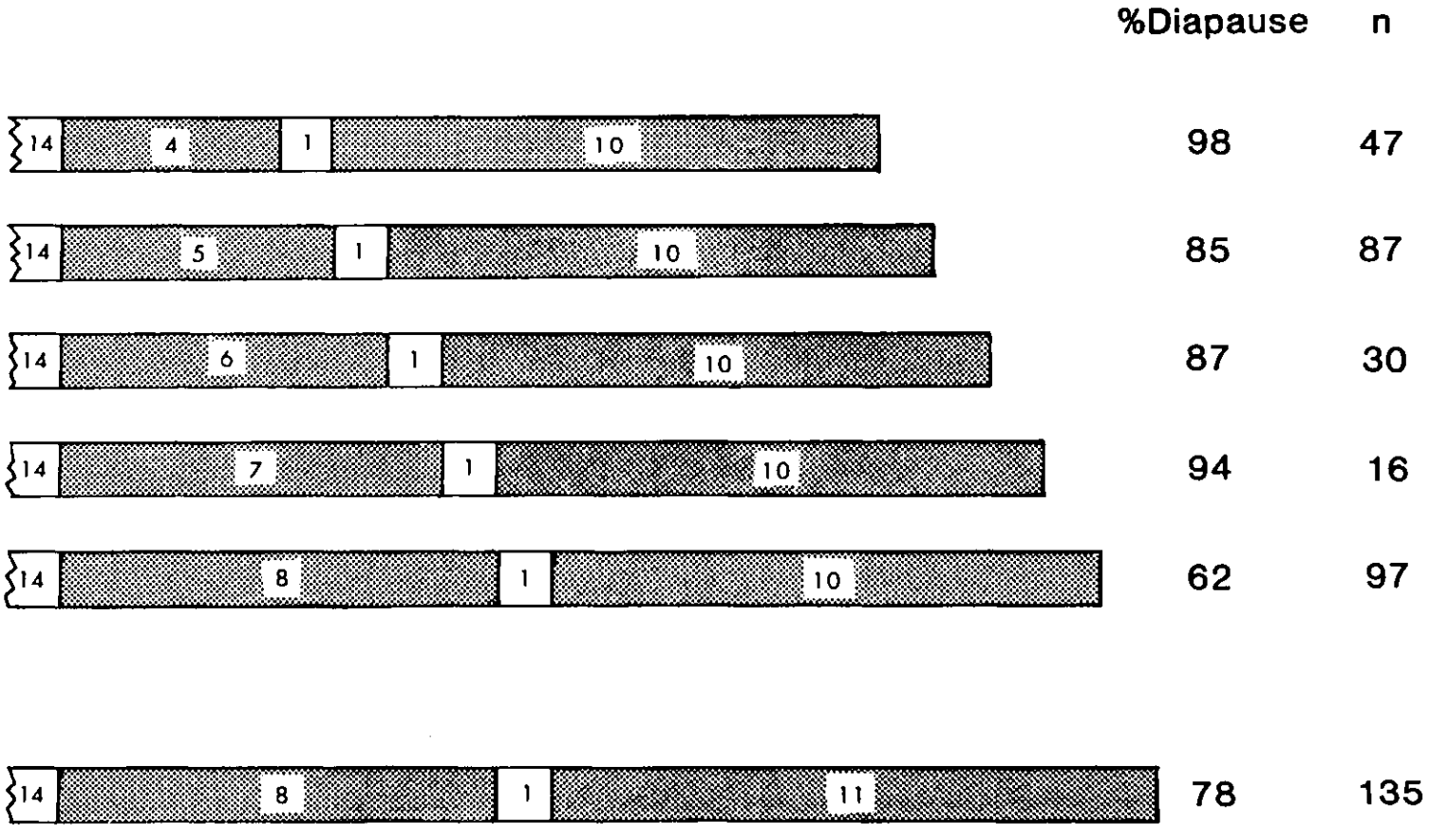
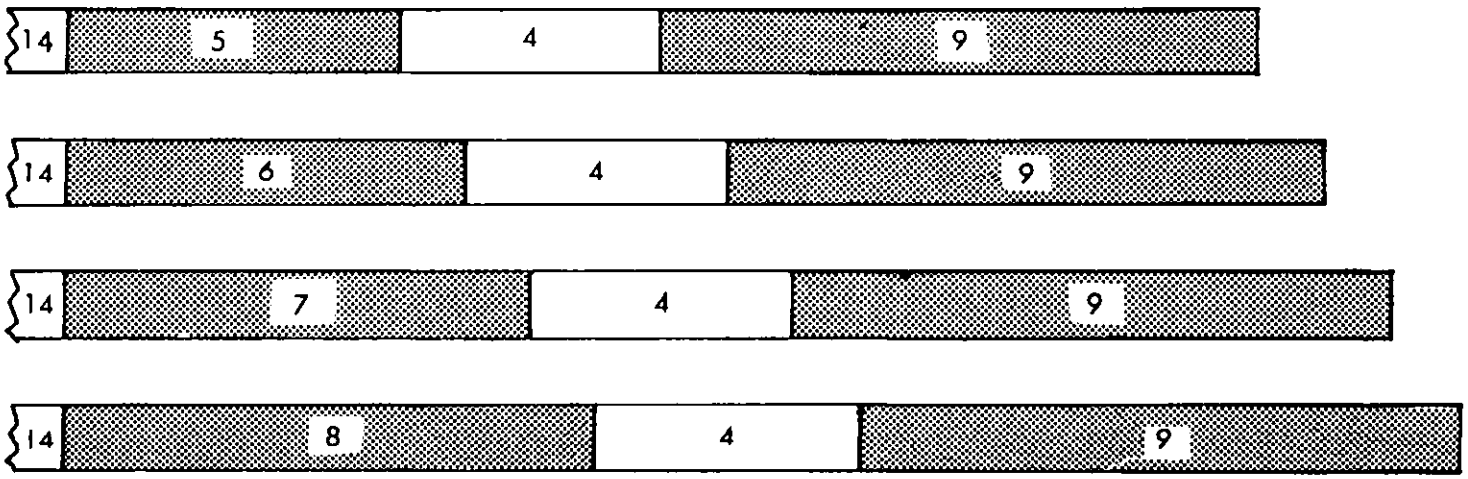

%Diapause
n
14
4
1
10
98
47
14
5
1
10
85
87
14
6
1
10
87
30
14
7
1
10
94
16
14
8
1
10
62
97
14
8
1
11
78
135

FIGURE 20.

Extended Light Interruption Experiments. The duration of the light break was increased to 4 hours commencing 5 to 8 hors into the scotophase. A dark period of 9 hours followed each light interruption. The females were dissected 21 days after emergence and the incidence of diapause was assessed from the condition of their ovaries.

FIG. 20.



120

were most sensitive to blue light in the 410-430nm range, with a 50% response at around 1.5uWcm^{-2}, at dusk. It is possible that peak sensitivity extends into shorter blue and ultra-violet wavelengths but these were not investigated. Sensitivity declined sharply from 430nm to a slight "shoulder" between 500nm and 550nm and a negligible response at 572nm and 618nm. Peak sensitivity for dawn extensions was also in the blue region of the spectrum (Fig.22). The most effective wavelength appeared to be 411nm with a 50% response at 2.5uWcm^{-2}although, once again, the effect of shorter wavelengths is unknown. A second peak of sensitivity was apparent at 471nm although the results at 428nm and 471nm are inconsistent and the reality of this peak is debateable. As before, a yellow-green shoulder was present at 500-550nm with a 50% response at 15-20uWcm^{-2}. It is clear that the two action spectra are similar in almost every respect.

In Table 5 the results of the control experiments using a dusk extension of infra-red (i-r) radiation are shown. In both controls, the incident radiation exceeded the amounts measured when the 470nm interference filter was in place. When the incident radiation was 50-100 times that experienced in the action spectra experiments, some intermediates resulted. This may be due to a local heating effect and a consequent increase in developmental rate. However, 90% diapause was recorded with an incident radiation of nearly 10 times that measured for the experimental larvae and it is clear that i-r energy did not have any influence upon the outcome of the action

FIGURE 21.

Action Spectrum for Dusk. The photophase of a LD14:10 15°C was extended, at dusk, by two hour period of spectral light. The incident energy reaching the leaf surface was measured for each treatment and was varied at each wavelength in order to obtain a 50% response curve. The small figures represent the percentage of diapausing females from each experiment. The 50% response curve has been drawn by eye.

FIG. 21. Dusk Extension

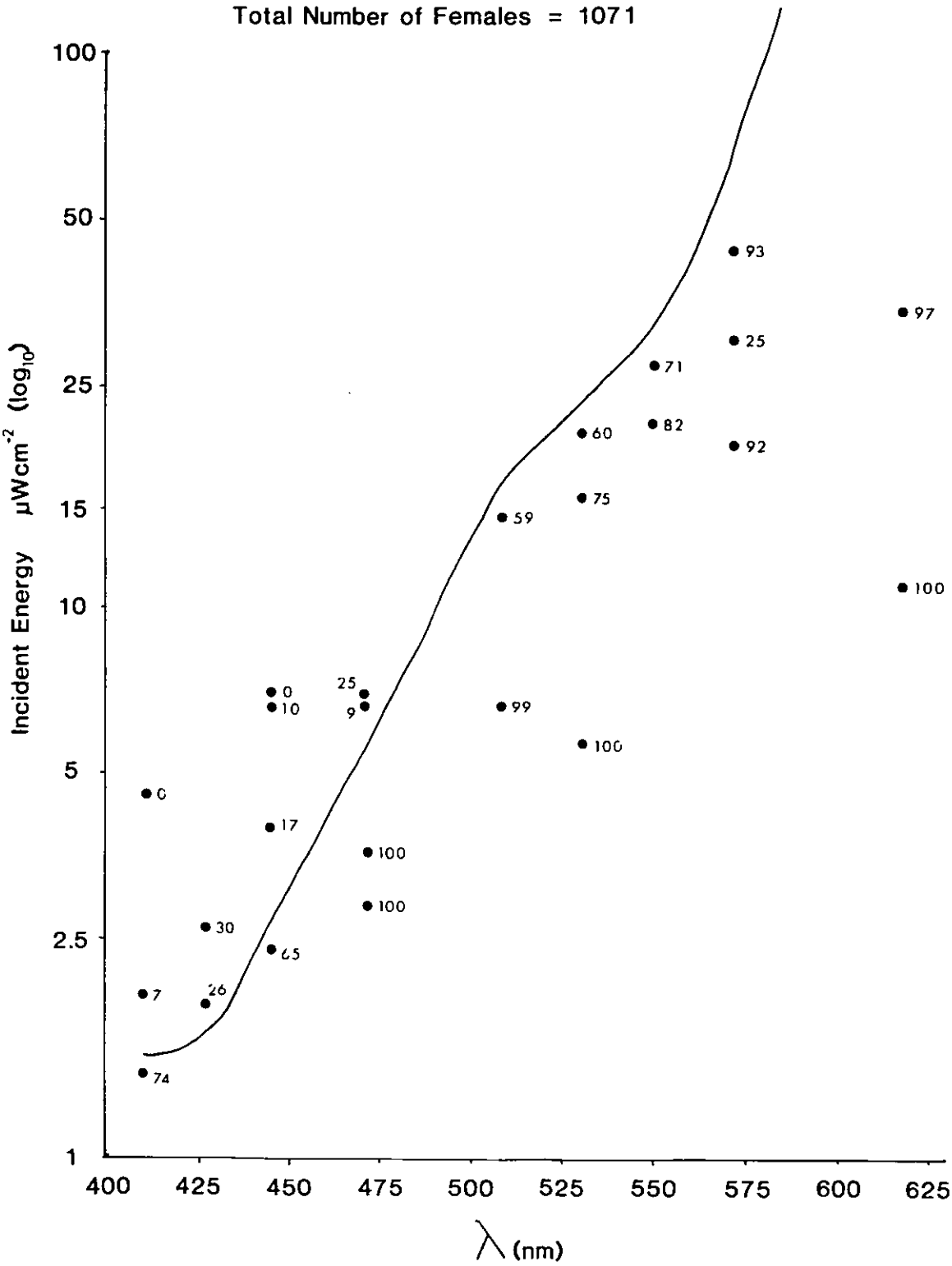


FIGURE 22.

Action Spectrum for Dawn. The photophase of a LD14:10 15°C regime was immediately preceded by two hours of spectral light of known incident energy. The energy was varied in an attempt to obtain a 50% response at each wavelength. The small figure represent the incidence of diapause in each experiment. The 50% response curve has been drawn by eye.

FIG. 22. Dawn Extension

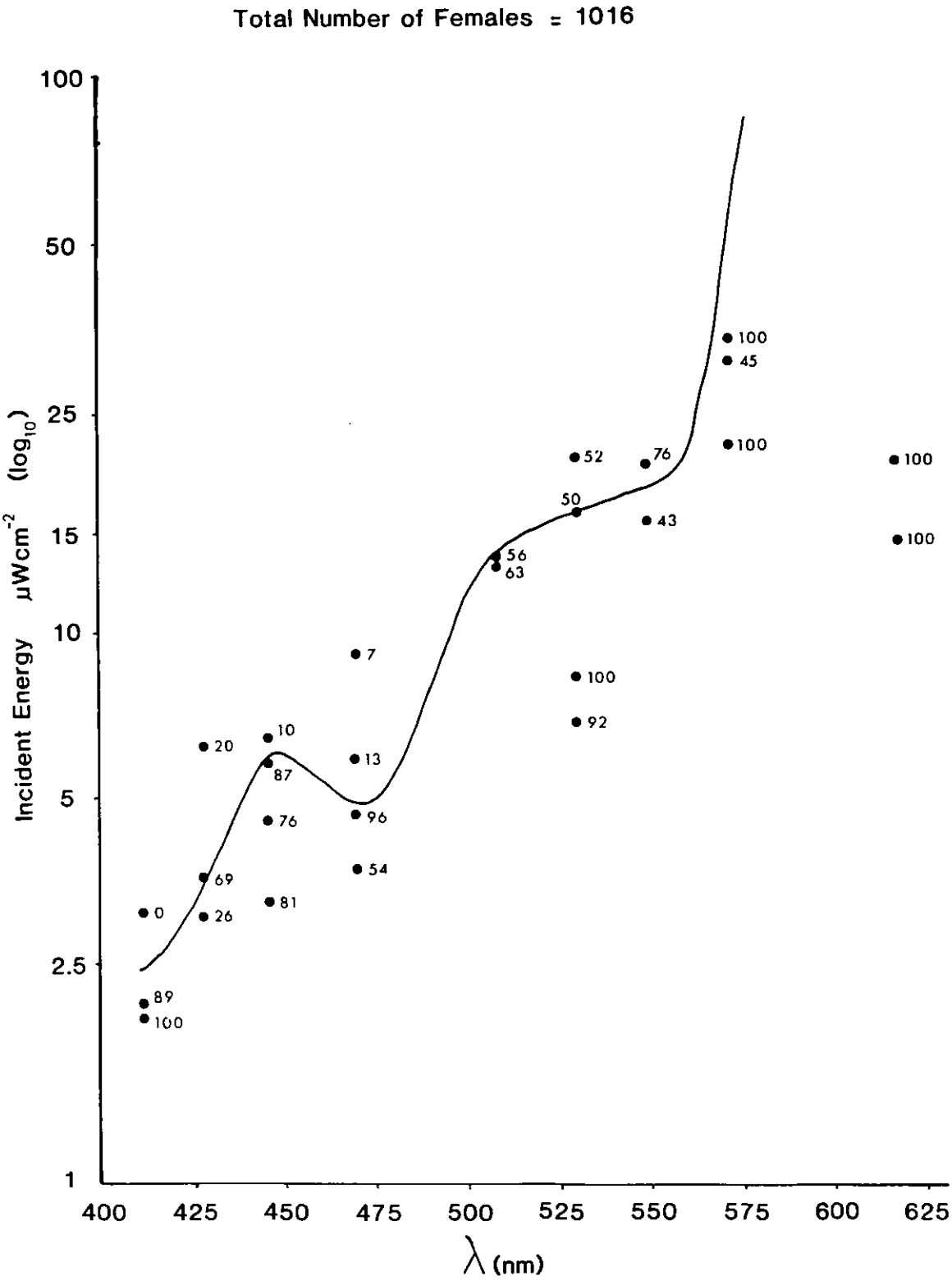


TABLE 5. The Incidence of Diapause when the Photophase of a LD14:10 15°C Regime is Extended from Dusk by two hours of Infra-red energy.

Filter	Irradiance ($uWcm^{-2}$)	n	Diapause (%)
Balzer 470nm	274	44	9
Wratten 87c i-r	10,238	64	32
Wratten 87c i-r	1,489	69	90

spectra experiments.

An extensive series of "resonance" experiments were performed in order to establish whether photoperiodic time measurement was influenced by the circadian system. Figs.23, 24 and 25 show the percentage of diapausing females, mean ovarian score and fat body score respectively when 8, 12 and 16 hour photophases were coupled with scotophases of up to 62 hours in length at a temperature of 15°C.

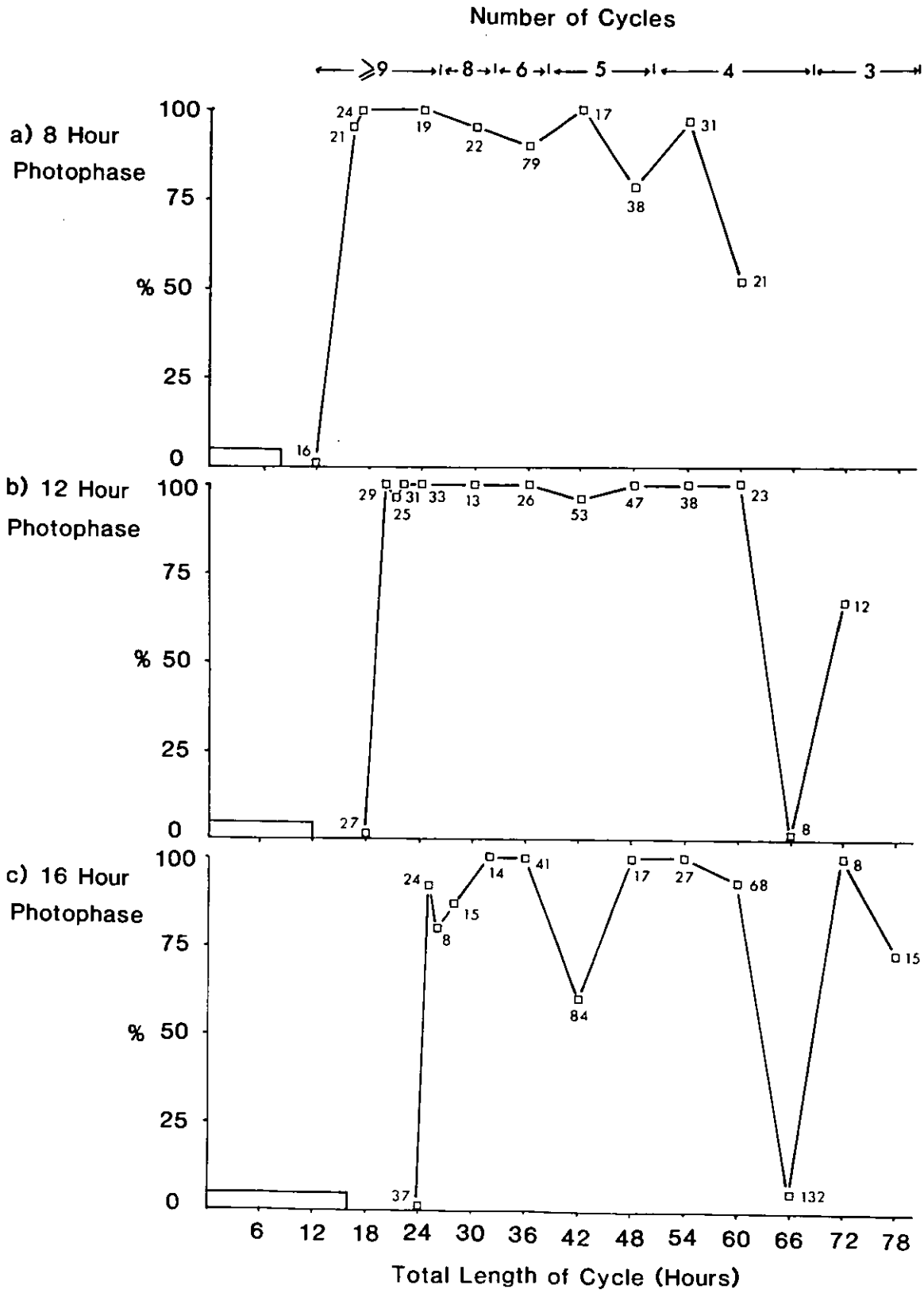
Once the CNL had been exceeded in Fig.23a and b the percentage of diapausing females remained high up to a night length of 48 hours with no indications of any periodic maxima or minima. However, two diapause minima were detected in Fig.23c at T42 and 66. This suggests a circadian influence but the absence of corresponding minima with 8 and 12 hour photophases and the low magnitude of the effect at T42, when a stronger effect would be expected compared to that at T66, renders this apparent positive resonance result somewhat equivocal.

It has been suggested that "careful selection of the "right conditions" may reveal circadian rhythmicity in photoperiodic responses" (SAUNDERS 1982b p256). Thus, higher temperatures may reveal resonance effects that are masked at lower temperatures. Another possible approach is to look at the response in a more detailed manner at the lower temperatures in an attempt to detect effects that are not expressed in the whole animal response. Ovarian and fat body scores provide means of detecting

FIGURE 23.

Resonance Experiments at 15°C. Photophases of 8, 12 and 16 hours were coupled with dark periods of up to 62 hours. The incidence of diapause was assessed by dissecting the females 21 days after emergence and recording the condition of their ovaries. The small figure indicate the number of females dissected in each condition.

FIG. 23. Incidence of Diapause



hidden resonance effects.

The data in Fig.24 were obtained by scoring the ovaries of a representative sample of the insects dissected to give the results shown in Fig.23. Any circadian based maxima or minima that were not apparent using the ovary condition criterion for diapause assessment would be expected to show up in this more precise measure of the effect of the test photoperiod. However, there was no evidence for resonance, indeed, the diapause minimum for T42 in Fig.23c had an unexpectedly low score for a 60% response. This was due to the unusually large number of intermediates resulting from that particular treatment (in fact, only 5 out of 84 were classified as non-diapausing).

Fat body development is another potential measure of the effect of photoperiodic treatment. The fat body scores of the females scored for ovarian development is shown in Fig.25 and, once again, there are no trends that suggest any circadian influence.

The result of raising the temperature to 17°C or 20°C during a resonance experiment with a 12 hour photophase are shown in Fig.26. In all three cases the incidence of diapause declined as the duration of the dark period increased. The 20°C "standard" (i.e. the test period commenced at the same developmental age as at 15°C) was performed first. Some whitefly had emerged before the end of the experimental period and it was clear that some of the photoperiodic cycles under test had been experienced

FIGURE 24.

Resonance Experiments at 15° C. A sample of the females dissected for Fig.23 had their ovaries scored. In each case the proportion of non-diapausing/intermediate/diapausing females in the sample was the same as that in the total experimental population.

FIG. 24. Ovarian Score

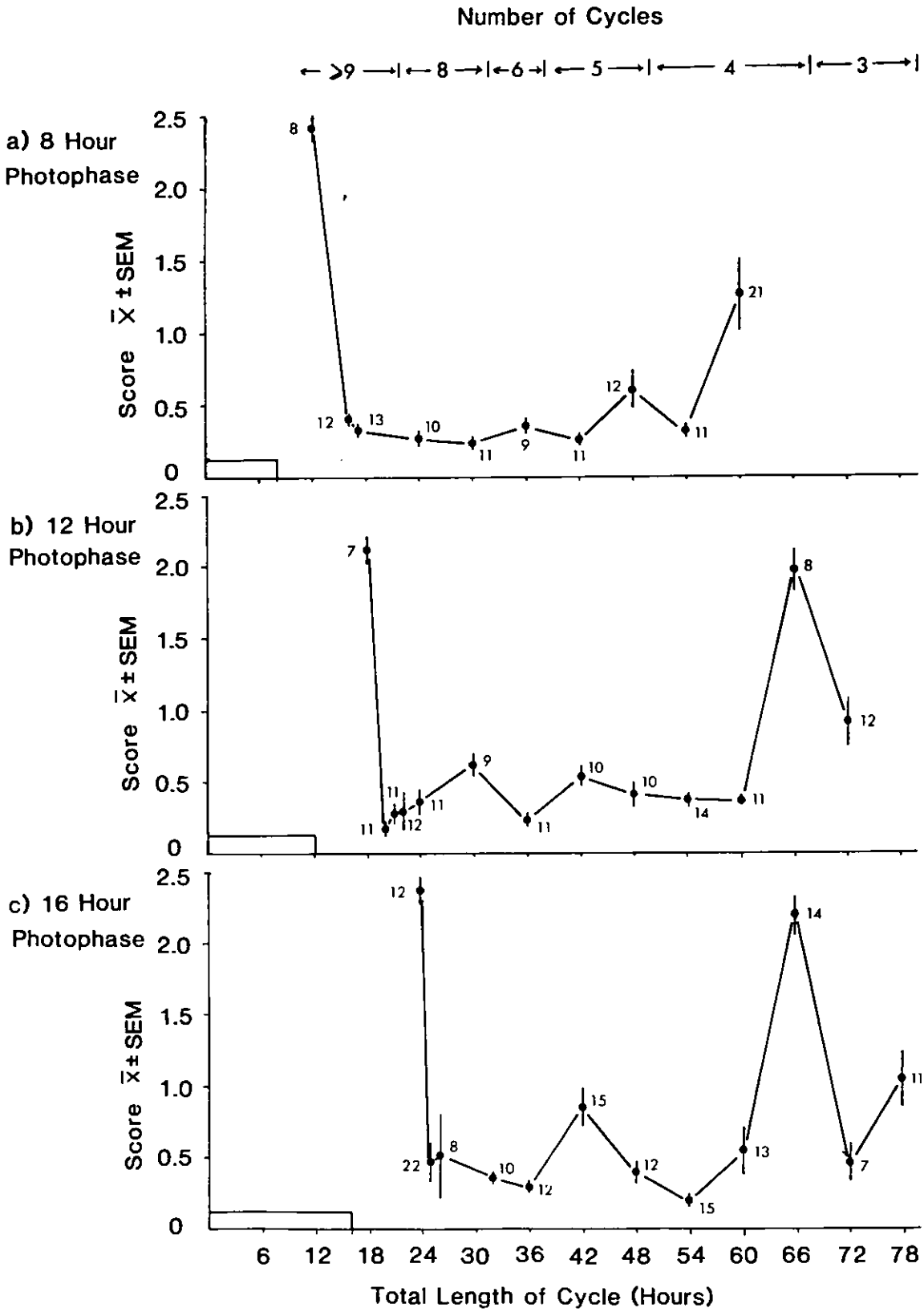


FIGURE 25.

Resonance Experiments at 15°C. The mean fat body scores of the females scored for ovarian development in Fig.24 were recorded.

FIG. 25 Fat Body Development

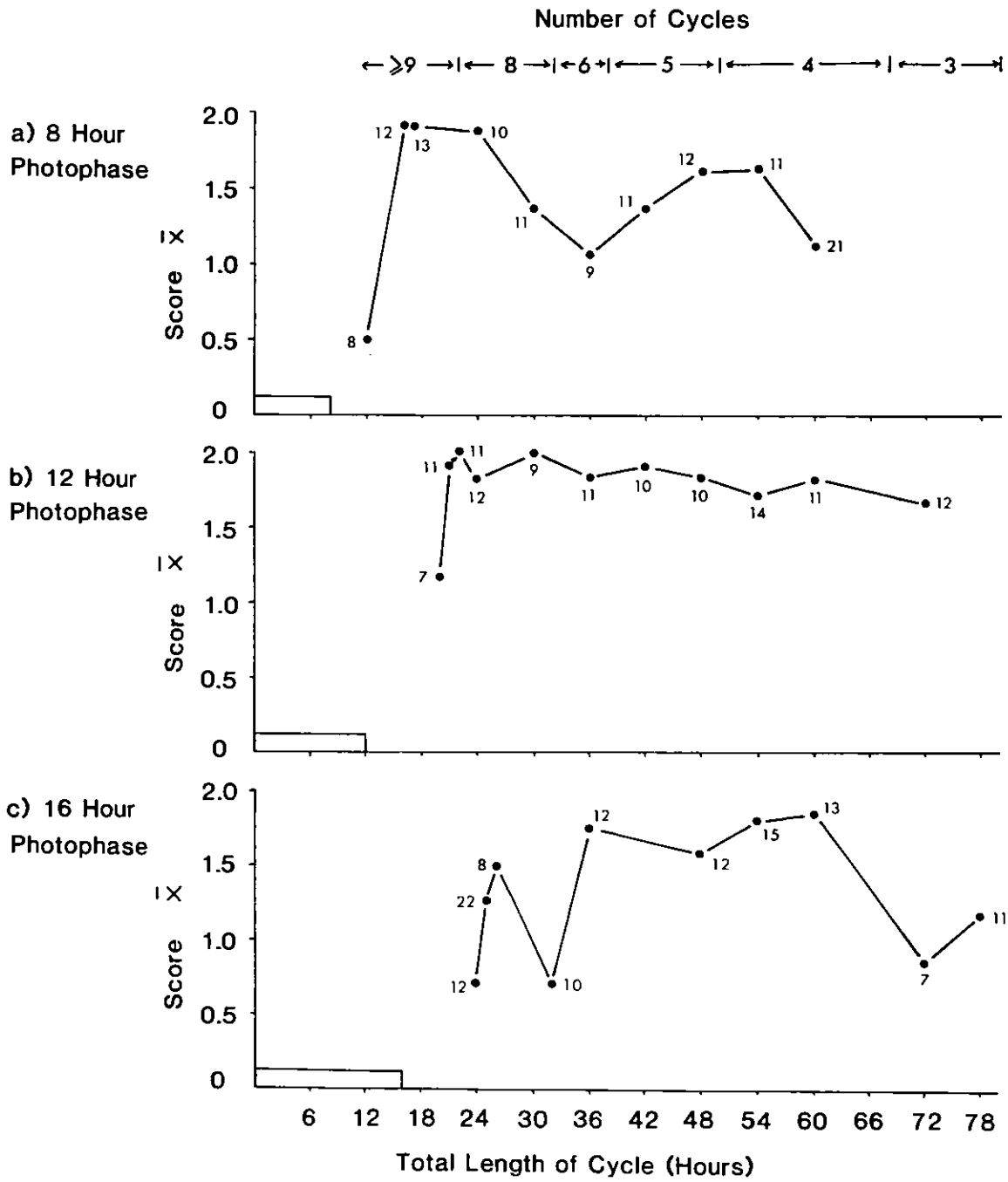
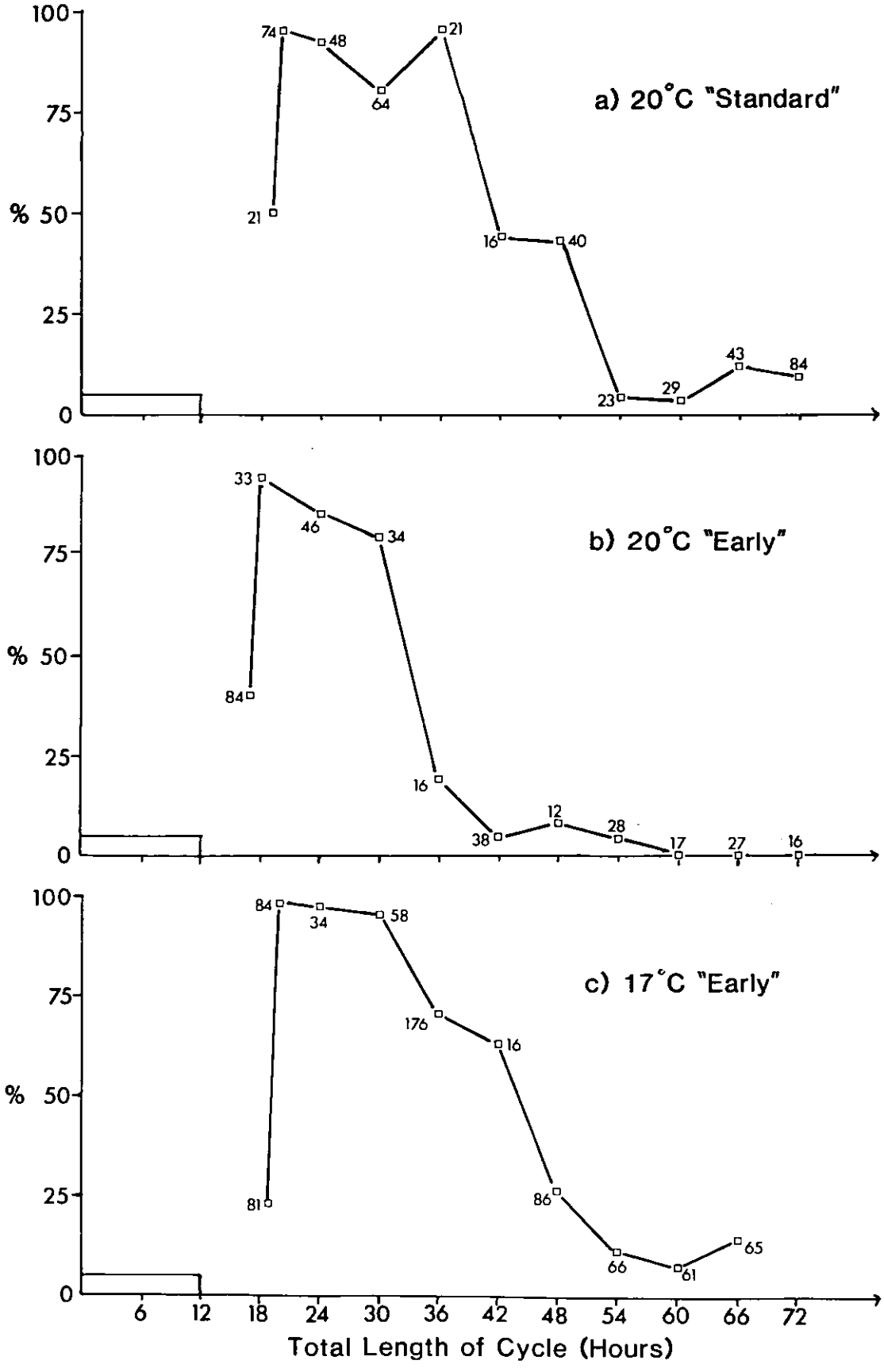


FIGURE 26.

Resonance Experiments at 20°C. a) larvae aged 2(4) to 3(1) were transferred to LD12:variable at 20°C for ten calendar days. b) and c) Larvae aged 1(5) to 2(2) were transferred to the experimental conditions. At the end of the experimental period, the insects were returned to LD16:8 15° C until 21 days after emergence when they were dissected. Diapause was assessed using the ovary condition criterion.

FIG. 26. Incidence of Diapause (12 Hour Photophase)



outside the sensitive period (see Fig.7). Consequently, a second series of 20°C experiments was completed with the experimental photoperiod starting around the time of the moult from the 1st to the 2nd instar (L1-E2). These results showed a diapause minimum at T42 but it was impossible to determine whether the subsequent maximum had been masked by the lack of sufficient cycles being experienced in longer cycles or whether the minimum was simply part of the trend for decreasing diapause incidence with increasing cycle length. Another experimental series, beginning at L1-E2, was performed at 17°C in order to clarify the situation. Fig.26c does not reveal a diapause minimum at T42 or provide any other evidence for circadian involvement. Instead, it appears that the percentage of diapausing females is related to the length of the cycle under test and the consequent counter effects.

DISCUSSION.

The nature of the photoperiodic clock in most insects is difficult to assess with any degree of certainty because, as the various hypotheses are refined and/or elaborated, further experiments become necessary to support or refute them. Consequently, very few species have been studied in sufficient depth for confident conclusions to be drawn. In a comprehensive review of insect clocks, Saunders (1982b) has described and discussed the numerous procedures available to the experimenter and it becomes readily apparent that this study has only covered a few of them in any detail.

If the photoinducible phase (ϕi) of a circadian based clock was able to free-run in long photophases, such as those in Fig.16, then periodic long day responses would result due to the illumination of this light sensitive phase. This was clearly not the case. However, it has been reported that the circadian oscillator underlying the rhythm of pupal eclosion in Drosophila pseudoobscura is "damped out" in photophases of more than 12 hours(PITTENDRIGH 1966). A similar effect has been recorded with the oscillator involved in the induction of diapause in Sarcophaga argyrostoma (SAUNDERS 1973b). If this is the case, the results presented in Fig.16 do not rule out the possibility of circadian involvement. However, the strong diapause inductive effect of a 10 hour scotophase, even when coupled with photophases of over 24

hours, suggests that time-measurement in A. proletella occurs during darkness. This has also been shown to be the case with Antheraea pernyi (TANAKA 1950), Panonynchus ulmi (LEES 1953a), Adoxophyes orana (BONNEMAISON 1978) and Ostrinia nubilalis (BONNEMAISON 1978).

Numerous studies on the spectral sensitivity of the photoperiodic photoreceptor have been performed. The earliest is that of Kogure (1933, see ANDREWARTHA 1952 p79 and LEES 1955 p20) working with Bombyx mori, which diapauses in response to long daylengths. He subjected eggs of the bivoltine race "showa" to continuous experimental light and found that violet and blue/green wavelengths (350-510nm) were equivelant to white light (98% diapause) with <10% diapause induced by similar relative energies of wavelengths >550nm. Peak sensitivity in the blue-green region of the spectrum was also recorded for Grapholitha molesta (DICKSON 1949). These, and many subsequent studies, paid little or no regard to the light intensities at different wavelengths and a wide range of "effective" wavelenths were identified for each species (see SAUNDERS 1982b p187).

A far greater degree of precision may be achieved if the energy is measured and varied for each wavelength and narrow band interference filters are used. An energy compensated action spectrum may then be determined. If the photosensitivity of the species throughout the light/dark cycle (usually the scotophase alone) is known, illumination of selected portions of the daily cycle may provide an insight into the underlying physiology of the

photoreceptor-pigment system.

The first study to include figures for different relative energies in the same, very broad, wavelength band is that of Lees (1953a) with the red spider mite Metatetranychus (=Panonychus)ulmi. A photoperiodic regime of 8hr white light: 8hrs spectral light: 8hrs darkness was used, Red light was ineffective and the threshold of sensitivity to blue (peak transmission around 425nm), green (540nm) and near ultra-violet (u-v) (365nm) was in the ratio 1:7:34 (LEES 1953a).

Extensive work with Megoura viciae has shown that the peak spectral sensitivity of early night interruptions (1hr of spectral light starting 1.5hrs into the scotophase) is in the 450-470nm range (LEES 1966, 1981). The incident energy required for a 50% response was $0.2\text{-}0.3^{-2}$ uWcm at peak. Sensitivity extended into the u-v but not into red wavelengths (>570nm). When 0.5 and 1.0hrs of spectral light started 7.5hrs into the scotophase (Stage 3 of the Megoura clock) sensitivity was much less specific and, although the peak still appeared to occur at 450-470nm, a 50% response resulted from similar energies between 365 and 550nm (LEES 1981). The major difference between the two late interruptions was that the 50% response at peak required 0.2-0.3uWcm^{-2} for 1.0hr and 2-3uWcm^{-2} for 0.5hrs.

Similar early night results were obtained for the photoperiodic termination of diapause in Laspeyresia pomonella where dusk extensions, rather than night

interruptions constituted the experimental regime (NORRIS et al. 1969). High sensitivity was found between 400nm and 500nm with a peak at around 450nm where 0.03uWcm^{-2} produced a 50% response (NORRIS et al 1969; HAYES 1971). The action spectrum for breaking diapause in Antheraea pernyi revealed greatest sensitivity between 400 and 500nm without a marked peak. The energy required for a 50% response was 0.01-0.05uWcm^{-2} in this range (NORRIS et al. 1969).

Action spectra for dawn and dusk extensions of a white light short-day have been determined for two species, Chaoborus americanus (BRADSHAW 1972, 1974) and Nasonia vitripennis (SAUNDERS 1975c). In the former, 4 hours of monochromatic light replaced either the first or the last 4 hours of the scotophase of an LD12:12 regime and peak sensitivity was located around 540nm in both instances at energies of 10^{-1} ergs cm^{-2}sec^{-1} (=0.01uWcm^{-2}) and 10^{-2}ergs cm^{-2} sec^{-1} (=0.001uWcm^{-2}) for 50% dusk and dawn responses respectively (BRADSHAW 1974). The Nasonia results, from 3 hours of spectral light showed peak sensitivity to lie between 550nm and 620nm for dusk and dawn extensions with 50% responses at 0.46uWcm^{-2} and 0.02uWcm^{-2} respectively (SAUNDERS 1975c).

The results presented for A. proletella in Figs.21 and 22 are for 2 hours of spectral light at dusk and dawn respectively. Peak sensitivity is at around the same wavelength in both cases (420nm) and a similar incident energy is required for a 50% response at both peaks.

Although it is unjustifiable to generalise on the basis of results from just three species it is striking that dawn and dusk extensions (as opposed to night interruptions of spectral light) have peak sensitivities at similar wavelengths and it has been suggested that "similar or identical photoisomers of a single protein complex underlie both the dawn and dusk responses."(BRADSHAW 1974). It was suggested that the differences in the early and late night action spectra of M. viciae reflected configurational changes of the pigment molecule during the scotophase rather than the existence of two pigment systems (LEES 1981). Some of the differences between these four species may be due to the different physiological effects of either extending the photophase or interrupting the scotophase. It has also been shown that the length of exposure to spectral light may influence the final action spectrum with regard to threshold (LEES 1981) and possibly sensitivity as well.

It is significant that neither Bradshaw (1974) nor Saunders (1975c) speculated upon the identity of the photopigment and, even in the action spectra of M. viciae (LEES 1981) that had been corrected for cuticular absorption (HARDIE et al. 1981) Lees went no further than to comment that "predominantly blue maxima are consistent with the view that a caroteno-protein (but not rhodopsin itself) is the photoreceptor." (LEES 1981). A similar tentative suggestion may be made for A. proletella in view of the results presented in Figs.21 and 22. In addition the slight shoulder that is apparent in the

green-yellow (500-550nm) band in the dawn and dusk extensions may be due to the presence of a second photoreceptor with a sensitivity that is approximately an order of magnitude lower than that of the blue receptor.

Recent work based on artificial diets and carotenoid deprivation and replenishment has shown that carotenoids are necessary for the photoperiodic response of three arthropod species (VEERMAN and HELLE 1978; VEERMAN 1980; VAN ZON et al. 1981; VEERMAN et al. 1983; SHIMIZU and KATO 1984) but the fact that the pigment is apparenly present in minute quantities (see HARDIE et al. 1981) has made positive in situ identification impossible.

Once the photoreceptor-pigment system has detected light/dark some measurement process is required to determine whether the photoperiod will induce or avert diapause. Two basic principles underly the various hypotheses of time measurement: an hour-glass, which starts timing at dusk and proceeds through certain biochemical phases until dawn; and circadian based oscillator(s) with phase relationships determining whether long or short day responses result from the lighting regime.

The external coincidence model (PITTENDRIGH and MINIS 1964; PITTENDRIGH 1966) was derived from Bunning's principle of endogenous oscillators being involved in time measurement (BUNNING 1960; BUNNING and JOERRENS 1960). In this model, light entrains the rhythm and induces the photoperiodic response if it then coincides with the

photoinducible phase, ϕi (PITTENDRIGH 1966). It is necessary to know the entrainment properties of the oscillator in order to predict the position of oi in a range of experimental light regimes and compare this with the observed percentage of responding individuals. However, the hypothetical oscillator is not observable so an overt rhythm is studied on the basis that its entrainment properties are comparable with those of the rhythm underlying photoperiodism (PITTENDRIGH and MINIS 1964, 1971; SAUNDERS 1982a,b). The entrainment behaviour of the pupal eclosion rhythm in Drosophila pseudoobscura (PITTENDRIGH 1965, 1966) combined with experimental data obtained with Pectinophora gossypiella (ADKISSON 1964, 1966) formed the basis of this model. The D. pseudoobscura oscillation is damped out in photophases of 12 hours or more and when it is set in motion by the onset of darkness it always starts at ct12 with ϕi positioned just short of the CNL (say ct12 + C) (PITTENDRIGH 1966). If the photophase is less than (24 - C) hours long, a scotophase of C hours or more will commence at ct12 and oi will coincide with darkness resulting in short day effects. Early night interruptions result in a new dusk position in the photoperiodic cycle, but the circadian time is re-set to ct12 such that oi falls at the same point relative to ct12, but later in the photoperiodic cycle where it may be illuminated to effect a long day response. A late night interruption may illuminate oi and this will also be "read" as a long day. Interruptions in the middle of the night cause a phase jump to occur (SAUNDERS 1982b; VAZ NUNES and VEERMAN 1982a).

In many species, the incidence of diapause is low in short photoperiods and DD (LEES 1968; SAUNDERS 1982b) whereas external coincidence predicts maximum diapause because oi always occurs in the dark. A multi-oscillator version of the external coincidence model has been developed for Sarcophaga argyrostoma (SAUNDERS 1982a, b) however an alternative hypothesis is that of internal coincidence in which the light/dark cycle entrains endogenous oscillators which may then free-run in DD. In its most simple form this model consists of two oscillators, one is phase set by dawn and the other by dusk. As the photoperiodic cycle changes, the phase relationship between the two oscillators alters such that long and ultra-short photophases cause the "active phases" of the oscillators to coincide and cause long day effects (TYSHCHENKO 1966, in DANILEVSKY et al. 1970; PITTENDRIGH 1972).

The hour-glass principle has been most intensively investigated in Megoura viciae (LEES 1966, 1971, 1973). A linear biochemical sequence is believed to commence at dusk and proceed through four dark stages that differ in their photoresponsiveness. Stage 1 is photosensitive and photoreversible and light re-sets the clock to commence timing in a normal manner at the end of the light interruption. Stage 2 exhibits some degree of photorefractoriness. Stage 3, like Stage 1, is photosensitive however, light stops the clock and does not re-set it correctly unless the light break lasts for a few hours, if it is too short then the subsequent dark timing

process is impaired. Stage 3 immediately precedes the CNL where Stage 4 starts. The timing process is completed by the end of Stage 3 and light falling in Stage 4 serves to "turn the hour-glass over" in preparation for the start of the next period of darkness.

Experimental lighting regimes in which the scotophase is interrupted by light at different points have provided evidence that has been interpreted as supporting one or more of the basic principles outlined above. Early work with Pieris brassicae where light breaks were applied during the scotophases of LD6:18 and LD12:12 produced one peak of photosensitivity (diapause inhibition/avoidance) although the interruptions were not continued into the late night (BUNNING 1960). The position of the peak was compatible with coincidence and hour-glass hypotheses. Subsequently, experiments with the entire night being scanned by light breaks, have been performed with numerous species and two periods of photosensitivity were often apparent. These have been termed the "A" and "B" peaks (SAUNDERS 1968) and their position relative to lights-on and lights-off in different cycles is a useful indicator of oscillator and/or hour-glass involvement.

Night interruption experiments with Pectinophora gossypiella showed maximum diapause inhibition when the light break occurred 14 hours after lights on (the A peak) and 14 hours before lights off (the B peak) (ADKISSON 1966; SAUNDERS 1982b). Alternatively, from an hour-glass standpoint, maximum inhibition occurred when the light break was positioned 10 hours after lights off (just short

of the CNL for this species) which would be Stage 3 of the timing process. Just as the results are open to either interpretation, "the type of experimentation chosen depends to some extent on the convictions of the investigator!"(LEES 1971).

In some species, including P. gossypiella (ADKISSON 1966), S. argyrostoma (SAUNDERS 1975b, 1979) Tetranychus urticae (VAZ NUNES and VEERMAN 1979b) and Aedes atropalpus (BEACH and CRAIG 1977) the A peak is not evident when long scotophases are interrupted by light. According to the external coincidence model, a light interruption at the A peak is read as a new dusk and if the post-interruption dark period exceeds the CNL, ϕi lies in the dark so diapause is not averted and the A peak apparently disappears. Similarly, an early night interruption is believed to re-set an hour-glass clock such that a subsequent long scotophase is measured and masks the effect of the earlier re-setting process.

There are a few species, including Carpocapsa pomonella (PETERSON and HAMNER 1968) and Pieris rapae (BARKER et al. 1964) in which the B peak is either minor or non-existent. This may be due to ϕi lying at A or ϕi at B requiring more energy to effect a long day response (SAUNDERS 1982b). Stage 3 of an hour-glass, in such cases, may require more energy to stop the biochemical process than is necessary in Stage 1, although it has been shown that the Megoura clock may be stopped in Stage 3 by a shorter light period than is necessary to re-set the clock in Stage 1 (LEES 1973, 1981).

Independently extending the light and dark components of night interruption studies has proved useful in determining whether coincidence or hour-glass processes are involved in photoperiodism. Interrupting a 10.5 hour scotophase with 1 hour light pulses produced similar profiles of photosensitivity in M. viciae when the accompanying photophase was 4, 8, 13.5 and 25.5 hours long (LEES 1966, 1973). This is compatible with a dark period hour-glass timer, but not with a coincidence model since the phase relationships would be different in the different cycle lengths (LEES 1971, 1973). Similar experiments with the parasitic wasp, Nasonia vitripennis, where the nights of LD11.3:10 and LD14:10 were scanned with light breaks suggested that the B peak was related to lights-off but the A peak was set by lights-on and, thus, exhibited rhythmic properties (SAUNDERS 1968). In the same species, however, night interruptions of LD14:10, LD12:12 and LD10:14 revealed a tendency for the A peak to "drift" towards dusk with the B peak remaining in a similar position with respect to lights on/off (SAUNDERS 1968). This was attributed to the rhythm being phase set by the entire light component and not just dawn (SAUNDERS 1968). However, since the two peaks of diapause inhibition were in similar positions relative to lights-off in all three regimes, an hour-glass argument could also be applied if these results were taken in isolation.

It is possible to assess the effect of light breaks during the scotophase by extending the post-interruption dark period to a value in excess of the CNL. Such experiments have been performed, to some degree, in three species, Megoura viciae (LEES 1966, 1971, 1973), Sarcophaga argyrostoma (SAUNDERS 1979, 1981a) and Sarcophaga crassipalpis (GNAGEY and DENLINGER 1984). The results with M. viciae and S. argyrostoma were almost identical in that early interruptions were overridden by subsequent dark periods longer than the CNL but late interruptions inhibited the long night response even when the post- interruption scotophase was 12 hours long (over 2 hours in excess of the CNL for both species). Unfortunately, the comparative value of the S. crassipalpis results is impaired by the small number of experiments performed and the choice of 10.5 hours (=CNL) as the post interruption dark period (GNAGEY and DENLINGER 1984) since a variable response would be expected in replicates of photoperiods including the CNL as the scotophase. Nevertheless, there were indications that light breaks late in Stage 1 and Stage 3 were not readily overridden by a long post-interruption scotophase.

In spite of the similarity of the M. viciae and S. argyrostoma results the former are interpreted as evidence for the hour-glass hypothesis (LEES 1973) and the latter as an example of external coincidence (SAUNDERS 1979). Indeed these two species only differ fundamentally in their response to resonance experiments which do not reveal any circadian rhythmicity in the M. viciae timer

(LEES 1973) but provide strong evidence in favour of a circadian basis to the S. argyrostoma clock (SAUNDERS 1973b). These results will be discussed later.

The results presented in Fig.17 show that, in A. proletella A and B peaks of photosensitivity are present during the scotophase of LD14:10 15° C. Without varying the lengths of the scotophase and photophase it is impossible to judge whether the peaks are related to different "reference points" in the photoperiodic cycle (such as dawn and dusk) or represent phases of an hour-glass clock set in motion at dusk. However, Fig.18 clearly indicates that the effect of light interruptions in the first 5 hours of the night may be overridden by a post-interruption scotophase that just exceeds the CNL Whilst longer periods of darkness were required to induce 100% diapause when the interruptions commenced 5 to 8 hours after dusk. The pattern of these results follows that of M. viciae (LEES 1973) and S. argyrostoma (SAUNDERS 1979) although the late interruptions applied to A. proletella are not as "resistant" to long scotophases as in these two species (see Fig.19). This may be due to the slightly different light sensitivities of the three species and it appears that an hour of light can either "turn over" the hour-glass more effectively in A. proletella than in M. viciae or phase set the oscillator better than in S. argyrostoma depending upon the nature of the timer.

It has been shown that the hour-glass of M. viciae is only turned over completely if a Stage 3 interruption comprises more than 6 hours of light (LEES 1973). This is the stage in which short nights in the ecological range of the species will fall and these will always be followed by photophases well in excess of 6 hours so this requirement is not prohibitive in any way. Extending the light break to 4 hours is sufficient to enable A. proletella to measure a 9 hour scotophase as "long" regardless of the position of the interruption (Fig.20). Although comparable experiments have not been performed for any other insects these results would be compatible with a coincidence based clock if the start of the long light break was accepted as dawn and the short nights preceding and following the long nights did not abolish their effect.

On the basis of night interruption experiments alone it is unnecessary to propose any circadian basis for the photoperiodic clock of A. proletella. However, as mentioned above, almost identical results have been obtained for two of the most intensively investigated species, M. viciae and S. argyrostoma and yet the models for their timers are based on fundamentally different hypotheses (LEES 1973; SAUNDERS 1973b, 1979).

The method most widely used to detect an underlying circadian influence in photoperiodic time measurement consists of coupling a constant length photophase with various scotophases up to 72 hours in length. These are termed resonance (see SAUNDERS 1982b) or Nanda-Hamner (see

PITTENDRIGH 1981) experiments and the rationale is that the periodicity (τ) of the supposed endogenous oscillator is close to 24 hours and free-runs in long scotophases. If the cycle length is a multiple of 24 (T24, T48, T72 etc), then darkness will always occur where the scotophase of LD12:12, for example, would occur and long night effects will result. However, with cycle lengths far away from modulo , various degrees of illumination will be experienced during the subjective night i.e. in LD12:24 (T36) the second cycle will be 12 hours out of phase with LD12:12 and the second photophase will occur where a 12 hour scotophase would be "expected". Consequently, periodic diapause maxima and minima are seen as long and short night effects are produced. If the clock is an hour-glass then only one time measurement process will be completed once the night length is greater than the CNL and long night effects are predicted at all subsequent scotophases.

Both coincidence models can explain positive resonance results (i.e. those with periodic maxima and minima)(see PITTENDRIGH 1981, PITTENDRIGH et al. 1984; SAUNDERS 1982b). In the external coincidence model, oi free-runs in DD and will be illuminated when light falls in the subjective night (T36, T60)(PITTENDRIGH 1966; VAZ NUNES and VEERMAN 1982a) whilst internal coincidence is implicated if the ascending and descending slopes of the maxima and minima are seen to be related to different phase setting points (SAUNDERS 1974, 1982b; PITTENDRIGH et al. 1984).

Positive resonance results constitute very strong evidence in favour of circadian involvement however, negative results do not necessarily rule out an oscillator role. It has been shown that the positive resonance result with S. argyrostoma is masked at 16° C (SAUNDERS 1973b) where the results are almost identical to the negative results presented for M. viciae (LEES 1973) and it was suggested that a higher temperature might reveal a resonance effect in Megoura (SAUNDERS 1981, 1982b). It has since been shown that this is not the case (LEES 1984 and personal communication) and the Megoura clock does behave like an hour-glass.

Negative resonance results have been observed at one temperature for diapause induction in Pectinophora gossypiella (ADKISSON 1966), Carpocapsa pomonella (PETERSON and HAMNER 1968) and Ostrinia nubilalis (BONNEMAISON 1978). Simlarly, no resonance effect was detected in diapause termination of O. nubilalis (SKOPIC and BOWEN 1976) or a Swedish stock of Pterostichus nigrita (THIELE 1977b). It is possible that higher temperatures would implicate the circadian system in all or some of these cases particularly in the case of dipause induction in O. nubilalis where scanning a long scotophase with light breaks did produce periodic diapause minima (BONNEMAISON 1978).

In Pieris brassicae, τ is closer to 22 hours (CLARET et al. 1981) and, in Tetranychus urticae it is about 20 hours (VEERMAN and VAZ NUNES 1980). Closer inspection of the latter example in particular reveals another possible

explanation for apparently negative resonance results as well as suggesting great care when "choosing" an overt rhythm to construct a phase response curve: diapause minima were observed at T26, T46 and T64 whereas a periodicity of 24 hours would be expected to produce minima at T36 and T60. In order to detect periodic minima when $\tau \neq 24$, it is essential that the length of the scotophase is increased in small increments. Whilst the variable scotophase in work with M. viciae was increased by 4 hours at a time (LEES 1973), the scotophase length of all the other "negative" species mentioned above was increased in 12 hour increments and this might also mask the effects of an oscillator if one is present.

A series of resonance experiments has been completed with A. proletella at 15°C and using scotophase increments of 6 hours (see Figs 23, 24 and 25). Three photophases were used and ovarian and fat body scores were calculated for each treatment since it was possible that these detailed measures might reveal maxima and minima of responsiveness to the photoperiodic regime that were not expressed at a whole animal level without necessitating further experiments at higher temperatures. There was a suggestion of diapause minima 24 hours apart at T42 and T66 in the LD16:variable experiment. However, this was not supported in any of the other experiments even at higher temperatures of 17°C and 20°C (Fig.26). Thus, in spite of an extensive search, no evidence to suggest a circadian role in the photoperiodic clock of A. proletella has been found.

One of the problems inherent in the resonance procedure is the effect upon the number of cycles experienced during the sensitive period. For example such experiments are impractical in S. crassipalpis where peak sensitivity is confined to a 48 hour period (GNAGEY and DENLINGER 1984). Temperature effects on the absolute duration of the sensitive period and, thus, the chances of the RDN being exceeded are well known (SAUNDERS 1981) and apply whether time measurement is executed by an hour-glass or an oscillator based clock. However, an important difference between these two principles of timing is that an hour-glass may only measure one night in long scotophases whilst a free-running oscillator may record several events. It is this feature of the hour-glass that probably explains the "tailing off" in diapause incidence shown in Fig. 23. Constant darkness, to an hour-glass, should also represent one night which is unlikely to produce enough inductive information to pass to the counter and induce diapause. However, DD did induce over 80% diapause (see Chapter 1 Fig.10). In the best studied example of an hour-glass clock it was shown that a highly variable result was obtained from DD in M. viciae (LEES 1973) and some sort of internal disorganisation may be responsible. Alternatively, spontaneous dark reactions may take place that effectively turn the hour-glass over and initiate another timing process in the absence of light (see VAZ NUNES and VEERMAN 1982b).

Using overt rhythms to construct phase response curves (PRC) and, hence, predict the likely incidence of diapause in different photoperiodic regimes assumes that the selected rhythm and the hypothetical photoperiodic oscillator are "connected" in the organism's multioscillator system and behave in a similar manner. It is ironic that the most well known overt rhythm is that of pupal eclosion in Drosophila pseudoobscura, a species which does not diapause (PITTENDRIGH 1965, 1981). Another PRC based on a pupal eclosion rhythm has been constructed for S. argyrostoma (SAUNDERS 1976) whilst an entirely theoretical PRC was calculated for T. urticae (VAZ NUNES and VEERMAN 1982a). In both cases, predicted and observed diapause values were in close agreement for most photoperiodic regimes. However, several experimental findings serve to emphasise the care that must be taken when using this approach. For example, a normal eclosion rhythm was reported in carotenoid deprived Bombyx mori although the photoperiodic response was affected suggesting that either the two systems had different carotenoid requirements, or that they were not part of the same system (SHIMIZU and KATO 1982). The entrainment of the D. pseudoobscura pupal eclosion has also been shown to be insensitive to carotenoid deprivation (ZIMMERMAN and GOLDSMITH 1971). Oviposition, egg hatch and pupal eclosion rhythms in P. gossypiella have different periodicities that were inconsistent with the external coincidence model (PITTENDRIGH and MINIS 1971) and would produce different predictions of diapause incidence depending upon which rhythm formed the basis of the PRC.

Some degree of reconciliation between hour-glass and circadian influences within photoperiodism has been suggested in the most recent model for time measurement. The "hour-glass timer - oscillator counter" model developed for T. urticae showed very close agreement between predicted and observed results in all experimental lighting regimes tested (VAZ NUNES and VEERMAN 1982b; VEERMAN and VAZ NUNES 1984). It comprises an hour-glass clock based on the principles developed for M. viciae (LEES 1973) and an oscillator that modifies the inductive value of the clock's output which acts as a fine adjustment on the amount of inductive information passing to the counter. Appropriate values for the variable components in the model have been shown to account for experimental results obtained with, amongst others, M. viciae and S. argyrostoma (VAZ NUNES 1983).

No evidence for circadian involvement has been detected in the process of photoperiodic time measurement by A. proletella, and the reduction in diapause incidence in extended scotophases can be accounted for if an insufficient number of cycles, regardless of whether they are inductive or not, are experienced during the sensitive period. In addition, the value of a particular photoperiodic cycle changes as it is presented to the whitefly at different times during the sensitive period and is optimal during the 3rd instar (see Chapter 1). Clearly, this will also influence the total amount of inductive information accumulated by the counter before the end of the sensitive period and yet, although a range

of sensitivities during this period has also been identified in other species (SAUNDERS 1971; VEERMAN 1977b; SAUNDERS and BRADLEY 1984)) none of the models proposed to date have incorporated this particular variable. Further support for, and refinement of, this proposal requires much more precise knowledge of the relative inductive value of each day of the sensitive period. Until such investigations have been carried out, a more formal construction of a model incorporating this factor cannot be constructed. However that does not affect the overall conclusion that the results presented here are consistent with the photoperiodic clock of A. proletella operating on the hour-glass principle.

CHAPTER FOUR.

DIAPAUSE MAINTENANCE AND TERMINATION.

INTRODUCTION.

The term "physiogenesis" was first used by Andrewartha (1952) to describe the physiological processes that took place between the induction of diapause and its termination. Probably the most important aspect of its definition was the recognition that diapause terminated, physiologically, some time prior to any apparent changes, such as behavioural activity or oviposition. It was followed by a period of post-diapause morphogenesis, the rate of which was determined by the prevailing environmental conditions. In very general terms, the dormancy as a whole could be regarded as diapause followed by quiescence (HODEK 1971d) since, although the second stage does not occur as a direct response to environmental factors, it is directly responsive to them. In Mansingh's (1971) scheme, diapause induction is immediately followed by a "refractory" stage (=physiogenesis) and then an "activation" phase (=post-diapause morphogenesis), whilst "termination" is used to refer to the apparent resumption of normal activity, such as pupation, pupal eclosion, or first oviposition. Throughout this Chapter, Mansingh's definition of termination will be observed and the other aspects of the classifications will be considered to be

interchangeable.

Detailed knowledge of physiogenesis and post-diapause morphogenesis is lacking and the relevance of some termination factors, identified in the laboratory, to naturally occurring conditions is often indirect. The role of the photoperiod is particularly confusing, which is probably a direct result of its great significance as an inducing factor. For example, a role for photoperiod may be inferred if termination results from a transfer of diapausing adults to long day conditions. In a series of experiments with the lacewing, Chrysopa carnea, the terminating effect of long days was recorded (MACLEOD 1967; TAUBER et al. 1970a; TAUBER and TAUBER 1970a). However, when diapausing adults were taken from the field and transferred to one of a range of photoperiods, the long day termination effect was seen to "disappear" more than two months before termination (first oviposition) was recorded in the field (TAUBER and TAUBER 1973c, 1976a). The authors concluded that natural photoperiods were not responsible for the termination of the dormancy, but the short photoperiods of autumn and winter might retard the rate of post-diapause morphogenesis and thus delay termination (TAUBER and TAUBER 1973b,c, 1976a).

One of the most intensively studied insects that exhibits an ovarian diapause is the Linden bug, Pyrrhocoris apterus. Bugs that were transferred from the field to long days in the laboratory during the early period of their reproductive arrest oviposited sooner than those transferred to short days (HODEK 1971d). However,

the difference in pre-oviposition time declined as field collections were made further into the dormancy until, by mid December, the photoperiodic effect was no longer significant (HODEK 1971b). In the field, termination was recorded in April, and it was suggested that low temperature served to maintain diapause through its effect upon the rate of morphogenesis after photoperiodic sensitivity was lost (HODEK 1971d). Recently, a photoperiodic induction-termination asymmetry was demonstrated for this species with the critical photoperiod for termination being one hour longer than that for induction (SAUNDERS 1983). It was also shown that 12-16 weeks chilling at 4°C, which would probably be experienced in nature, followed by favourable temperatures, at any photoperiod, resulted in termination so the photoperiodic effect formed: "...part of an ecologically redundant mechanism." (SAUNDERS 1983). In addition, it is now known that diapause may be re-induced in P. apterus if it was terminated by photoperiod, but not if low temperarures were experienced (HODEK 1983). Thus, it is clear that the rate of post-diapause morphogenesis in nature is largely the result of temperature and the role of photoperiod is relatively insignificant since daylengths will be short throughout the period when long days could exert an effect. Similar conclusions may be drawn from studies with some other insects (eg. KAMM 1972; McMULLEN and JONG 1976; VEERMAN 1977b).

Data in support of a major role for photoperiod in diapause termination have been presented for several species in which the time to termination is related to the photoperiod over a wide range of temperatures (see: McLOUD and BECK 1963; WILLIAMS and ADKISSON 1964; HODEK 1971c; LUMME et al. 1974; HODEK and HONEK 1981).

In some beetles, there appears to be a short day - long day requirement, for termination which is analogous to the long day - short day diapause induction and intensity effect seen in some insects (eg. NORRIS 1962, 1965; TAUBER and TAUBER 1970b; see also Chapter 1). The carabid, Pterostichus nigrita requires a period of short days in order for pre-vitellogenesis to take place and a subsequent period of long days in which vitellogenesis may be completed (THIELE 1966, cited in THIELE 1977a; FERENZ 1977). Similar photoperiodic treatment has also been shown to terminate the dormancy of P. cupreus and P. coerulescens (KREHAN 1970), whilst another carabid, Agonum assimile, is believed to require decreasing daylengths to induce diapause and increasing daylengths to bring about reproductive development (NEUDECKER and THIELE 1974).

In temperate latitudes, insects almost inevitably experience very cold and sometimes frosty conditions during the winter, and diapause has evolved as a means of enhancing the chances of surviving these adverse conditions. The functional significance of chilling in physiogenesis and/or post-diapause morphogenesis is far from clear in most cases (TAUBER and TAUBER 1976a). Some species are known to have low temperature optima for

physiogenesis (see: ANDREWARTHA 1952; LEES 1955; DANILEVSKY et al. 1970) which provides circumstantial evidence in favour of a role for cold temperatures. Over 100 years ago, it was demonstrated that the diapausing eggs of Bombyx mori failed to survive unless they had experienced a period of chilling (DUCLAUX 1869, 1876 cited in ANDREWATRHA 1952). However, just as cold temperatures may reduce the rate of activation and, hence, delay termination (see above), so the effect of chilling during physiogenesis may retard the rate of that process if higher temperatures are more favourable (TAUBER and TAUBER 1976a), as has been shown to be the case in Leptinotarsa decemlineata (DE WILDE 1949, 1953 cited in LEES 1955).

Juvenile hormone (JH) is known to play a central role in insect reproductive physiology through its effects upon vitellogenin synthesis, release and uptake by developing oocytes, and follicle cell development (eg. ENGELMANN 1970; ADAMS 1974; ABU-HAKIMA and DAVEY 1977; RANKIN and JACKLE 1980; RIDDIFORD 1980; KOEPPE et al. 1981; DE KORT 1981; DE KORT and GRANGER 1981; KELLY and HUNT 1982). The influence of ecdysone upon these processes has been most extensively studied in mosquitoes where the interaction with JH has been shown to be important (eg. FUCHS and KANG 1981; REDFERN 1982). It is not surprising, therefore, that the role of these hormones (particularly JH) in ovarian diapause has received a great deal of attention. The first firm evidence that JH was involved came from experiments with L. decemlineata in which removal of the corpora allata (allatectomy), which

synthesise JH, from long day beetles resulted in diapause (DE WILDE and DE BOER 1961). If removal of the sourse of JH resulted in diapause, it seemed logical to expect artificial raising of the JH titre to break diapause. This has been shown to be the case in numerous species in which JH or a juvenile hormone analogue (JHa) was either applied topically or injected into diapausing females (BOWERS and BLICKENSTAFF 1966; CONNIN et al. 1966; KAMM and SWENSON 1972; HERMAN 1973; HODEK et al 1973; FERENZ 1977; JAMES and HALES 1983). However, the break in dormancy was only temporary indicating that endogenous production/release of JH was not stimulated. When the hormone treatment was accompanied by a transfer to long days, termination was observed in L. decemlineata (SCHOONVELD et al. 1977). Nervous inhibition of the corpora allata has been shown to be responsible for the low JH titre in diapausing females of Pyrrhocoris apterus (HODKOVA 1976, 1977, 1979) and Tetrix undulata (PORAS 1982) whilst the absence of a humoral stimulatory factor is believed to be responsible in L. decemlineata (DE WILDE and DE BOER 1969) and Anacridium aegyptium (GIRARDIE et al. 1974).

There is very little previous work on diapause maintenance and termination in Aleyrodes proletella. A short period of chilling does, apparently, facilitate termination whilst transfers of field collected diapausing females to long or short days in the laboratory have revealed a photoperiodic effect, especially during the first three months of the overwintering period but this

decreases some time before termination in nature (IHEAGWAM 1976, 1977).

In this Chapter, ovarian development in the field during the autumn and winter will be monitored and the rate of ovarian development when field collected females are transferred to long or short days, during this period, will be recorded. This will be used to assess the effect of laboratory conditions in preference to pre-oviposition time which is a measure of overt termination of dormancy since it provides a dynamic physiological picture of the response. In addition, the significance of chilling laboratory induced, diapausing females will be shown. Finally, the dormancy breaking/terminating effect of topical application of JH will be investigated.

MATERIALS AND METHODS.

Culture, Diapause Criteria, Ovarian Development, Fat Body Development and Field Samples.

See Chapters 1 and 2.

Rate of Ovarian Development.

This was calculated using the formula:

$$\text{Rate} = \frac{\text{Increase in Ovarian Score}}{\text{No. Days}}$$

Chilling.

A refrigerator, set at 5°C, was fitted with a Mazda 12V 5W light bulb controlled by a Venner time-switch to provide an illumination cycle of LD12:12. The temperature fluctuation was ± 2 C over a period of six months.

Juvenile Hormone (JH) Application.

Juvenile Hormone I (JHI, Calbiochem), dissolved in hexane, was diluted in Analar acetone. Whitefly were anaesthetised with chloroform, which was preferred to ether and carbon dioxide because it caused the wings to lock at either the top of the upstroke or the bottom of the downstroke and this made the abdominal cuticle more

accessible. The hormone was topically applied to the abdomen in 0.1µl quantities using a graduated 1µl microcap. In all the experiments presented here, the dose was 0.1µg hormone in 0.1µl, although one lower dose, 0.01µg, was applied in preliminary studies. Similarly, two of the other natural juvenile hormones (JHII and JHIII, Calbiochem) were applied in preliminary experiments. Control insects were either anaesthetised and untreated, or anaesthetised and "dosed" with acetone.

During the autumn and winter of 1982-3, the ovarian score of field collected whitefly increased from around 0.7, at the beginning of November, to 1.9 in mid February, whilst the fat body score remained above 1.8 until the end of January (Fig.27). During November, very little ovarian development was recorded, but the score increased at a near uniform rate between early December and February. Over the same period in 1983-4, the ovarian score rose uniformly from 0.35 to 1.6 and the fat body score was above 1.7 throughout that period and did not start to fall until early March (Fig.28). In both sets of records it is apparent that the ovarian score was increasing while the field photophase was still decreasing. The mean daily temperatures were similar for both periods, and are also comparable with the corresponding data published previously (IHEAGWAM 1977). When the periods of rising and falling temperature are compared with the corresponding rates of ovarian development, there is some indication of a relationship, particularly during the 1983-4 period (Fig.28). It can be seen that the decreasing temperatures of early November, mid January and early February, are concurrent with relatively low ovarian developmental rates.

A series of experiments was conducted to investigate the effect of chilling diapausing females. The results, shown in Fig.29 and Table 6, suggest that chilling has a

Figure 27.

Ovarian and Fat Body Development in the Field during the autumn and winter 1982-3. Weekly field collections were taken and a representative sample of the females were scored for ovarian and fat body development. The small figures indicate the number of females scored on each date.

FIG. 27.

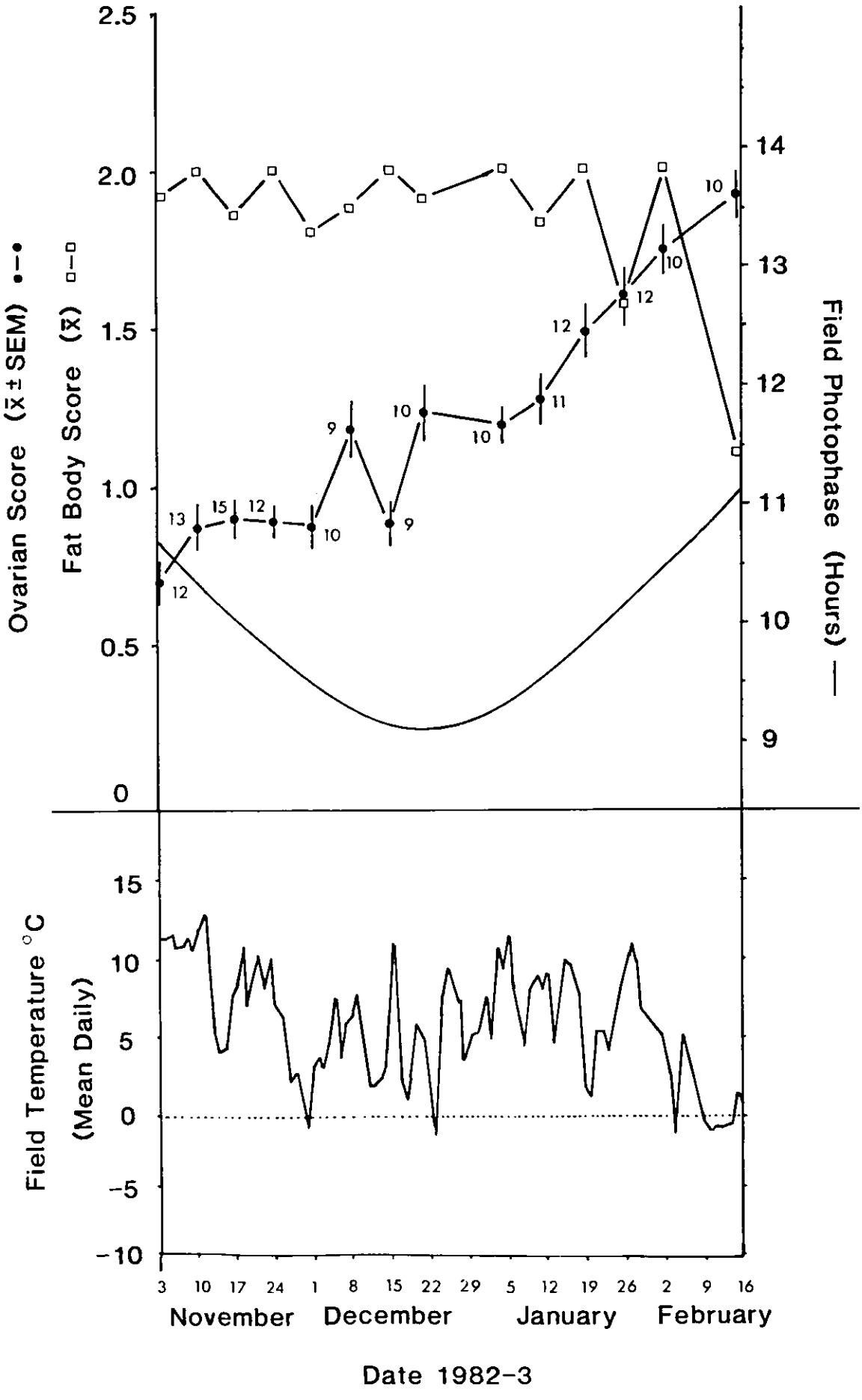
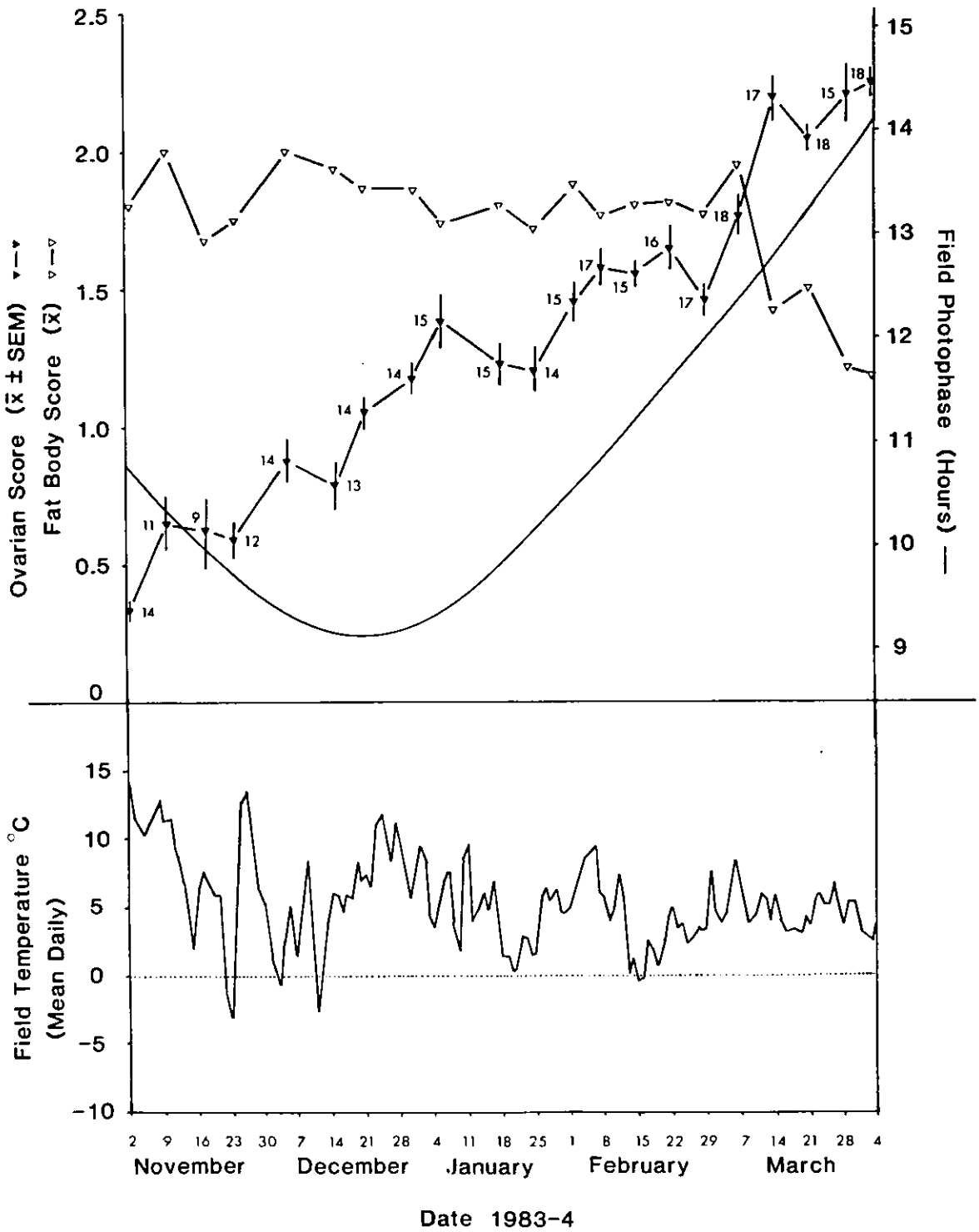

Ovarian Score (x̄ ± SEM) •—•
Fat Body Score (x̄) □—□
Field Photophase (Hours) —
2.5
2.0
1.5
1.0
0.5
0
14
13
12
11
10
9
13
15
12
10
9
12
10
10
11
12
12
10
Field Temperature °C
(Mean Daily)
15
10
5
0
-5
-10
3 10 17 24 1 8 15 22 29 5 12 19 26 2 9 16
November December January February
Date 1982-3

FIGURE 28.

Ovarian and Fat Body Development in the Field during the autumn and winter of 1983-4. Weekly field collections were taken and a representative sample of the females were scored for ovarian and fat body development. The small figures indicate the number of females scored on each date.

FIG. 28.


Ovarian Score (x̄ ± SEM)
Fat Body Score (x̄)
Field Photophase (Hours)
Field Temperature °C
(Mean Daily)
2.5
2.0
1.5
1.0
0.5
0
15
14
13
12
11
10
9
15
10
5
0
-5
-10
2 9 16 23 30 7 14 21 28 4 11 18 25 1 8 15 22 29 7 14 21 28 4
November
December
January
February
March
Date 1983-4

slight "priming" effect upon the rate of ovarian development recorded in warmer conditions. All the females were in an intense diapause induced by a transfer from LD16:8 15° C to LD12:12 15°C during the first or second instar (see Chapter 1, Table 2 and Fig.8). If the induced females were returned to LD16:8 15°C at emergence, a score of 1.8 was reached in six weeks (Fig.29) at an ovarian developmental rate of 0.041 (Table 6), which is significantly higher ($P<0.5$) than the rate of 0.016 recorded when females remained in LD12:12 15° C (Table 6). Induced females that had been chilled for six weeks from emergence had a developmental rate of 0.004 which is much lower than in standard long or short day conditions. However, after either six or ten weeks of chilling the ovarian developmental rate observed upon transfer to LD16:8 15° C and LD12:12 15° C is significantly higher ($P<0.5$) than that recorded without chilling, and long day treatment produced a substantially higher post-chilling rate than short days (Table 6). This suggests that both temperature and photoperiod play a part in post-diapause morphogenesis, and that chilling may permit certain biochemical changes to take place during physiogenesis.

Further investigations into the role of the photoperiod during dormancy were conducted by transferring field collected adults to either LD16:8 15°C, LD12:12 15°C or LD12:12 5° C during the overwintering period and monitoring the subsequent rate of ovarian development. The results are shown in Figs.30-36 and Table 7. In Fig.30, there is an increase in ovarian development after

FIGURE 29.

The Effect of Chilling on the Ovarian Developmental Rate of Induced Females. Females that had been transferred to short days during the 1st or 2nd instar were treated in one of the following ways: Transferred to long days at emergence; chilled in short days for 6 or 10 weeks and then transferred to either long or short days at 15° C. Periodic dissections were made to assess the ovarian developmental rate in each condition. The arrows indicate the time of transfer to 15° C. The small figures represent the numbers of females dissected and scored.

FIG. 29.

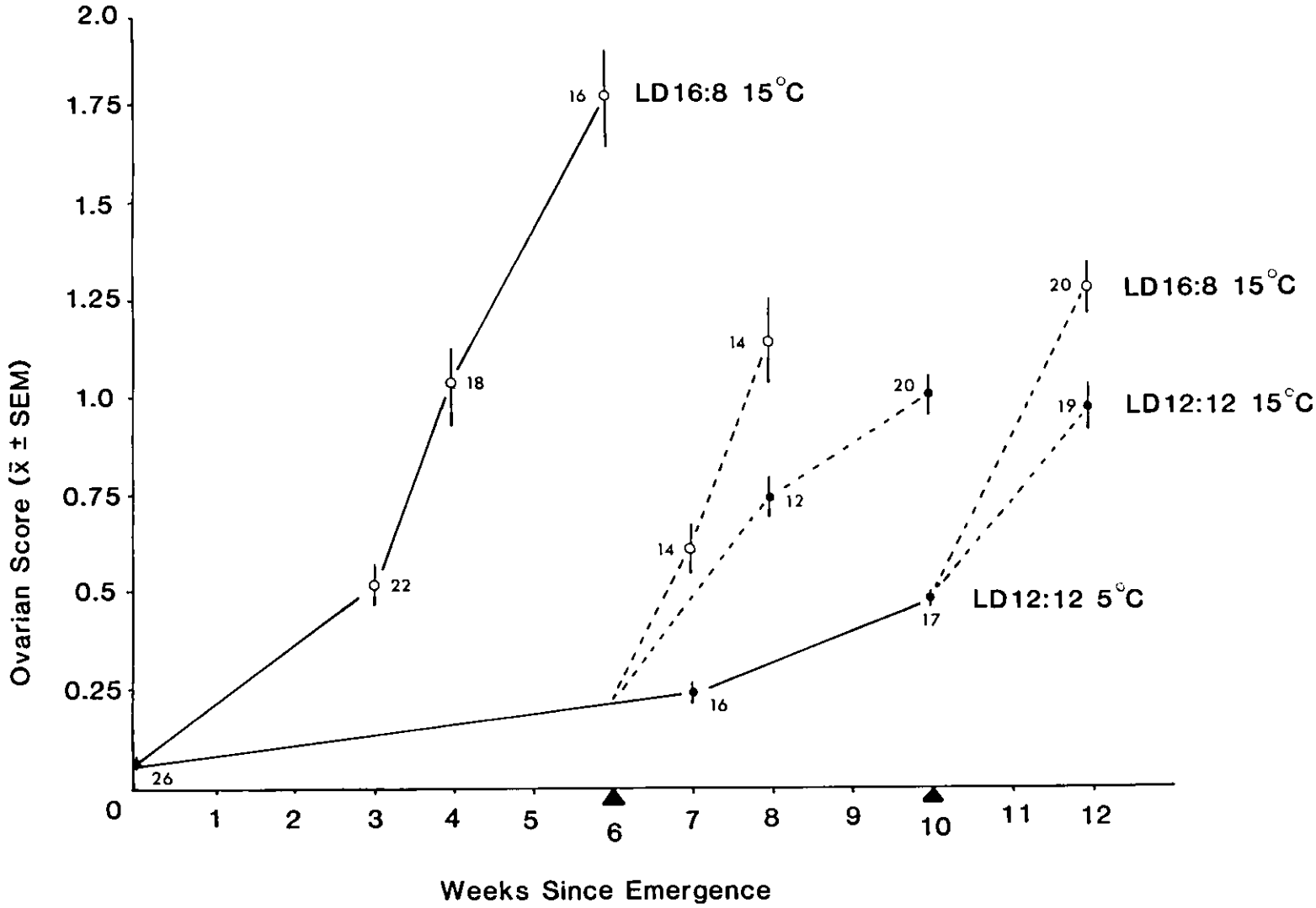

2.0
1.75
1.5
1.25
1.0
0.75
0.5
0.25
0
Ovarian Score ($\bar{x}$ ± SEM)
1
2
3
4
5
6
7
8
9
10
11
12
Weeks Since Emergence
LD16:8 15°C
LD12:12 15°C
LD12:12 5°C
16
18
22
26
14
20
12
14
16
20
19
17

TABLE 6. Comparative Rates of Ovarian Development for *A. proletella*.

Ovarian Score at start of conditions.	Conditions and their Duration		Rate
0.68	Winter 1982-3	Nov 2 to Feb 16	0.012
0.33	Winter 1983-4	Nov 2 to Feb 15	0.012
		Nov 2 to Apr 4	0.012
0.05	Induced. Chilled from Emergence	6 weeks	0.004
		10 weeks	0.006
1.21	LD16:8 15°C. 3 Days from Emergence		0.433
0.05	Induced. 6 weeks from Emergence		0.016
0.05	Induced. LD16:8 6 weeks from Emergence		0.041
0.05	Induced. Chilled 6 weeks,		
0.22	then to:	LD16:8 15°C 2 weeks	0.066
		LD12:12 15°C 2 weeks	0.037
		LD12:12 15°C 4 weeks	0.028
0.05	Induced. Chilled 6 weeks,		
0.37	then to:	LD16:8 15°C 2 weeks	0.056
		LD12:12 15°C 2 weeks	0.035

transfer to laboratory conditions, but the scores recorded in the field and laboratory two weeks later do not show any significant differences which suggests that photoperiod and temperature are ineffective in the early stages of the dormancy. In contrast, field samles collected on 25th October, 18th November, 24th December and 6th January showed a much higher rate of development under laboratory conditions, with no significant photoperiodic differences, than that recorded in the field (Figs.31,32,33 and 34). An inspection of Table 7 reveals that the rate of ovarian development tended to increase with each collection, which may reflect the priming action of greater exposure to chilling.

Adults collected from the field on 18th January and 3rd February, were transferred to one of three conditions and each produced similar results for the two samples. In Fig.35 it is clear that ovarian development occured at a rapid rate over the first five days in the laboratory under both long and short day conditions at 15°C (0.104 and 0.080 respectively, see Table 7), whilst chilling resulted in a rate of 0.014-0.022 which was not significantly different from that recorded in the field over the same period. Over the next week in long and short days, the rate of development declined and there was some indication that long days caused a higher rate of oogenesis. The same trends were apparent in the last field collection except that the photoperiodic effect was not significant at either dissection date (Fig.36).

FIGURE 30.

Females collected from the field on 5th October were transferred to long and short day conditions at 15°C in the laboratory. Samples were dissected 5 and 15 days later and ovarian scores were calculated. The numbers represet the number dissected for each point.

FIG. 30.

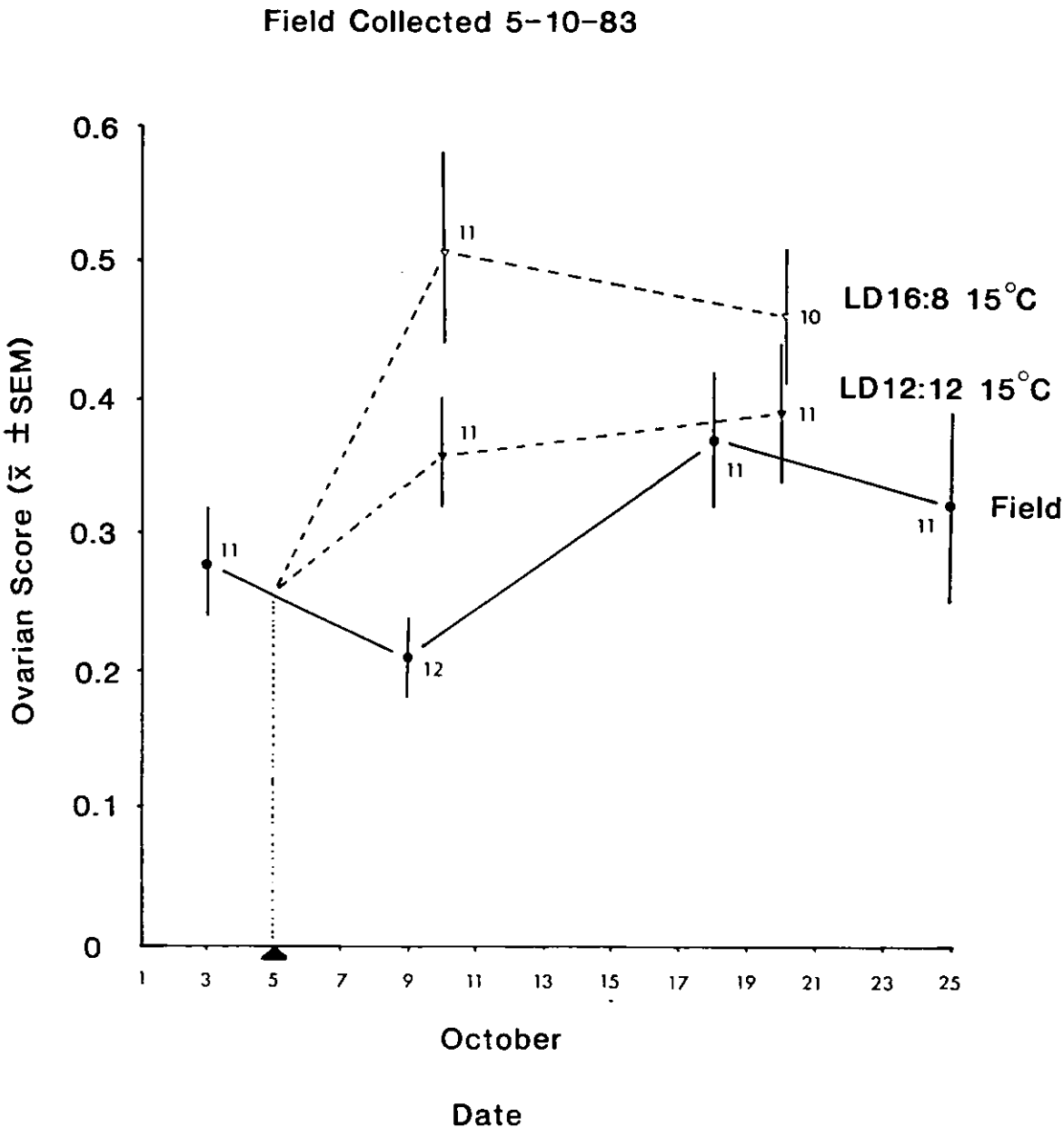

Field Collected 5-10-83
Ovarian Score (x̄ ± SEM)
0.6
0.5
0.4
0.3
0.2
0.1
0
11
10
11
11
11
11
12
LD16:8 15°C
LD12:12 15°C
Field
1
3
5
7
9
11
13
15
17
19
21
23
25
October
Date

FIGURE 31.

Females were collected from the field on 25th October and transferred to long or short days at 15°C in the laboratory. Samples from each condition were dissected, and their ovaries scored, 9 and 28 days later. The figures represent the number of females scored for each point.

FIG. 31.

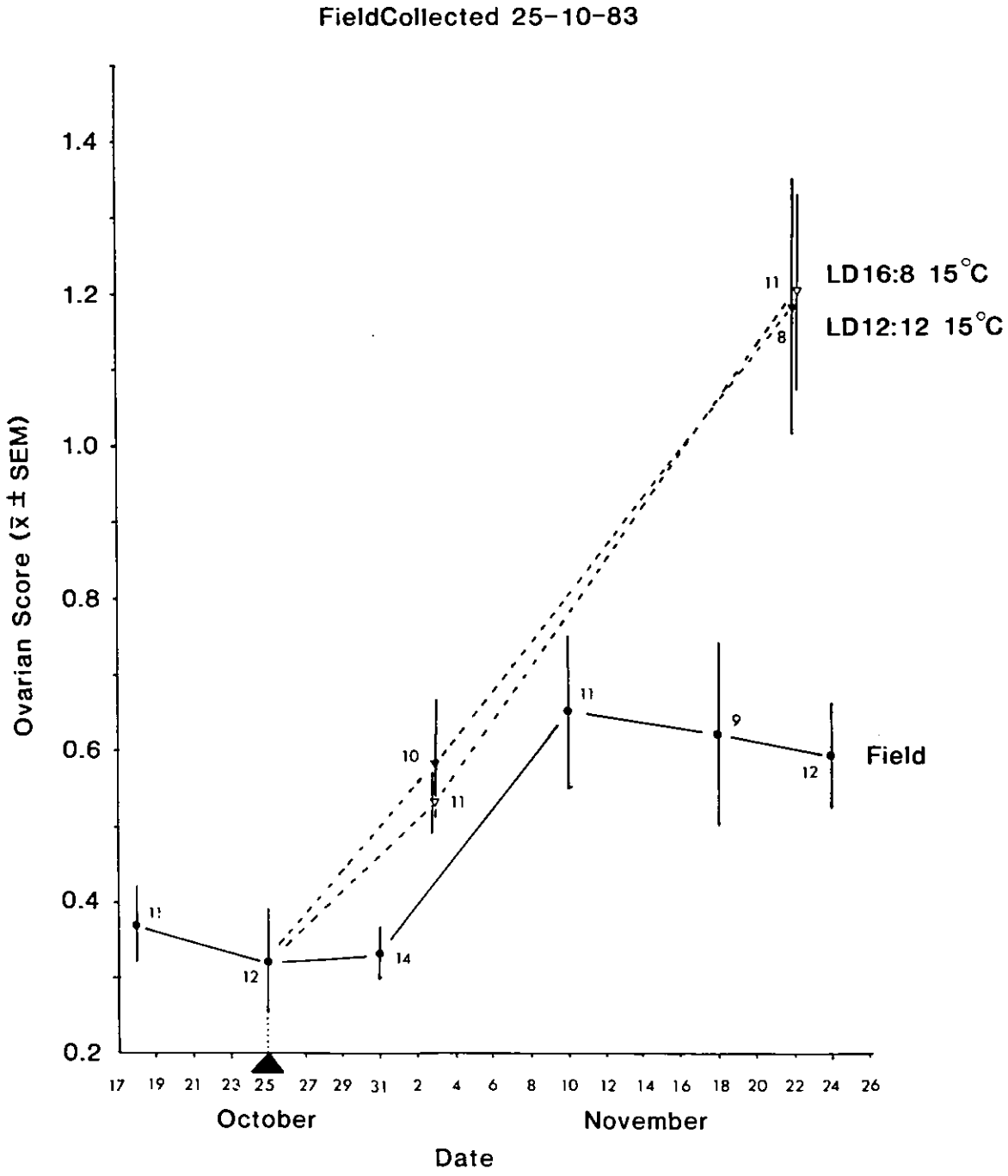

FieldCollected 25-10-83
Ovarian Score (x̄ ± SEM)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
LD16:8 15°C
LD12:12 15°C
Field
11
8
10
11
11
9
12
11
12
14
17 19 21 23 25 27 29 31 2 4 6 8 10 12 14 16 18 20 22 24 26
October
November
Date

FIGURE 32.

Females were collected from the field on 18th November and transferred to either long or short days at 15°C in the laboratory. Samples from each condition were dissected, and their ovaries scored, 18 days later. The figures represent the number of females scored for each point.

FIG. 32.

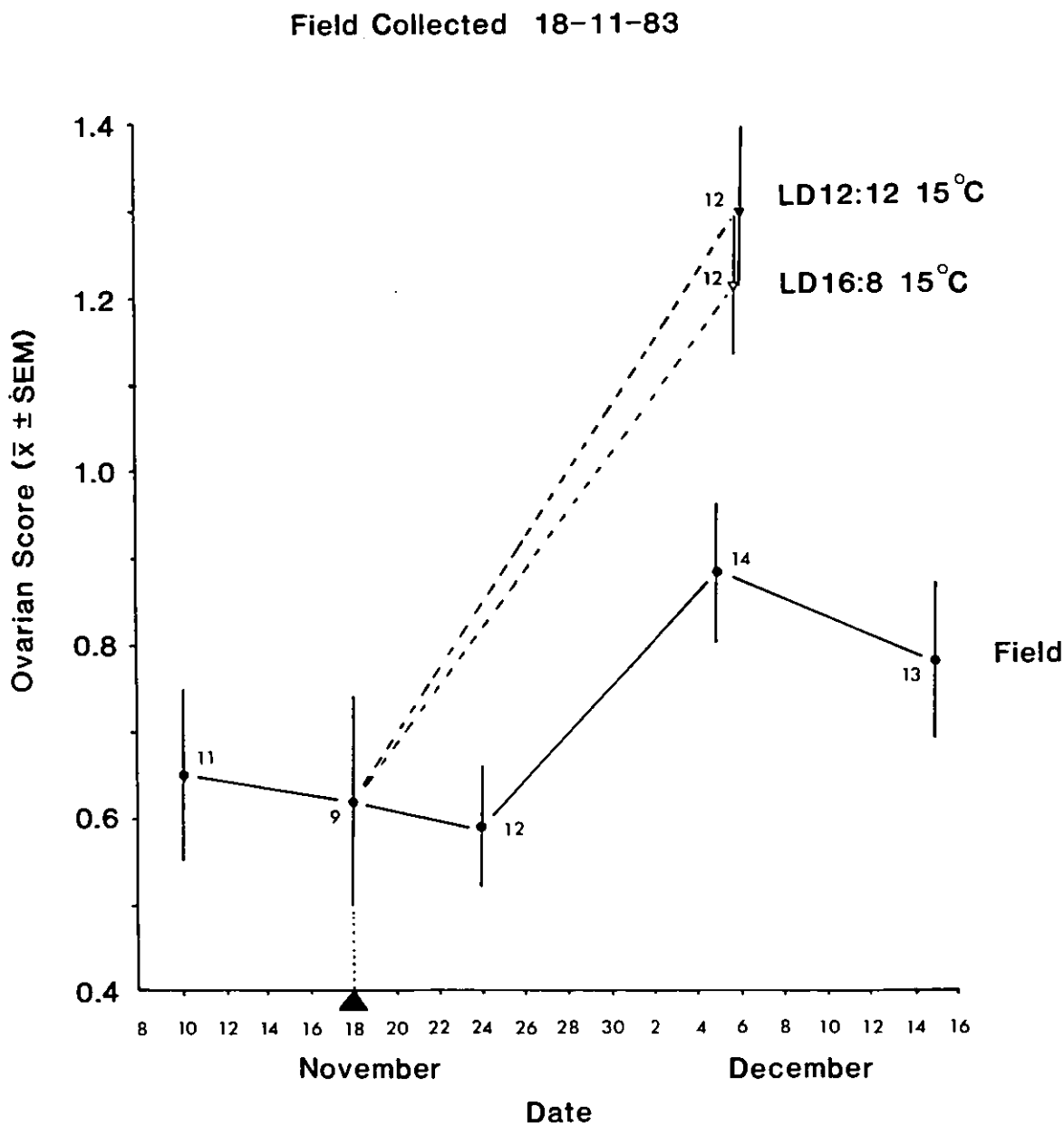

Field Collected 18-11-83
Ovarian Score ($\bar{x}$ ± SEM)
1.4
1.2
1.0
0.8
0.6
0.4
LD12:12 15°C
LD16:8 15°C
Field
12
12
14
13
11
9
12
8 10 12 14 16 18 20 22 24 26 28 30 2 4 6 8 10 12 14 16
November
December
Date

FIGURE 33.

Females were collected from the field on 24th December and transferred to long or short days at 15°C in the laboratory. Samples from each condition were dissected, and their ovaries scored, 11 days later. The figures represent the number of females scored for each point.

FIG. 33.

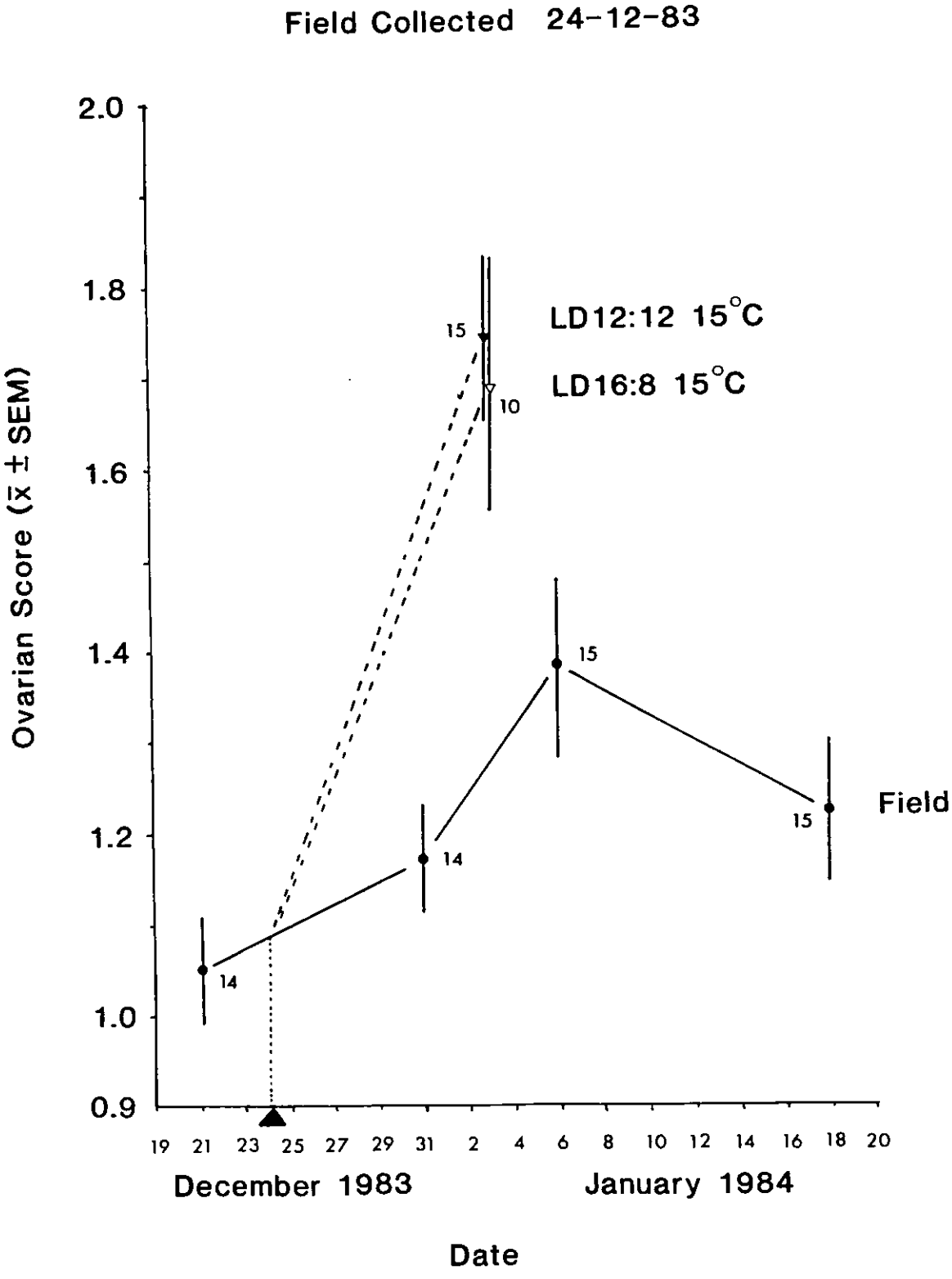

Field Collected 24-12-83
2.0
1.8
1.6
1.4
1.2
1.0
0.9
Ovarian Score ($\bar{x} \pm$ SEM)
LD12:12 15°C
LD16:8 15°C
Field
15
10
15
14
15
19 21 23 25 27 29 31 2 4 6 8 10 12 14 16 18 20
December 1983
January 1984
Date

FIGURE 34.

Females were collected from the field on 6th January and transferred to long or short days at 15°C in the laboratory. Samples from each condition were dissected, and their ovaries scored, 14 days later. Since the ovarian score of the field sample dissected on 6th January was high compared to the previous and subsequent dissections, the rate of development in the laboratory was calculated from a starting score of 1.25 at Z. The figures represent the number of females scored for each point.

FIG. 34.

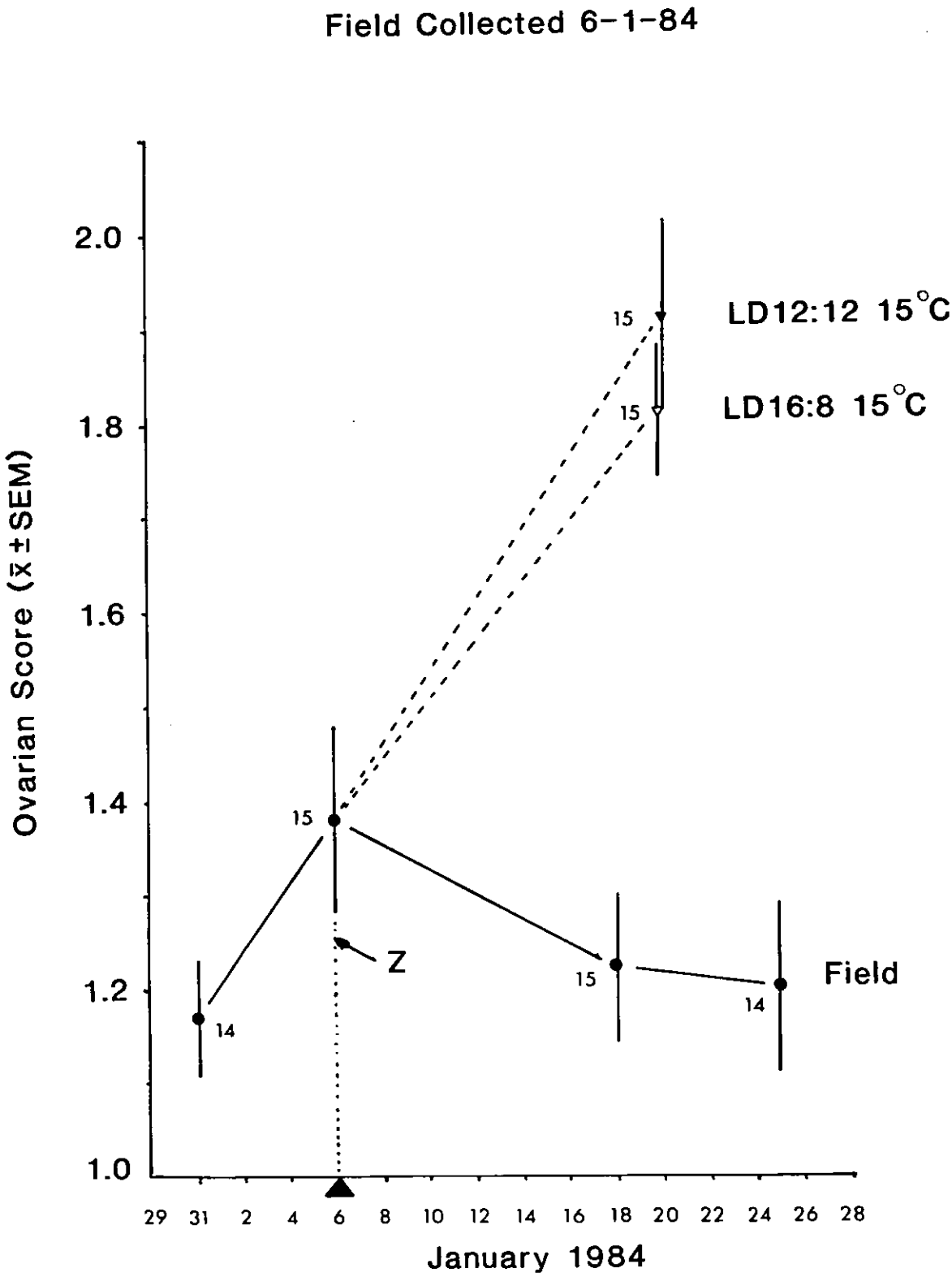

Field Collected 6-1-84
Ovarian Score (x̄ ± SEM)
2.0
1.8
1.6
1.4
1.2
1.0
LD12:12 15°C
LD16:8 15°C
Field
Z
15
15
15
15
14
14
29 31 2 4 6 8 10 12 14 16 18 20 22 24 26 28
January 1984

FIGURE 35.

Females were collected from the field on 18th January and transferred to long or short days at 15°C, or short days at 5° C, in the laboratory. Samples from each condition were dissected, and their ovaries scored, 5 and 12 days later. The figures represent the number of females scored for each point.

FIG. 35.

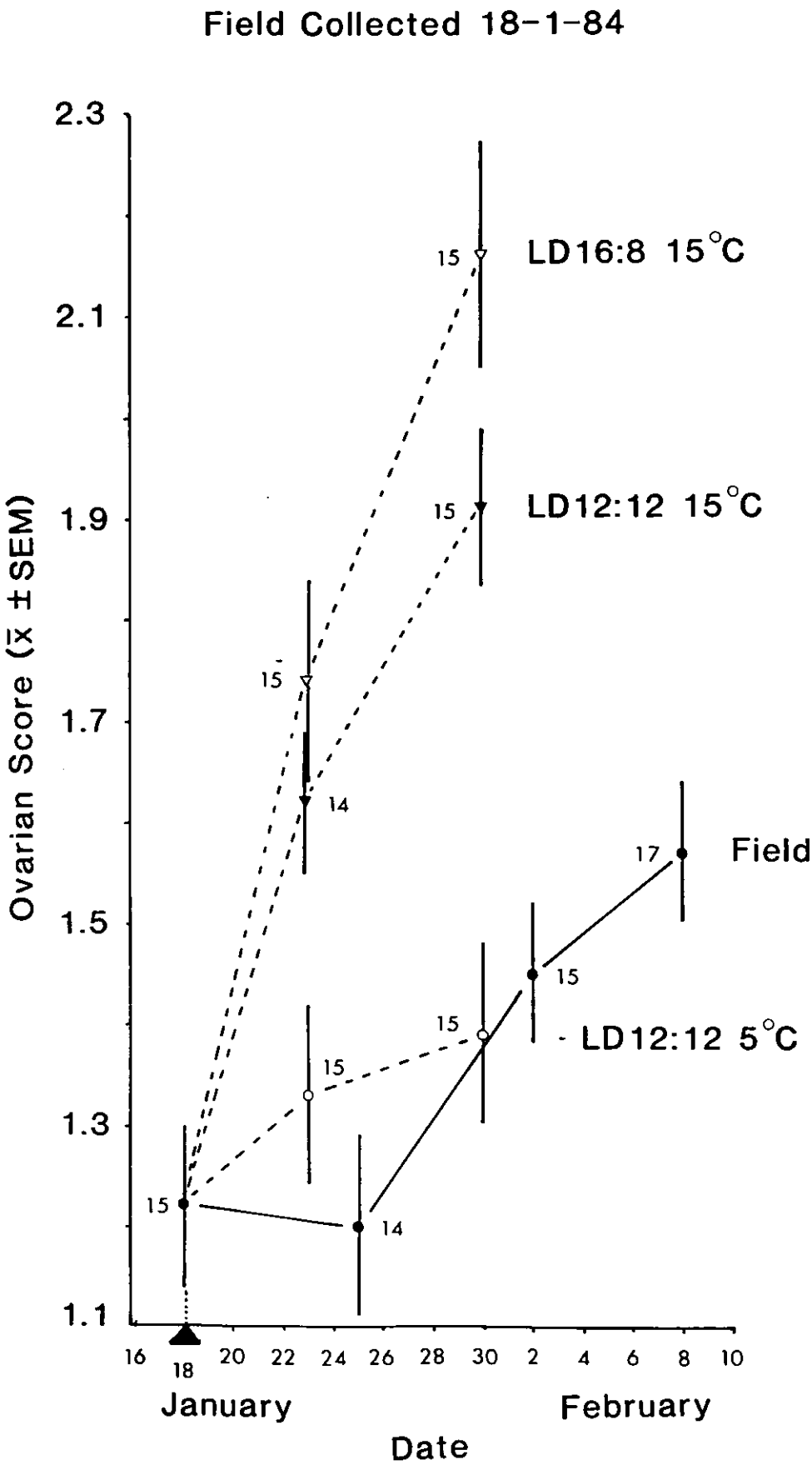

Field Collected 18-1-84
Ovarian Score ($\bar{x}$ ± SEM)
2.3
2.1
1.9
1.7
1.5
1.3
1.1
LD16:8 15°C
LD12:12 15°C
LD12:12 5°C
Field
15
15
15
14
17
15
15
15
14
16
18
20
22
24
26
28
30
2
4
6
8
10
January
February
Date

FIGURE 36.

Females were collected from the field on 3rd February and transferred to long or short days at 15° C, or short days at 5° C, in the laboratory. Samples from each condition were dissected, and their ovaries scored, 7 and 11 days later. The figures represent the number of females scored for each point.

FIG. 36.

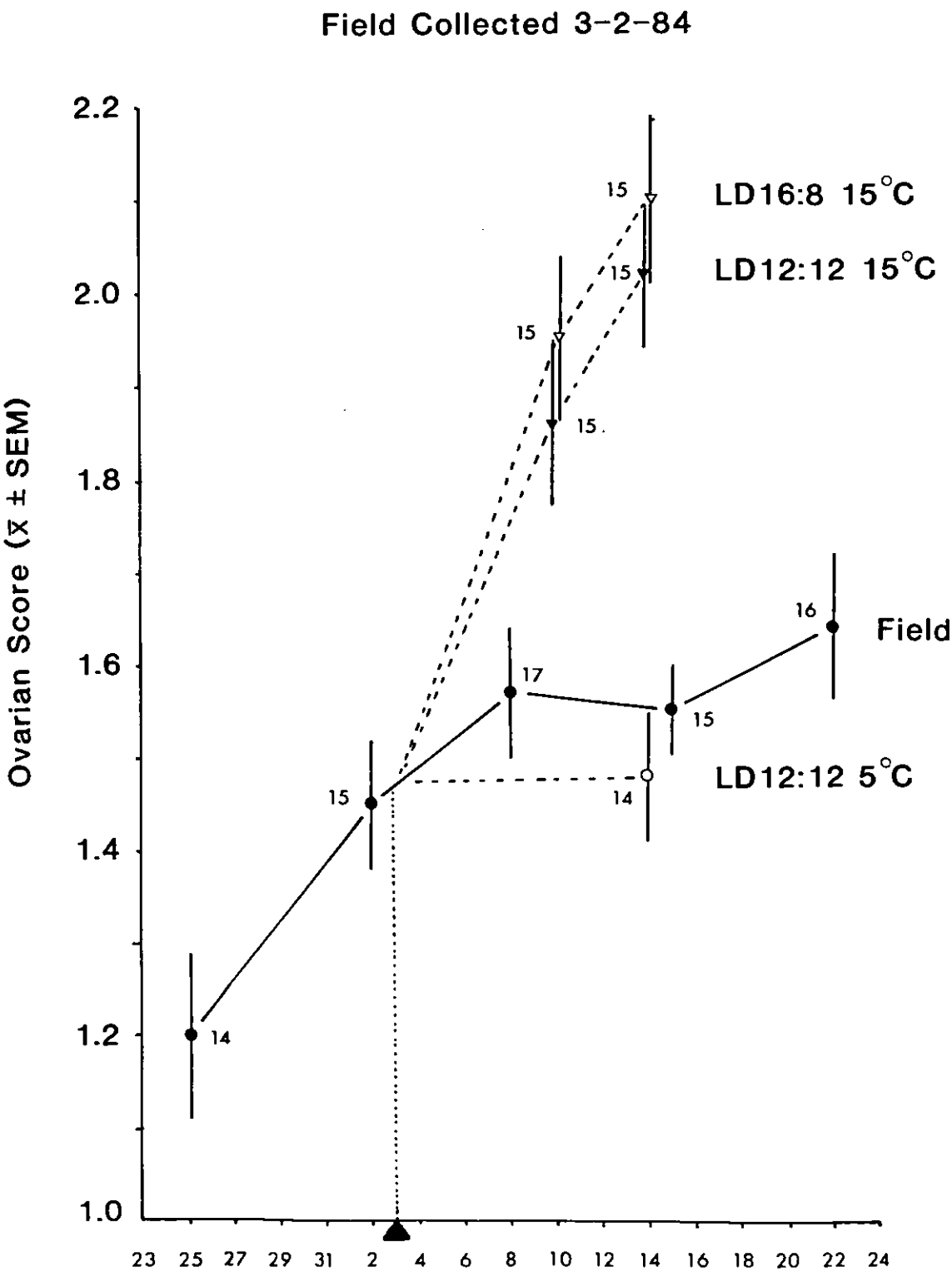

Field Collected 3-2-84
Ovarian Score (x̄ ± SEM)
2.2
2.0
1.8
1.6
1.4
1.2
1.0
23 25 27 29 31 2 4 6 8 10 12 14 16 18 20 22 24
LD16:8 15°C
LD12:12 15°C
Field
LD12:12 5°C
15
15
15
15
16
17
15
15
14
14

TABLE 7. Rates of Ovarian Development of Field Collected Whitefly Transferred to Laboratory Conditions during the Autumn and Winter of 1983-4.

Date of Collection.	Score when Collected.	Lab. Conditions	Rate	
			5 Days	15 Days
5th Oct.	0.26	LD16:8 15°C	0.030	0.014
		LD12:12 15°C	0.020	0.009
			9 Days	28 Days
25th Oct.	0.32	LD16:8 15°C	0.023	0.031
		LD12:12 15°C	0.027	0.031
			-	18 Days
18th Nov.	0.62	LD16:8 15°C		0.033
		LD12:12 15°C		0.038
			10 Days	-
24th Dec.	1.09	LD16:8 15°C	0.060	
		LD12:12 15°C	0.065	
			-	14 Days
6th Jan.	1.25	LD16:8 15°C		0.041
		LD12:12 15°C		0.047
			5 Days	12 Days
18th Jan.	1.22	LD16:8 15°C	0.104	0.078
		LD12:12 15°C	0.080	0.058
		LD12:12 5°C	0.022	0.014
			7 Days	11 Days
3rd Feb.	1.47	LD16:8 15°C	0.069	0.057
		LD12:12 15°C	0.056	0.050
		LD12:12 5°C	-	0.001

In an attempt to determine if and when topical application of JH to diapausing, field collected, A. proletella females was effective at breaking the dormancy, six field samples were taken during the autumn and winter of 1983-4. The treated and control females were transferred to either LD16:8 15°C or LD12:12 15°C, and were dissected a few days later. An inspection of Tables 8 and 9 shows that the results obtained were inconsistent and, as a consequence, inconclusive. In only one case, the collection made on 6th December, was the JH treatment significantly more effective than the controls (Table 8). None of the treatments produced a substantially higher rate of ovarian development than that recorded in the corresponding photoperiod and temperature experiments previously described (Figs.30-36 and Table 7), so there was no evidence to suggest that exogenous JH was capable of breaking the dormancy.

TABLE 8. Ovarian Development of Female Whitefly Collected from the Field during Autumn 1983 and treated with Juvenile Hormone.

Date of Collection	Treatment	Photoperiod	Days to Dissection	n	Ovarian Score $\bar{x}$±SEM
6th Oct.	JHI 0.1µg	LD16:8 15°C	4	10	0.40±0.01
	Acetone	LD16:8 15°C	4	4	0.27±0.01
	Chloroform	LD16:8 15°C	4	10	0.26±0.03
	JHI 0.1µg	LD12:12 15°C	5	10	0.35±0.04
	Acetone	LD12:12 15°C	5	12	0.28±0.03
	Chloroform	LD12:12 15°C	5	10	0.30±0.03
6th Dec.	JHI 0.1µg	LD16:8 15°C	8	13	1.58±0.13
	Acatone	LD16:8 15°C	8	12	1.43±0.09
	Chloroform	LD16:8 15°C	8	6	1.17±0.13
9th Dec.	JHI 0.1µg	LD12:12 15°C	7	17	1.54±0.09
	Acetone	LD12:12 15°C	7	8	1.28±0.06
	Chloroform	LD12:12 15°C	7	13	1.28±0.06

TABLE 9. Ovarian Development of Whitefly Collected from the Field during Winter 1984 and Treated with Juvenile Hormone.

Date of Collection	Treatment	Photoperiod	Days to Dissection	n	Ovarian Score $\bar{x}$±SEM
3rd Jan.	JHI 0.1µg	LD16:8 15°C	9	5	1.89±0.09
	Acetone	LD16:8 15°C	9	4	1.38±0.11
	Chloroform	LD16:8 15°C	9	15	2.02±0.09
9th Jan.	JHI 0.1µg	LD12:12 15°C	8	16	1.91±0.14
	Acetone	LD12:12 15°C	8	15	1.76±0.09
	Chloroform	LD12:12 15°C	8	12	2.04±0.10
13th Feb.	JHI 0.1µg	LD16:8 15°C	7	13	2.24±0.14
	Acetone	LD16:8 15°C	7	16	2.26±0.08
	Chloroform	LD16:8 15°C	7	15	2.29±0.07

DISCUSSION.

Weekly dissections of overwintering *A. proletella* females have shown that ovarian development continues, albeit at a very low rate, from the time of induction until termination the following spring, without any apparent period when development is completely at a standstill (Figs.27 and 28). The definitions of physiogenesis (ANDREWARTHA 1952) and refractory stage (MANSINGH 1971), both state that morphological changes do not take place, instead biochemical changes occur and, upon their completion, post- diapause morphogenesis (activation) may proceed. This is not apparently compatible with the results obtained with the cabbage whitefly. However, the absence of a clear-cut refractory stage may be attributable to the fact that the field samples represent a cross section of the entire population at any one moment in time so the post-diapause morphogenesis of the older females may have masked the refractory stage of more recently emerged individuals. If this is the case it would account for the slow rates of ovarian development during October 1983 (see Fig.12) in which some diapausing females were still emerging, and others were two months old. In view of the post-emergence rate shown for induced females kept in LD12:12 15 C (see Fig.8), it seems likely that a total arrest of ovarian development does not occur in this species, but some degree of refractoriness is indicated by the low rate of development during late September and October 1983,

compared to the following five months when the temperature was lower, and the photophase was shorter (see Figs.12, 27 and 28). Continuous, cyclical growth and resorption of pre-vitellogenic oocytes has been recorded in diapausing Leptinotarsa decemlineata (DE WILDE et al. 1959), but resorption has rarely been recorded in hundreds of A. proletella dissections. Instead, progressive development to vitellogenic stages takes place.

Ovarian scores provide a means of monitoring ovarian development that is both straightforward and detailed. The fact that small morphological changes may be detected is reflected in the continuum of scores in Figs.27 and 28. A scheme based upon a non-gravid/gravid or diapause/intermediate/non-diapause scheme using the same females would have indicated that a refractory stage was present. Clearly, the appearance of the results is dependent upon the nature of the records made as well as what actually happens.

Dissections of Aelia acuminata collected from the field between August and February did not reveal any signs of pre-vitellogenesis (HODEK 1975 cited in HODEK and HONEK 1981). A similar phenomenon was recorded when monthly field samples of overwintering Drosophila subobscura females were dissected and their ovaries classified into one of five groups. During November and December, 90% and 100% of the females, respectively, had undistinguishable ovaries (BEGON 1976). These two species could certainly be considered to possess a true refractory stage.

From Table 6, it is clear that when induced adults are transferred, at emergence, to long days, their subsequent ovarian development occurs at a significantly higher rate than if they remain in short day conditions. It follows that the pre-oviposition times would show corresponding differences. This kind of photoperiodic effect has also been demonstrated in Chrysopa carnea (TAUBER et al. 1970a), Aelia acuminata (HODEK 1971c; HODEK and HONEK 1981), Neoseiulus fallacis (ROCK et al. 1971), C. downesi (TAUBER and TAUBER 1976b), Notonecta undulata (VANDERLIN and STREAMS 1977), Tetranychus urticae (VEERMAN 1977b) and Riptortus clavatus (NUMATA and HIDAKA 1982). In two of these species, the photoperiodically terminated diapause may be re-induced by short day treatment (HODEK 1971c; NUMATA and HIDAKA 1982) and it appears that continuous photoperiodic stimulation of the neuroendocrine system is necessary for ovarian development to proceed (HODEK 1971c). In another species, Pyrrhocoris apterus, photoperiodic termination and re-induction of diapause is also possible (HODEK 1983) but, in nature, termination is believed to be in response to warm temperatures and not to photoperiod (HODEK 1971d; SAUNDERS 1983).

Previous work with A. proletella has shown that diapausing adults collected at the end of September and immediately transferred to LD16:8 15°C had a significantly longer pre-oviposition time than if they were chilled for five days and then transferred to long day conditions (IHEAGWAM 1977). The data in Fig.29 and Table 6 are in

agreement with this finding, although longer periods of chilling were used, and indicate that chilling is a significant factor in the progression of dormancy in this species and does not simply retard development. It is possible that closer investigation of the role of chilling in some other species in which it is not believed to be necessary for diapause termination, such as Drosophila littoralis (LUMME et al. 1974) and Notonecta undulata (VANDERLIN and STREAMS 1977), will reveal some form of priming effect. If the low temperatures of winter do favour the completion of certain biochemical changes, the fact that chilling does not always appear to be a necessary pre-requisite for activation and termination may hide a more subtle role which could have a bearing on subsequent fecundity and viability of offspring although this has not been investigated.

There have been several studies in which diapausing adults have been transferred from the field to laboratory conditions during the overwintering period. In Pyrrhocoris apterus, females in the early stages of diapause were responsive to photoperiod and had a significantly shorter pre-oviposition time in long days compared to short days (HODEK 1971d). However, this effect was less marked in later collections until no differences were observed in mid December. Very similar results were recorded for another hemipteran, Psylla pyricola (McMULLEN and JONG 1976). Previous work with A. proletella also revealed a photoperiodic effect that decreased during the autumn and winter (IHEAGWAM 1976,

1977). These results suggest that photoperiodic sensitivity is gradually lost during the autumn and winter. Whilst short photoperiods may help to maintain the reproductive arrest, there is no significant role for the photoperiod in post-diapause morphogenesis and termination in nature.

Undoubtedly the most extensive series of field to laboratory transfers has been performed with species in the Chrysopa genus of the Neuroptera. Laboratory photoperiods between LD9:15 and LD16:8 have been used and a differential photoperiodic terminating effect, especially early in diapause, has been demonstrated with C. carnea (TAUBER and TAUBER 1973c), C. harrisii (TAUBER and TAUBER 1974) and C. downesi (TAUBER and TAUBER 1976c). As with the three hemipteran species, the photoperiodic effect declined during diapause but, although the results for the Chrysopids appeared to be similar, in principle, subtle differences in the activation and termination processes were proposed. In C. carnea, it was suggested that decreasing daylengths decellerated diapause development (=physiogenesis) (TAUBER and TAUBER 1973c, 1976a) whilst, in C. harrisii, daylengths below the critical photoperiod served to maintain diapause (TAUBER and TAUBER 1974, 1976a). In both cases, photoperiod was believed to play no active role in termination. In contrast, increasing daylengths were believed to terminate the dormancy of C. downesi (TAUBER and TAUBER 1976a, c). It seems certain that many of the intricacies of physiogenesis and post-diapause

morphogenesis will remain hidden until much more detailed analyses, especially of the progress of the two stages in the field, are completed.

The results of the field to laboratory transfer experiments conducted with A. proletella are shown in Figs.30-36 and Table 7. They are all based upon the observed rates of ovarian development and not pre-oviposition time. Thus, they may be expected to provide a more dynamic picture of the response to different photoperiodic and temperature treatments than previous studies with a "black box" approach.

In October, mean daily temperatures are generally between 10 and 15°C and the daily photophase falls from 12.75 to 11 hours. In view of this, the transfer of field collected females to 15°C would not be expected to produce a markedly different rate of ovarian development to that observed in the field if temperature was the most influential factor. However, a photoperiodic effect might be revealed since LD16:8 represents a substantial increase over the field photophase whilst LD12:12 is only slightly longer. Two weeks after the transfer, neither laboratory condition had produced a significantly higher rate of ovarian development than that recorded in the field at the same time (Fig.30). In addition, the rates of development were around 0.009 - 0.014 (Table 7) which is the same as the overall rate of development of 0.012 for the period between November and February in the field in 1982-3 and 1983-4 (Table 6). In view of the high temperatures and long photophase in October compared to the following

period, it is clear that a high degree of refractoriness to these two environmental variables is exhibited in early October. However, by the end of October, no such signs of refractoriness were apparent and the rate of development after a transfer from the field to long and short day conditions in the laboratory was 0.031 (Table 7). Since the field scores recorded on October 5th and October 25th were 0.26 (Fig.30) and 0.32 (Fig.31) respectively, it is unlikely that the differences in response of the two collections are due to morphological development and a consequent increase in competence to respond. Instead, it is more plausible to infer that physiogenesis was completed between the two dates. This means that small morphological changes probably do accompany physiogenesis in A. proletella which is contradictory to the original definition of the term (ANDREWARTHA 1952). Nevertheless, the refractoriness of the early part of the dormancy period to temperature and photoperiod is clearly substantial.

By the end of October, the mean field temperature is around 10°C and the daily photophase is 11 hours (Figs.27 and 28). Consequently, LD16:8 and LD12:12 represent an increase in photophase and this holds true for all the remaining field collections since the field photophase does not increase to over 12 hours until the end of February. This means that photoperiodic sensitivity based upon an "above or below critical photoperiod?" system would not be revealed in Figs.31 to 36, although differences would be expected if the absolute duration of

the photophase was important. The latter can be discounted because significant differences between the two photoperiodic treatments were not recorded. However, in the absence of data from experiments in which a shorter photoperiod, such as LD9:15 (which is shorter than the shortest field conditions), was used, the former suggestion must remain a possibility. Alternatively, the similarity between the results in the two laboratory conditions could be accounted for by the identical temperatures. This seems to be the most likely explanation and it is much more simple than to propose that the critical photoperiods for induction and termination differ by four hours. Further changes in critical photoperiod would be necessary later in the winter when the field photophase increased to over 12 hours in late February, since termination is believed to occur in April or May (BUTLER 1938a).

Some of the apparent inconsistencies in the rate of ovarian development following transfers to the laboratory between October 1983 and February 1984 (Table 7) may be explained with reference to the ovarian scores. Since a score of around 2.3 is maximal in LD16:8 15°C (see Fig.5), a reduction in ovarian developmental rate might be expected as this score was approached. Similarly, pre-vitellogenesis may occur at a different rate to vitellogenesis. Consequently, if the ovarian scores from which the rate was calculated were either too high or too low, the developmental rate might not be directly comparable with one in which the two scores were in the

intervening period of rapid growth. This may be seen in Figs.35 and 36 in which the developmental rate tailed off as the score approached 2.0 and it may account for the differences recorded from the 24th December and 6th January samples (Table 7). The trend is for increasing rate of post-diapause morphogenesis with later collections which may be due to a combination of longer exposure to chilling and a consequent increasing degree of priming and the morphological development of the ovary providing an increasing capacity for the uptake of yolk proteins.

Studies on the rate of ovarian developmemt of overwintering A. proletella transferred from field to laboratory conditions have not shown any significant effect of photoperiod. This is apparently contrary to earlier work in which the pre-oviposition time was consistently shorter if overwintering females were transferred to long day conditions compared to short days (IHEAGWAM 1976, 1977). It is possible that the duration of stage 4 and/or 5 of ovarian development (see Chapter 1) is partly related to the duration of the photophase. If this were the case, then the rates of ovarian development in LD12:12 and LD16:8 would only differ as the score approached 1.8-2.0 when the proportions of the last two ovarian stages were rising. There is some sign of this occurring in the later field samples (Figs.35 and 36) but the dissections of earlier samples were not continued for long enough to cover the developmental period where any such differences might be revealed. It may be significant that the equilibrium ovarian score in LD16:8 15° C was

around 2.3 (Fig.5), whilst it was only 1.7 in LD12:12 15°C (Fig.6). However, further investigations are clearly necessary in order to determine the precise interactions of photoperiod and temperature during the development of an oocyte.

In contrast with work on numerous other species with an ovarian diapause, A. proletella does not exhibit a significant diapause breaking response to exogenous JH (Tables 8 and 9). There could be a number of reasons for this including: wrong dose; wrong JH; JH not reaching haemocoel; JH degraded rapidly; ovary and/or fat body not competent to respond to JH; temperature masks JH effect or that JH does not have a significant role in the ovarian diapause of this species.

In the aphid, Megoura viciae 0.001µg of JHI applied, topically, to a third instar larva produced a juvenilizing effect of over 90% and increasing the dose to 0.01µg resulted in 100% effect (LEES 1980). Adult whitefly are approximately one fifth of the size of a third instar Megoura so, allowing for different sensitivity and an ovarian rather than morphological effect, 0.1µg should be high enough to cause some response if JH is influential. With other, larger species that diapause as adult females, the topically applied doses that elicited a response were much higher. In the weevil, Hypera postica, 50-100µg of a JH analogue were necessary to break the diapause (BOWERS and BLICKENSTAFF 1966) and 100µg JHI were required with the Colorado beetle, Leptinotarsa decemlineata (SCHOONVELD et al. 1977). An injection of JH (10µg in 2 µl) was

effective at stimulating some ovarian development in the carabid, Pterostishus nigrita and a second dose had an even greater effect (FERENZ 1977). One other dose (0.01µg) has been applied to whitefly without any apparent effect, but it is clear that more extensive investigations with different doses are necessary in A. proletella.

Different natural JH molecules and analogues have been shown to have different activity (eg. SCHOONVELD et al. 1977; LEES 1980) so the response does not appear to be specific for the natural hormone. Indeed, it has been demonstrated that, whilst JHIII is the natural hormone in a large number of species (see DE KORT et al. 1982; BAKER et al. 1982; LOHER et al. 1983), JHI is much more potent (LEES 1980; BAKER et al. 1983). Amongst the Hemiptera, in vitro investigations have revealed that JHIII is the natural JH in Dysdercus fasciatus (BOWERS et al. 1983) and JHIII has also been detected in another hemipteran, Aphis fabae (HARDIE and LEES personal communication; HARDIE 1984). JH effects have been demonstrated in aphids (LEES 1977, 1980; HARDIE 1981), Oncopeltus fasciatus (SMITH and NIJHOUT 1982) and Rhodnius prolixus (ABU-HAKIMA and DAVEY 1977; NIJHOUT 1983). Therefore, it seems unlikely that the use of the "wrong" JH is responsible for the lack of response. A few preliminary experiments with JHII and JHIII were also performed with A. proletella without effect.

Since JH is a surface active molecule, it is possible that adsorption onto the abundant waxes overlying the whitefly cuticle prevents any hormone from reaching the haemolymph. In addition, a high level of circulating JH esterases might degrade any JH that did reach the haemolymph before it had a marked effect upon ovarian development (see DE KORT and GRANGER 1981).

It has been shown that vitellogenic competency in O. fasciatus results from a precise sequence of endocrinological events (RANKIN and JACKLE 1980; KELLY and HUNT 1982), and the interaction of JH and ecdysone has been discussed for mosquitoes (FUCHS and KANG 1982). It seems unlikely that competency is the only reason for the lack of JH effect in A. proletella since the field samples cover a range of initial ovarian scores. In addition, the fact that morphogenesis occurs without hormone treatment (Figs.30 to 36) shows that the ovary is capable of responding.

An exogenous JH effect may be masked by the "high" temperature that the field samples are transferred to if that temperature stimulates endogenous JH production. If this is the case, experiments at lower temperatures should reveal an effect.

Finally, it is possible that the JH titre is not the central endocrinological factor causing or maintaining the low rate of ovarian development. In view of the extent of the literature in support of a role for JH in ovarian diapause, including topical application and injections of

hormone as well as corpora allata denervation, extirpation and implantation studies, this would make A. proletella an exceptional insect.

The results presented here suggest that a short refractory stage follows the induction of ovarian diapause in the cabbage whitefly, Aleyrodes proletella. Some slight morphological changes within the ovary occur during this stage but it does exhibit a high degree of refractoriness to photoperoid and temperature is exhibited. Subsequently, the rate of post-diapause morphogenesis appears to be largely temperature dependent, such that the cold winter temperatures retard the rate of development. However, chilling also appears to prime the ovary for subsequent development. In the field, the dormancy is probably terminated by the prevailing temperatures in April and May. At present, the role of JH, if any exists, is unknown. In a series of experiments, it did not exhibit any dormancy breaking effect.

Since the ovarian diapause of A. proletella is induced by photoperiod, influenced by chilling and ultimately terminated by temperature, it fits Muller's (1965, 1970; see THIELE 1973) definition of eudiapause.

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