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THE LEAFHOPPER, Agalliopsis abietaria OMAN

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Photoperiod controls the duration of both the fourth and fifth

stadia in Agalliopsis abietaria Oman. Development was arrested in

the fourth stadium unless short days were given during the second

and third instars; a short arrest of development occurred if the short

days did not begin until the fourth instar. A diapause of long duration

in the fifth instar, which overwinters, was induced by giving short

days for all nymphal instars. The fifth stadium was minimized by

short days during the first one or two instars and long days for the

rest of the nymphal period. The earlier in the nymphal period the

change from short days to long days was made, the shorter was the

duration of the fifth stadium. The ecological significance of the

fourth-instar arrest of development is discussed.

Photoperiodic Control of Development in the
Leafhopper, _Agalliopsis abietaria_ Oman

by

Robert David Brown

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INTRODUCTION

Diapause

The growth and development of insects is often greatly slowed
or even arrested at times. Such interruptions of the life cycle are
generally related to unfavorable changes in the environment. The
intensity of such dormancy, the method of its induction, and the kind
and degree of physiological changes connected with it vary according
to the degree of uncertainty and unsuitability of the environment.

Diapause is a physiological condition that is initiated in advance
of adverse conditions and permits the insect to survive these condi-
tions, e.g. the low temperatures of winter, or hot, dry summers.
Diapause is not directly brought about by the unfavorable conditions,
as is the case in torpor, but rather by some dependable token stimu-
lus, such as changes in photoperiod or temperature.

Perhaps the simplest diapause is that of the univoltine insect.
Diapause occurs in every generation and persists until broken by the
events during diapause development and subsequent favorable tem-
perature or humidity. The univoltine insect is often considered to
have an "obligatory" diapause. Diapause is obligatory in its natural
environment; but under artificial laboratory conditions, a univoltine

insect can often be made to develop continuously (Mansingh and

Smallman, 1967). Such a finding underlines the concept of diapause

as an adaptive strategy which follows environmental cues rather

than a fixed developmental stage. Such a system, that allows only

one generation per year, permits survival, but does not permit the

insect to take advantage of growing seasons longer than what it needs

for the development of a single generation. Short-day insects, which

diapause in response to the long, warm days of summer, can be only

univoltine or bivoltine. Long-day insects are often multivoltine,

completing several to many generations during the favorable grow-

ing season. Diapause in multivoltine insects is initiated only by

specific environmental stimuli, usually photoperiod or temperature

or a combination of both.

There are instances of insect life histories in which there is

a mechanism that insures a univoltine life cycle even under conditions

that seem favorable to more than one generation per year. K. F.

Geispits found that the larvae of the Tussock moth, Dasychira

pudibunda, developed to pupae in 2 to 2 1/2 months near Leningrad,

but near the Black Sea development took over four months. Extra

instars occurred in the insects at high temperatures, and develop-

ment was more rapid under short-day than long-day conditions

(Danilevskii, 1961:204). Similarly, Kamm (1971) found that the

rate of development of early-instar sod webworms (Crambus tutillus)

was slow during the longer photoperiods of midsummer and quickened under short fall days. The webworms molted to their diapausing instar only when exposed to short days.

The termination of diapause involves a physiological change of some sort during a time of otherwise arrested development. Andrewartha (1952) has called this physiological change "diapause development." Others have emphasized that the breaking of diapause involves the removal of a block and prefer the term "reactivation" (Danilevskii, 1961). Diapause induced by photoperiodic means may be terminated by a different environmental factor (Tauber, Tauber, and Denys, 1970). In fact, Beck (1968:152) has said, "As far as is currently known, the diapause of most species is not terminated by photoperiod." Diapause induced by photoperiodic conditions is often broken by temperature. A period of cold temperatures (often 0-15° C; Danilevskii, 1961) is often effective. In the codling moth, Laspeyresia pomonella, diapause was induced by short days but could be terminated in nearly 100 percent of the insects by either long days or chilling (at 4. 5° C) followed by room temperatures (Peterson and Hamner, 1968). The effect of the cold or light is on the insect brain: specifically (in the termination of larval diapause) the neurosecretory cells are "reactivated" to produce prothorocotropic hormone which in turn causes the thoracic glands to produce ecdysone (Highnam and Hill, 1969). Other hormones, such as juvenile hormone, may be

involved too (de Loof, 1972). "Spontaneous" termination of diapause

occurs in some individuals or species, but only after an extended

period of time has passed under unchanging conditions (Tauber and

Tauber, 1972; Kisimoto, 1958, 1959; Beck, 1968:152; Danilevskii,

1961:50).

In the target organs ecdysone acts directly on the gene and

stimulates RNA synthesis (Sekeris, Karlson and Congote, 1971).

Ecdysone brings about a chromosome puffing pattern typical of late

larval stages; this suggests that ecdysone stimulates the activity of

certain genes to produce their specific m-RNAs (Highnam and Hill,

1969). Zdarek and Fraenkel (1970) and Ohtaki, Milkman and Williams

(1968) have shown that ecdysone works through small, covert effects

that are cumulative, but which may be lost, at least in part, if

ecdysone ceases to be present in the target organs. That is, small

amounts of ecdysone must be present continuously over a period of

time to produce molting.

"[Diapause] is an adaptive phenomenon, comparable with migra-

tion, and it enables the insect to live in areas which at times are

unsuitable for it" (Chapman, 1969:717). In both diapause and

migration animals are able to exploit periodically unsuitable envi-

ronments because changes in physiology or behavior brought on by

token stimuli annul the effects of an environment that would other-

wise be unsuitable. The ancestors of today's diapausing insects

presumably survived conditions that were (periodically) unfavorable while their non-diapausing relatives did not; they passed on the genes that are ultimately responsible for the diapause response, whether "obligatory" or "facultative."

Areas may be climatically unsuitable for a non-diapause insect annually or only in some years. Non-diapause insects may not always be wiped out: insects in diapause may only survive at a higher rate than those without, and perhaps only in some years. Non-diapause genes that were recessive or hypostatic would remain in a population even in years in which only diapausing insects survived. In such cases, genes for development without diapause will be present in the population, and a percentage may survive without diapause in some or most years. More common, perhaps, is the case in which part of a population of long-day insects will start and perhaps complete an extra generation. The diapause-determining thresholds (including critical daylengths or the necessary number of photoperiodic cycles) are lower in this part of the population. For example, the European corn borer, Ostrinia nubilalis, was univoltine or bivoltine in different parts of the United States, and in some areas it was reported that the population was changing from one generation per year to a bivoltine life cycle (Beck and Apple, 1961). Such a change, of course, may change an insect's status as a pest and require changes in the pest management recommendations

(McClanahan and Founk, 1972).

In addition to permitting an insect to survive periods of harsh climate, diapause serves as a synchronizing factor in the lives of insects. The active stages are, of course, coincident with periods of favorable climate. In some insects (such as monovoltine species or those with low population densities) the mechanism of breaking diapause may be very exact (with selection for little genetic heterogeniety) so that adult emergence or the breaking of reproductive diapause is synchronized and species survival is enhanced. In the semivoltine insect Dendrolimus pini (Geyspits, 1965) diapause termination is by photoperiod; this may be the most common means of termination for semivoltine insects. Corbet (1956) found that a very precise photoperiodic control of diapause-breaking was responsible for the very limited period of adult emergence occurring in the emperor dragonfly, Anax imperator, which takes one or more years to mature.

Diapause has been studied mostly in insects that are pests, often in introduced species that have proven themselves to be adaptable rather than in insects that are adapted to a more restricted niche. The latter are more typical of insects in general, and these often innocuous species may be more helpful in deriving general principles, whether of physiology or population dynamics (Clark et al., 1967:167). The frequently-studied pest species are often

multivoltine insects with a long-day type response. They are pro-
grammed to produce one generation after another under long days
or high temperatures, and are able to build up high populations that
univoltine species rarely attain. The potential for success with this
strategy is great, but the large numbers invite the evolution of spe-
cific predators and parasites. When introduced into a new area
without its specific control organisms, the long-day insect may
easily become a pest if other factors permit. As a pest, it becomes
a candidate for scientific study, and so the preponderance of refer-
ences to long-day type insects in the literature may not reflect the
actual proportion in nature.

The primitive insect presumably lived in a climate lacking
extremes; it survived with an uninterrupted life cycle and without
special protective devices such as a highly-variable metabolism.
Such insects might survive the worst of a mild climate in torpor,
but were too complex to have an anabiosis that would permit them
to survive in a harsher climate. For an insect to survive, extensive
changes in physiology must occur, which take time. Therefore the
change must occur well before the adverse conditions arise. Dia-
pausing might be considered as a malfunctioning of the primitive
insect endocrine system, such that continuous development was
interrupted. This interrupted development would be a disadvantage
if insects adapted to the same niche reproduced continually, unless

a harsh environment killed off the non-diapausing insects.

Biology of the leafhopper

Agalliopsis abietaria Oman did not complete its life cycle,

except after extended periods of time, under greenhouse conditions.

The mechanisms controlling this apparent diapause was unknown, and

it was desired to rear the insect in the greenhouse for virus trans-

mission experiments. Black's wound tumor virus (Marmorosch,

1970) had been discovered in a member of the same genus, Agalliopsis

novella (Say) (Black, 1944). It was thought that A. abietaria might

also be able to transmit the virus. If so, this leafhopper could be

used to study the effects of a vector's diapause on virus transmission.

Field collections were made and experiments were undertaken to

learn more about the life cycle in the field and in the laboratory cul-

tures.

A. abietaria is a fuscous-colored leafhopper, about four mm

long in the adult stage. Females are somewhat larger (4. 0-4. 5 mm)

than males (3. 8-4. 24 mm) (Oman, 1970). This insect is presently

believed to live on wild strawberry in proximity to fir trees. The

laboratory insects were descended from insects collected in McDonald

Forest, Benton County, Oregon. This is in the mid-Willamette Valley

at an elevation of 500 feet on the eastern side of the Coastal Range.

Winters are mild and wet; summers may be hot and dry in July and

August. In this area 70 percent of the annual precipitation occurs
during the period from November to March, with only about five
percent occurring during the three summer months (Bates and
Calhoun, 1970). See Figure 1. Vegetation, in general, is most
abundant and succulent in spring and fall. Adult insects have been
collected in the above location from very late April to mid-July.
No first or second instars have been collected either by sweeping
or from samples put in Berlese funnels. I have collected one third
instar on September 15 and fourth instars from September 15 to
October 6. Fifth instars have been collected on October 18 and 25,
November 13, and in the spring. Nymphs are difficult to collect,
and the scarcity of information makes it difficult to make exact
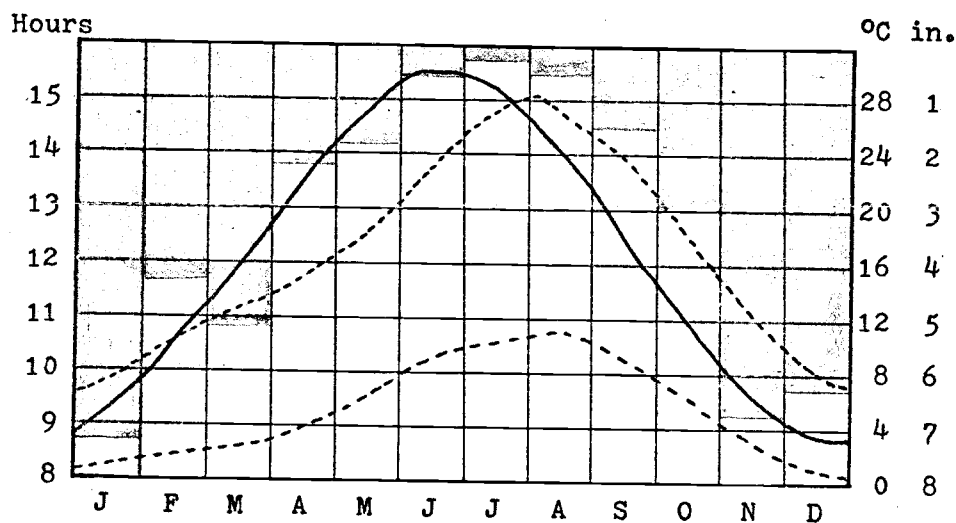statements about the life cycle in the field.

Figure 1. Photoperiod in hours (———), for 45° latitude,
including civil twilight (Beck, 1968). Average
monthly maximum and minimum temperatures
(----), and average monthly precipitation
(shaded, inverted scale), 1959 to 1968, for
Corvallis, Oregon (from Bates and Calhoun,
1970).

MATERIALS AND METHODS

The leafhoppers were raised in the entomology greenhouse at
Oregon State University. Long days (LD 16:8) were maintained with
florescent lights supplementing sunlight. A daytime temperature of
about 24°C was maintained, usually for about 11 hours, but occa-
sionally the temperature did not go down at night. The night temper-
ature was 18°C. Temperatures were monitored on a Leeds and
Northrup multipoint recorder which printed the temperature from
each thermocouple every 20 minutes. Mean temperatures and
standard deviations were calculated from these printouts.

During experiments the leafhoppers were kept in the green-
house or in one of several temperature-controlled cabinets, or in
a walk-in, temperature-controlled chamber. Temperatures were
monitored in the cabinets by the thermograph as above. A United
Electric Controls pen and 24-hour circular graph recorder was
used to monitor temperatures in the chamber, and in the cabinets
during two periods when the multipoint recorder was broken. High
intensity florescent lamps were used for lighting in the chamber and
cabinets, and for supplemental lighting in the greenhouse. Approxi-
mately one-half of the lights were "wide spectrum" output. Light
intensity varied from 40 to 55 foot-candles at the plant level in the
chamber and cabinets, and varied from a low of 25 foot-candles in

the greenhouse on a cloudy day.

Insects were raised on potted red clover plants (Trifolium

pratense L. cultivar Kenland) in wooden frame cages (43 x 20 x 20

cm) covered with plastic screening and having one side (43 x 20 cm)

enclosed by glass. During an experiment insects were kept in

Corning #2 lamp chimneys (21.6 cm high x 7 cm in diameter; vol-

ume 950 cc), which were placed over a clover plant in a "four inch"

pot. Cheesecloth was placed over the top of the chimney. The insects

in chimneys were transferred to new clover plants (approximately 21

days old and about three inches high) at seven day intervals, to reduce

variation in clover age and humidity. In later experiments plants

were changed every 10 or 14 days.

Several leafhoppers were put on each clover plant to conserve

space which was particularly important in the growth cabinets. In

order to keep track of the insects individually, a small dab of non-

toxic, water-soluble, tempera paint was applied to the insect. It

is generally agreed (Highnam and Hill, 1969; but see also Beck, 1964,

and Beck and Alexander, 1964), that light acts on the insect head,

so the paint was put on the abdomen. The painted insects had no

difficulty molting and showed no significant difference in stadium

length when compared to unpainted insects (paired t-test, $P < 0.2$,

for the fourth stadium, 12 pairs of insects. In later experiments

newly hatched nymphs were put on a single plant each day and not painted until the second instar.

TEMPERATURE-RELATED EXPERIMENTS

Nymphal Diapause

Two variations of an experiment were made to test the hypothesis that diapause development and the breaking of diapause is temperature controlled in A. abietaria. One hundred-fifty insects believed to be in diapause due to the infrequent molting in the group were transferred from rearing cages to Stender dishes lined with moistened filter paper and kept at 3° C in darkness. At the end of 2, 3, 4, 5 and 6 weeks, 30 insects were removed and 10 were put on one clover plant under each of the following conditions: (a) LD 16:8 (22° day, 11° night); (b) LD 16:8 (24° day, 18° night--greenhouse); and (c) LD 10:14 (15° constantly). Insects in (a) were moved to (b) after six weeks. Exuviae and adults were counted weekly for each group.

A second group of leafhoppers (d) were removed from their rearing cages and put on individual clover plants in the greenhouse. I checked for exuviae every three days and changed plants every ten days. Of the 72 insects so treated, all eventually went for a period of 14 days without molting, at which time I assumed that they were in diapause and chilled them at 3° C as above for periods of from two to seven weeks, except a control group of ten insects was not chilled.

TABLE I. Exuviae of leafhoppers after return to higher temperature following exposure to 3° C for a period of weeks.

Time (after end of cold exposure) in weeks	GROUP			
	A (22°/11°)	B (24°/18°)	C* (15°)	D (24°/18°)
3	32	37	17.25	38
6	45	47	45	42
9	45	51	49	42

* figures corrected for fewer insects in this group.

TABLE II. Emergence of adults ten weeks after return to higher temperatures following exposure to 3° C for a period of weeks.

Weeks Chilled	GROUP			
	A (22 /11)	B (24 /18)	C (15)	D (24 /18)
2	0	7	0	0
3	0	3	0	3
4	0	5	0	1
5	0	2	0	1
6	0	3	–	0
7	–	–	–	0
Control	–	–	–	5
Total	0	20	0	10
Number Insects	50	50	40	63

After the cold treatment, the insects were returned to the greenhouse and exuviae and adults were counted weekly.

Molting was similar in groups (a) and (b): 47 and 45 skins cast in the first six weeks respectively (not including skins cast in the final molt). Group (c) lagged significantly (Chi^2) in molting at three weeks, but equalled the other groups by the end of the six weeks (45 exuviae, corrected for fewer insects, compared to 45 and 47). See Table 1. The lag in molting in group (c) was very likely due to the lower temperature.

Nine adults had emerged by the end of six weeks and twenty by the end of ten weeks in group (b). Ten adults emerged in group (d) by the tenth week, but none in group (a). See Table 2. The reason for this difference is not apparent; it may have been due to the somewhat higher average temperature in the greenhouse (group b), or perhaps to the greater light intensity or spectrum range of natural sunlight. No adults emerged from group (c). This may have been due to the low temperature, but more likely to the short photoperiod or a combination of both.

Chilling at 3° C for the durations used in this experiment had no effect on the breaking of diapause. However, the molting patterns indicate that most, if not all, of the chilled insects were in the fourth instar at the time of chilling. The insects were raised under constant long-day conditions, which, it will be shown below, causes

an arrest of development in the fourth instar. Field data and photo-periodic experiments (reported below) indicate that the fifth instar overwinters; it would undergo the cold conditions in the field. It is therefore not too surprising that chilling had no effect on the fourth instar. However, the fact that the insects lived up to seven weeks at 3°C without food, indicates that diapause may have been present in the fourth instar.

At the conclusion of this set of experiments it was apparent that more significant results were coming from concurrent experiments involving the effect of different photoperiods on the insects. Further experiments investigated the photoperiodic control of diapause in the leafhoppers.

Egg hatching

The possibility of an egg diapause occurring in the field in summer in this leafhopper was suggested by the failure of some eggs to hatch in the laboratory, by the absence of data about the first three instars in the field, and by the fact that if eggs hatched about 20 days after being laid, as occurred in the laboratory, the early instars would be in a hot, dry environment, seemingly more hostile than if hatching were delayed. Some insects, including an entire laboratory culture, developed without fourth-instar diapause under long-day conditions. This suggested that the fourth instar

diapause may not be necessary for survival and may not occur in the field. If there was an egg diapause through the hottest part of the summer, the third and fourth instars might perceive short days, and hence there would be no fourth-instar diapause.

Leafhoppers were mated, a pair to a small clover plant, which was kept in the greenhouse (LD 16:8, 24°:18°). After 10 or 14 days the clover plant (leaves and stems) was put in a Petri dish lined with moistened filter paper, and nymphs were counted as they appeared. The adults were moved to new clover plants as long as the female lived. Experiments with 13 pairs showed that the first nymph hatched an average of 23.08 ± 1.14 days after the male and female insects were placed together. After the adults were moved to new plants the first nymph appeared after an average of only 18.86 ± 1.64 days: so the eggs hatch in about 19 days under these conditions.

Insects raised under long days were moved during the fourth instar to short days. Fifty-two days later one-half were chilled at 3° for 22 days. Both groups were then moved to long days (after 74 days in short days). Ten females that had been chilled as nymphs laid more eggs on the average (81.9±19.7) than eight unchilled females (61.3±41.1). This is not a significant difference (t=1.32: P < 0.21). Twenty-six other unchilled females put singly or in pairs on clover plants laid an average of 48.3 eggs per female (maximum of 131).

No evidence of an egg diapause was found. It is possible that drier conditions are necessary for egg diapause in the field; humid conditions may prevent or break egg diapause (Danilevskii, 1961:15). Large clover plants, enclosed in the wooden frame cages offer a somewhat drier environment. It was in such a cage that the apparent egg-hatch failure was observed; but in other similar cages nymphs hatched within at least 30 days. However, the collection of a third instar on September 15 indicated that the early instars do survive in the driest part of the summer.

It was thought that clover was not the host in the field and that the host plant might affect egg hatching. Nymphs hatched within about a month when previously-mated adults were placed on Fragaria vesca variety semperfloreus ("Alpine") plants. Adults did not survive well on these strawberry plants, probably due to water droplets on the plant and glass. The moisture problem was particularly great because the plants were not changed every 10 to 14 days as with clover. The experiments with leafhoppers on strawberry plants were continued long enough to determine that the insects could complete a generation on the plants even under the humid conditions.

PHOTOPERIODIC EXPERIMENTS

The First Set: Standard Photoperiodic Conditions

Leafhoppers were put in groups of four, or individually, on clover plants in controlled-environment cabinets. One group was subjected to long days (LD 16:8), the other to short days (LD 10:14). The long day chamber ran slightly warmer, 24.7°:15.3° compared to 23.7°:14.7° for the first two months; and 24.0°:15.7° compared to 23.7°:14.4° for the following three months during which some insects were still in the fourth or fifth instars. During the first two months the standard deviation (S.D.) was less than 1°C for any single day calculated, except slightly above 1° for the short-day dark cycle; for the next three months the S.D. was about 1° and always less than 2° except in the last month of the short-day dark cycle. The S.D. from the average temperatures cited above was less than 1° (except slightly above 1° for the short-day dark cycle) for the first two months; and less than 2° (except for parts of both short day cycles) for the next three months. There were several irregularities in the temperature control in the short-day cabinet. The temperature failed to drop one night during the first month. The temperature dropped to 7°C for two hours at the end of the night cycle once during the second month, and for an entire night

TABLE III. Duration of the stadia under standard long-day and short-day conditions.

Stadium	LONG DAYS (LD 16:8)		SHORT DAYS (LD 10:14)	
	Days ± S.D.	Number of Insects	Days ± S.D.	Number of Insects
1	10.4 ± 1.5	101	12.2 ± 1.5	103
2	10.0 ± 1.6	92	9.1 ± 1.5	90
3	13.3 ± 2.5	80	10.6 ± 1.7	77
4	52.5 ± 46.7	22*	16.0 ± 2.9	74
5	36.9 ± 24.5	16	123+ ±(23.5)	23**

Ranges:
   4th stadium, long days: 19 to 175 days.
   5th stadium, long days: 13 to 80 days.
   5th stadium, short days: 77 to 153+ days, including
                  8 insects terminated at 141-153 days.

* One-half of the long-day insects were moved to short days during the fourth stadium (a few were in the fifth). The median fourth-instar insect molted to the fifth instar 29 days after this change.
**One-half of the short-day insects were moved to long days during the fifth stadium. These 38 insects became adults an average of 17.7 ± 2.0 days later.

at the end of the third month.

The results of this first set of photoperiodic experiments are given in Table III. The sharp decline in the number of insects used in the calculations between instars three and four under long days, and between instars four and five, short days, was due to the dividing of each group in half. One-half of each group was moved to the other photoperiodic regimen, giving four groups: long days (LD), short days (SD), insects moved from long days to short days during the fourth instar (LD-SD), and insects moved from short days to long days during the fifth instar (SD-LD).

Short days were necessary for development to proceed directly to the fifth instar. Under constant short days (SD) the fourth stadium lasted 16.0 ± 2.9 days, while under constant long days (LD) there was an arrest of development and the stadium lasted 52.5 ± 46.7 days with a range of 19 to 175 days. The arrest of development was broken by moving insects to short days. The fourth stadium was completed by all insects in the LD-SD group within 33 days of the change to short day conditions. The median insect took 29 days. The pattern of molting (see Figure 2) suggests the hypothesis that it takes about 30 days to _break_ fourth-instar diapause in this insect. Under this hypothesis some insects (particularly those insects num- bered 1-5 in Figure 2) are assumed to have started the physiological changes that control the resumption of development previously to the
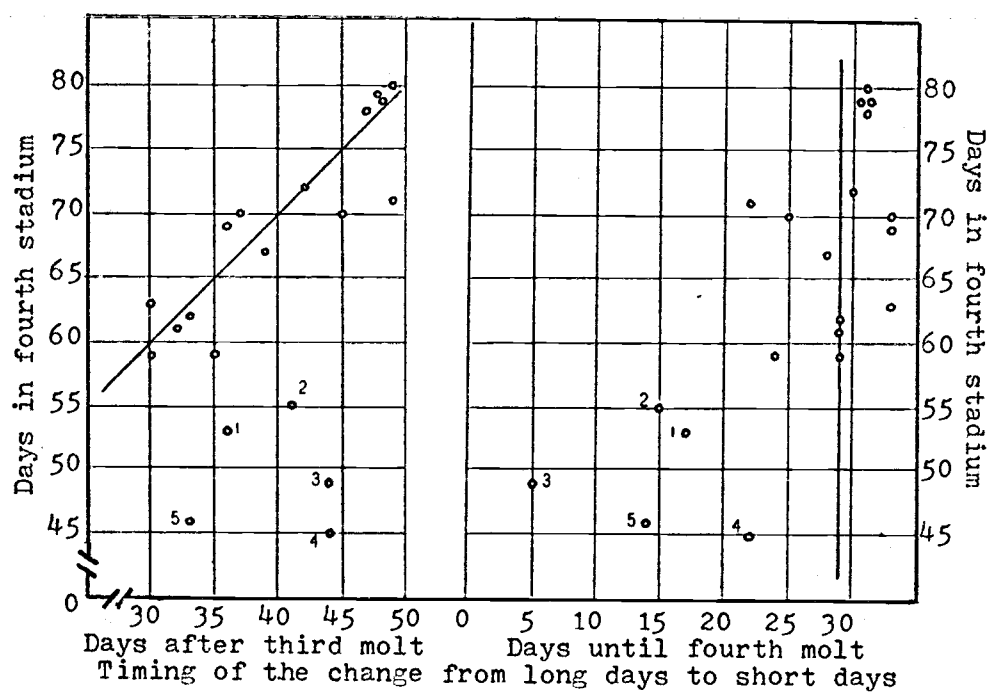
Figure 2. Resumption of development following the change from long days to short days (LD-SD) during the fourth stadium.

change in photoperiod; the change to short days may have speeded

up their development. It is possible, but does not seem probable,

that the insects were in the same physiological state, but reacted

differently to the change and so proceeded with diapause development

at different rates.

The left side of Figure 2 shows that reaction to short days

was the same over a period of 20 days in the fourth stadium. For

the 15 unnumbered points, the linear regression equation was

$Y = 0.919 X + 32.49$, which is very close to the $X + 30$ drawn on

the graph. The correlation coefficient, $r = 0.897$; so eighty percent

of the variance of Y (days in the fourth stadium) can be attributed to

its regression on X (timing of the photoperiodic change in relation

to the third molt) (Snedecor and Cochran, 1967:176).

The fifth stadium averaged 38.1 ± 29.6 days under long days,

somewhat shorter than the fourth stadium under these conditions.

But the fifth stadium of insects raised under constant short days

showed the most definite and intense arrest of development. Of 23

insects, 15 terminated diapause spontaneously after an average of

109.5 ± 18.2 days in the fifth stadium. The remaining eight were

terminated (by moving them to long days) after five months. If

these eight are included in the calculations, an overall average of

123+ days (± 23.5 days) is obtained for the fifth stadium under short

days.

The insects raised under short days were divided into two groups at a point when both groups had been in the fifth instar for an average of 20.7 days. Adults emerged from the SD-LD group 13 to 21 days after the change to long days (average 17.7 ± 2.0 days) compared to at least 57 days (after the division) for the SD insects. Thus the breaking of fifth-instar diapause is photoperiodically controlled too.

The most important discovery to come out of this set of experiments is that _Agalliopsis abietaria_ can diapause in two instars, with the induction and termination being by different photoperiodic regimens in each: the fourth-instar diapause is induced by long days and terminated by short days, while it is just the opposite for the fifth. In general, diapause occurs only at a specific growth stage, and few insects are able to diapause at more than one point in their life cycle (Beck, 1968:136). Insects that do diapause more than once during a year usually do so in well-separated stages: the winter moth _Operophtera brumata_ and the leafroller _Exapate congelatella_ both diapause in summer as pupae and in winter as eggs (Danilevskii, 1961). The leafhopper _Draeculacephala crassicornis_ overwinters as eggs and the McDonald Forest population also has a summer reproductive diapause in the adults (Kamm and Swenson, 1972). (This leafhopper is abundant in the area where _A. abietaria_ was collected.) Because the induction of diapause in the fourth and fifth instars of

A. abietaria is by opposite photoperiodic signals, it cannot be considered to be at one point in the life cycle even though the instars are adjacent. Whether the arrest of development this leafhopper showed in the fourth instar should be considered to be a true diapause, and whether this arrest occurs in the field will be discussed later.

The laboratory data rules out the possibility of the leafhopper overwintering as a fourth instar. When raised under short days, the fourth stadium was short. If moved from long days to short days during the fourth stadium, the insect molted to the fifth instar within about 30 days. The insect in the field would molt to the fifth instar with the coming of short days in the fall. The arrest of development in the fourth instar under long days was not as intense as that in the fifth instar under short days: only three insects of 22 had not molted to the fifth instar within three months, and the average duration of the fourth stadium was less than two months. This fourth-instar arrest of development may simply be a mechanism for preventing the commencement of a second generation that probably could not complete development before winter. The fourth stadium is lengthened enough so that short days are sure to be perceived. The short days apparently trigger the last nymphal molt and initiate the fifth-instar diapause. The short days maintain the fifth-instar diapause until the coming of long days in the spring which control termination of diapause and adult emergence. The short-day insects

that became adults in the laboratory in less than five months were
kept at temperatures well above winter field temperatures--tempera-
ture compensation as well as genetic diversity may be involved. High
temperatures complement the effects of long days and tend to act
oppositely to short days, to a degree that varies from species to
species (Saunders, 1966, 1971; Beck, 1968).

The standard deviations were nearly as large as the means in
both the fourth and fifth stadia under long days. The division of the
long-day and short-day groups was done with this great variability
in mind. Danilevskii (1961) noted that variation in development of
the lepidopteran Arctia caia was much less under changing conditions.

The leafhoppers responded quite uniformly to the abrupt change
in photoperiodic conditions: the fifth-instar insects moved from
short days to long days became adults in 17. 7 days on the average,
and the fourth instars moved to short days from long days molted
to the fifth instar in about 30 days. It seems doubtful that the varia-
tion that occurred under constant long days would occur in the field,
although there is great heterogeneity of diapause in many populations
(Mansingh, 1971). Increased variability in diapause induction, dura-
tion and intensity may be caused by environmental conditions not
normally experienced in the field (Danilevskii, 1961; Krunic and
Hinks, 1972). Very long or very short daylengths often give highly
variable response curves. Diapause may occur only within a limited

temperature range regardless of the photoperiod (Dickson, 1949),
and humidity may affect diapause response (Danilevskii, 1961). In
this case, it may be that the continuous unchanging long days are the
unnatural stimulus that results in great variation; the insect popula-
tion does not perceive a precise environmental signal.

Sets with Various Photoperiodic Regimens

The data from the first experiments show that fourth-instar
diapause was prevented or terminated by short days and fifth instar
diapause was terminated by long days. Further experiments were
carried out to determine which instars were sensitive to photoperiod
and what regimen would minimize the duration of the fourth and fifth
stadia. Long days were LD 16:8 and short days were LD 10:14 as
before. Temperatures were also set as before: the average temper-
atures were 24.3°:15.2°C for the long-day cabinet and 24.0°:15.1°C
for the short-day cabinet. The standard deviations of the calculated
temperatures were less than 1°C on any single day, and were less
than 2°C for the averages given above. After the first three months
temperatures were monitored on the UEC pen and graph recorder
for the most part: temperatures were generally within 1° of the
desired level, and always within 2°.

The insects were subjected to the different light regimens
shown in Figures 3 and 4. In most cases the duration of the fifth

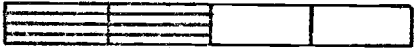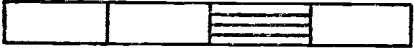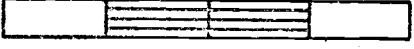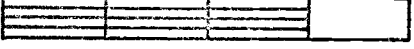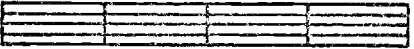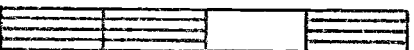| Reg-imen	INSTAR 1	2	3	4	Set	x̄ ± S.D.	Range	Number of Insects	
A					3	40.3 ± 49.8	7-160	11	abcdefghi
					4a	25.6 ± 30.1	11-146	18	abcdefg
					4b	39.9 ± 36.4	11-246	31	f h
B					5	18.5 ± 8.0	11-38	12	a
C					3	15.7 ± 3.2	12-22	7	abc
D					5	14.8 ± 2.6	12-26	53	b
E					3	13.3 ± 1.9	11-17	18	d
F					5	11.9 ± 1.7	9-15	28	e
G					3a	11.8 ± 0.8	11-13	21	e
					3b	11.8 ± 1.3	10-14	12	e
H					1	16.0 ± 2.9	13-28	74	c
					2	14.5 ± 2.7	11-20	12	abcd
					3	13.8 ± 1.6	12-17	16	b d
					5	15.6 ± 1.3	14-18	7	abc
I					3	23.5 ± 4.5	15-34	27	f
J					1	52.5 ± 46.7	19-175	22	ghi
					2	104.3 ± 68.0	41-261	9	j
					3	68.6 ± 44.0	18-176	20	ij
					4	70.7 ± 52.8	27-204	18	ij
					5	49+ ± (24.3)	24-88+	34	*hi

Figure 3.   Duration of the fourth stadium (in days) under various photoperiodic regimens with the general pattern of short days then long days.   Horizontal lines indicate short days. Treatments sharing a common letter are not significantly different at the 0.05 level.

*Regimen J-5 includes six insects terminated at 74 to 88 days.

Reg-imen	INSTAR 1	2	3	4	Set	x̄ ± S.D.	Range	Number Insects	
A					5	11.9 ± 1.7	9-15	28	
B					1	16.0 ± 2.9	13-28	74	b
					2	14.5 ± 2.7	11-20	12	ab
					3	13.8 ± 1.6	12-17	16	a
					4	15.6 ± 1.3	14-18	7	ab
C					2	15.7 ± 2.3	11-18	11	bc
					5	17.7 ± 1.0	16-19	7	cd
D					2	17.2 ± 2.2	14-22	26	cd
E			3		2	18.9 ± 2.1	16-22	7	de
					3	17.9 ± 2.5	14-24	15	d
F			6		2	20.6 ± 3.2	13-19	9	efg
					3	18.1 ± 1.2	16-20	7	cd f
G			9		2	23.9 ± 4.6	18-33	10	gh
					3	20.4 ± 2.7	18-27	13	e
H					3	23.5 ± 4.5	15-34	27	gh
I					2a	25.4 ± 3.8	18-31	10	h
					2b	32.7 ± 4.2	25-40	30	i
					3	27.9 ± 2.5	23-36	28	
J					1	52.5 ± 46.7	19-175	22	ij
					2	104.3 ± 68.0	41-261	9	k
					3	68.6 ± 44.0	18-176	20	jk
					4	70.7 ± 52.8	27-204	18	jk
					5	49+ ± (24.3)	24-88+	34	*j

Figure 4. Duration of the fourth stadium (in days) under various photoperiodic regimens with the general pattern of long days then short days. Horizontal lines indicate short days. Treatments sharing a common letter are not significantly different at the 0.05 level.
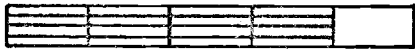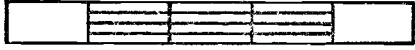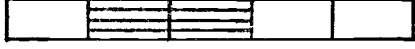*Regimen J-5 includes six insects terminated at 74 to 88 days.
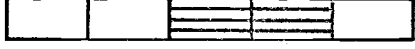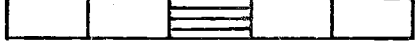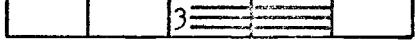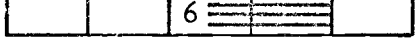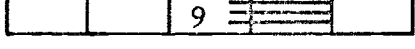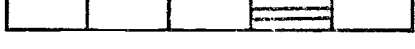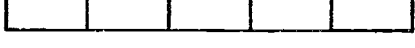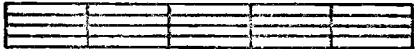
Reg-imen	INSTAR 1 2 3 4 5	Set	$\bar{x}$ ± S.D.	Range	Number of Insects
A		2	20.0 ± 1.0	18-21	4 ab
		3	20.8 ± 1.7	18-23	12 b
B		3	21.0 ± 2.8	16-26	25 b
C		2	24.9 ± 3.5	18-29	8 cd
D		5	24.2 ± 8.3	17-50	18 bcde
E		2	26.6 ± 2.6	22-31	15 cd
F		5	28.5 ± 13.6	12-59	11 cdef
G		3	28.5 ± 4.5	23-35	15 de
H		2	31.6 ± 4.6	27-38	3 efg
		3	28.2 ± 2.3	27-32	5 e
I		2	38.0 ± 5.8	30-45	5 fg
		3	29.3 ± 3.1	26-32	7 e
J		2	35.3 ± 5.3	26-42	12 fg
		3	37.5 ± 9.4	20-65	25 g
K		1	36.9 ± 24.5	13-80	16 efg
		3	29.9 ± 14.3	13-54	8 bcdefg
		4	16.3 ± 4.3	12-27	9 a
L		1	123+ ±(23.5)	77-153+	23

Figure 5. Duration of the fifth stadium (in days) under various photoperiodic regimens with the general pattern of long days then short days. Horizontal lines indicate short days. Treatments sharing a common letter are not significantly different at the 0.05 level. Regimens G, H, and I had 3, 6, and 9 long days respectively at the start of the third stadium. Regimen L includes 8 insects terminated at 141 to 153 days.

Reg- imen	INSTAR 1 2 3 4 5	Set	x̄ ± S.D.	Range	Number of Insects	
A		3	13.4 ± 1.9	12-18	8	ab
		4a	12.9 ± 1.8	10-16	17	a
		4b	17.4 ± 6.8	12-43	25	abcd
B		3	14.7 ± 1.6	13-18	6	abc
C		3	16.7 ± 2.0	13-20	16	de
D		3a	16.2 ± 1.5	14-20	12	cde
		3b	15.3 ± 2.7	12-23	17	cde
E		2	20.0 ± 1.0	19-21	4	fg
		3	20.8 ± 1.7	18-23	12	g
F		3	21.0 ± 2.8	16-25	25	gh
G		2	24.9 ± 3.5	18-29	8	i
H		5	24.2 ± 8.3	17-50	18	ghi
I		2	26.6 ± 2.6	22-32	15	i
J		5	28.5 ± 13.6	12-59	11	hij
K		2	35.3 ± 5.3	26-43	12	j
		3	37.5 ± 9.4	27-65	25	j
L		1	36.9 ± 24.5	13-80	16	ij
		3	29.9 ± 14.3	13-54	8	ghij
		4	16.3 ± 4.3	12-27	9	bcdef
M		1	123+ ±(23.5)	77-153+	23	

Figure 6. Duration of the fifth stadium (in days) under various photoperiodic regimens with the general pattern of short days then long days. Horizontal lines indicate short days. Treatments sharing a common letter are not significantly different at the 0.05 level. Regimen M includes 8 insects terminated at 141 to 153 days.

stadium under long days was noted also (Figures 5 and 6). Groups

of insects with the same number in the "set" column came from the

same leafhopper culture, and were started at about the same time.

Sets 2, 3, and 4 overlapped in time where elongated stadia were

involved. Set 1 (reported in Table III also) and set 5 did not overlap

with the others in time except for a few insects with extremely long

fourth and fifth stadia. The t-test was used to determine which means

were significantly different at the 0.05 level. When the F-test indi-

cated that variances were significantly different, a modified t-test

was used (Snedecor and Cochran, 1967:115).

The fourth-instar developmental rate varied according to the

photoperiod received during the first four instars. The duration of

the fourth stadium was minimized (to under 12 days) by a regimen

of short days during both the second and third stadia (or the first

three) with a change to long days at the time of the molt to the fourth

instar. Short days for the first three instars (plus long days during

the fifth stadium) also resulted in a uniformly short fifth stadium

(15.3 ± 2.7 and 16.2 ± 1.5 days). However, the fifth stadium was

minimized by a regimen of short days during the first two stadia

or during the first stadium only. The insects in sets 3 and 4a

(Figure 6: regimen A) were given short days for the first instar

only; they had significantly shorter average fifth stadia than those

given short days during the first three instars. The medians were

12 and 13 in sets 3 and 4a respectively; zero out of 12 and five

out of 17 insects had stadia of 13 days or less when short days were

given to instars 1-3 (regimen D).

However the few short days during the first stadium were

apparently not enough to affect all insects completely; the fifth

stadium average of 25 insects in set 4b was raised from 15.6 to

17.4 by two insects with long stadia (32 and 43 days long. The

median was 15 with or without them.) This pattern of a few to sev-

eral insects that were outliers, with much longer stadia than most

insects, was even more prevalent for fourth than for fifth stadia

among groups given short days for only the first or second stadia:

the standard deviations and ranges for regimens A and B in Figure 3

are very large. One insect in set 4b (regimen A) had a fourth stad-

ium of 246 days; seven insects in this group had a fourth stadium

of over 50 days. Note also the lack of effect of omitting short days

for the first instar compared to short days for instars 1-3 (F and

G in Figure 3). A possible explanation for this lack of sensitivity

in the first instar is that while a change to short days is easily sensed

by the insect, short days from the beginning are not perceived as

well, or the reaction to short days (without a change) is not clear

cut. It may be that the number of short days (24-hour cycles) was

not sufficient for all insects (McLeod and Beck, 1963).

A comparison of regimens A-C with D (in Figure 3) shows

that short days for the third instar gave a shorter and more uniform fourth stadium than short days for the first or second, alone or together. The standard deviations steadily became smaller and the averages got lower as the short days moved from the first only, to the second, to the third, and also as two or all three instars received short days (C, E-G) instead of only one. Table III indicates that the first three instars are of about the same duration; so the number of photoperiodic cycles was the same. This means that there is differential sensitivity to photoperiod among the first three instars, with the third being most sensitive.

The effects of short days in this insect are similar to the pattern found by Müller (1958) when he studied the delphacid Stenocranus minutus (F.). This is a naturally univoltine insect, but Müller found that the ovarial diapause was prevented by constant short days. Diapause induced by long days could be terminated by four weeks of short days, but diapause was prevented by only two weeks of short days acting at any time during the larval period except during the first stadium.

Tauber and Tauber (1970b:2075) have summarized:

When several stages sense the inducing stimulus, there may be no sharply defined period of sensitivity (Beck et al., 1963), differential sensitivity (Barker et al., 1963), or an apparent cumulative effect (Adkisson et al., 1963). . .

While there are differential and cumulative aspects to the photo-

periodic sensitivity of A. abietaria, and the ambiguity surrounding

the sensitivity of the first instar might be interpreted by some as

indicating "no sharply defined period of sensitivity," the insect is

apparently sensitive to the photoperiodic conditions at all times in

the nymphal stages. The question of whether the first instar is

actually much less sensitive to short days than the second or third

instars, or whether the great variation shown in this group in Figure 3

is due to the lack of a change to short days is an important one.

The Effect of a Change in Photoperiod

The variation in the duration of the fifth stadium in leafhoppers

raised in long days and moved to short days at various times during

the fourth and fifth stadia presents an interesting pattern. As can

be seen in Figure 7, the fifth stadium became longer as the change

from long days to short days occurred closer to the time of the fourth

molt (that is, the molt to the fifth instar); it continued to become longer

as the change occurred later in the fifth stadium. The same pattern

emerged when the insects were moved back into long days at the

time of the molt to the fifth instar: Figure 6 shows that the fifth

stadium became progressively longer as short days began progres-

sively later in the nymphal stages. Intervening periods of long days
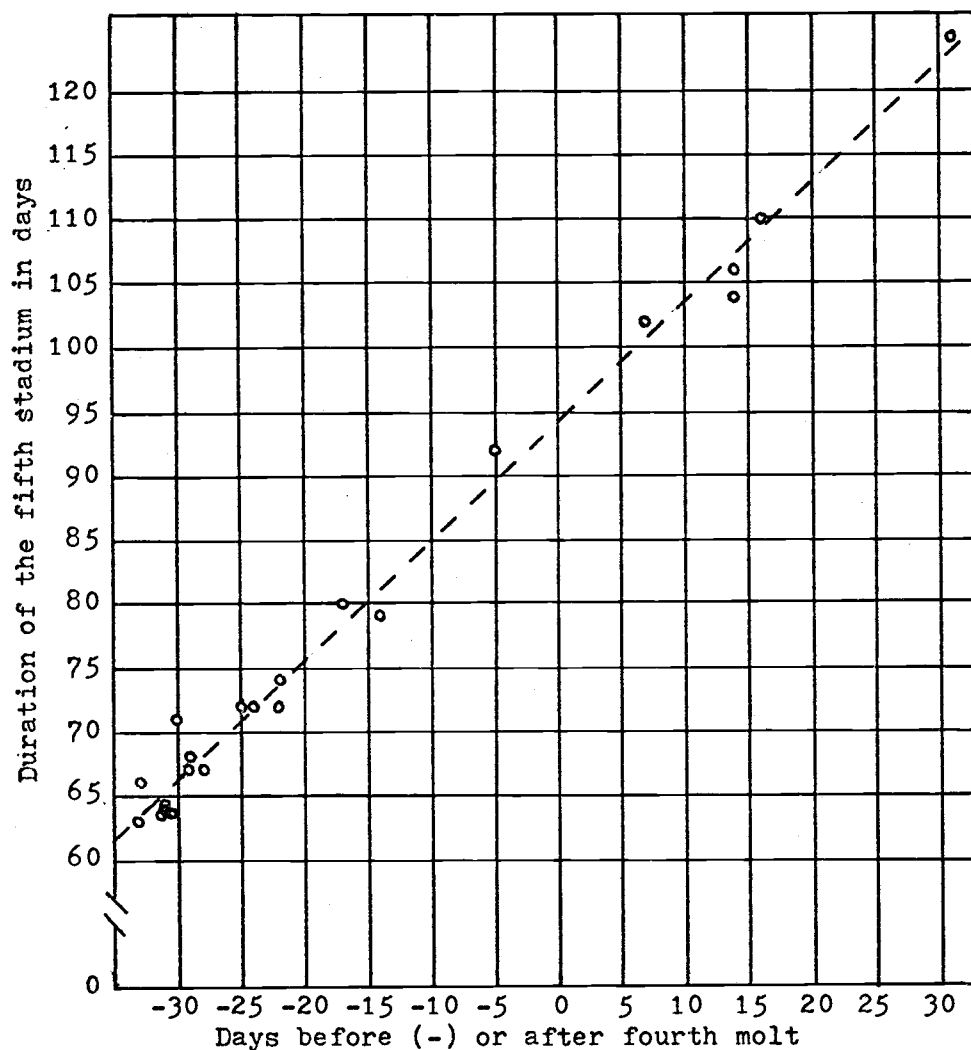
had little effect on the pattern.

Figure 7.   Duration of the fifth stadium of insects raised
under long days and moved to short days at
various times during the fourth and fifth stadia.
$Y = 0.924 X + 94.26.$ $r = 0.9951.$ $r^2 = 0.9902.$

The process controlling the termination of the fifth stadium

was evidently initiated or set to zero by the photoperiodic change,

for all insects completed the fifth stadium about 94 days after the

change to short days (Figure 7). Insects moved to short days about

30 days before the fourth molt spent about 65 days in the fifth stadium.

No insect raised under constant short days (Table III) completed the

fifth stadium in less than 77 days; all but five out of 23 took more than

100 days. A comparison of these two groups clearly indicates that

the change in photoperiods had an effect. It seems that the change

to short days started some process, which, however, was not started

at the beginning of short day treatment in insects raised in short days

from hatching (or if it was it did not proceed at the same rate). The

process was started "spontaneously" at some point in insects raised

under constant short days, for 15 out of 23 insects had completed

the fifth stadium at the end of five months. The large variance when

short days were given to the first instar only (Figure 6: regimen A,

4b; and Figure 3 A for the fourth stadium) was mentioned earlier.

This variance may reflect the fact that there was no change in photo-

periodic conditions for the nymphs to perceive. The eggs were

always kept in long days; it is possible that the change to short days

on the day of hatching was perceived. (There was a change in tem-

perature pattern as well as photoperiodic pattern.) If the eggs had

been kept in short days the variances might have been larger, or

even all insects might have had very long fifth instars.

In Figure 7 there are only five points representing insects in which the photoperiodic change occurred in the fifth stadium. In one insect the change occurred only five days before the molt: this is too short a period to imagine that the photoperiodic change caused the molt. These six points fit the curve Y = 0.878 X + 95.06, which lies very close to the curve based on all 20 data points, namely Y = 0.924 X + 94.26. The correlation coefficient for these six points, r = 0.9825. This is significant at the 0.01 level (Snedecor and Cochran, 1967:557); r^2 = 0.965.

It was previously shown with these same insects (in Figure 2) that the photoperiodic change to short days which controlled the duration of the fifth stadium also initiated the process terminating the fourth stadium: the arrest of development in this instar ended about 30 days after the change. This observation is reflected again in Figure 7 by the clump of data points in the vicinity of -25 to -35.

Discussion

From Figures 5 and 6 it can be seen that long days during the fifth stadium, or simply long days following short days, greatly reduced the duration of the fifth stadium. As in the fourth, short days at some point were necessary for an uniformly shortened fifth stadium: under constant long days the average duration of the fifth

stadium was about 36.9 days (range 13 to 80 days, n = 16), 29.9 days

(13 to 54 days, n = 8), or 16.3 days (12 to 27 days, n = 9). In each

case many of the insects that had had long fourth stadia died, or were

lost (or in the case of set 4, were terminated after completing the

fourth stadium). This may have biased the results for the fourth

and especially the fifth stadia, although there was no correlation

between duration of the fourth and fifth stadia in these groups (corre-

lation coefficients not significant at the 0.05 level). However, the

fact that a short days, then long days photoperiodic sequence resulted

in the minimal stadia for both the fourth and fifth instars indicates

that the processes may have been the same and insects that completed

the cycle once spontaneously might be more likely to do it again.

The fourth stadium can be as short as 12 days (for 28 insects,

11.9 ± 1.7 days, Figure 3: F). Anything longer than 12-13 days (or

14-15 days to allow a large margin for possible genetic differences)

must be considered to be an arrested development. Similarly, the

fifth stadium can be as short as 12 to 15 days. (The medians of sets

3, 4a and 4b are 13, 12 and 15 days respectively, Figure 3: A.)

Such low values are not attained in the field for the fifth stadium.

Values for the fourth stadium might be expected to be in the range

of 15 to 20 days for insects that were in the third instar in early

September. Such short arrests of development do not seem to be

a case of diapause so much as an artifact of there being a

physiological photoperiodic reaction in the insect. The reaction is such that a long arrest of development occurs in the fourth instar (in the laboratory) under long-day conditions. The situation is apparently analogous with "obligatory diapause": obligatory development in the fourth instar can be prevented by artificial conditions.

The question of whether "true diapause" is present in this insect should be considered. Danilevskii (1961:7) listed several characteristics of diapause, among them: abrupt lowering of metabolism; profound changes in the oxidizing enzyme system; a decrease in water volume and its transfer from a free to a colloidal state; large deposits of reserve food material; and the absence or extremely low level of feeding and movement. He noted that all these criteria need not be present in an insect said to be in diapause.

The criterion used in these experiments, the lack of molting, does indicate that development is arrested, but cannot of itself indicate what type of dormancy is involved. Leafhoppers that over-winter as fifth instars, by surviving, make it seem likely that the physiological changes of diapause do occur in that instar. Fourth-instar insects have survived seven weeks at 3°C with no mortality, and like fifth-instar insects, may exhibit little inclination to feed or even be on the plant.

Mansingh (1971) divided dormancy into quiescence (due to slight and short-term adverse variations in weather), oligopause

(due to mild, but seasonal adverse climatic changes), and diapause

(due to very adverse seasonal climatic changes). The latter two,

he said, could be either teleo-diapause (high-intensity) or ateleo-

diapause (low-intensity). Mansingh does not want to classify a

photoperiodically-controlled arrest of development as quiescence.

Photoperiod is not directly favorable or unfavorable to the insect and

is always seasonal in nature. It is a token stimulus of things to come

for the insect. It acts through complex nervous and endocrine path-

ways whether or not the diapause induced is intense or not.

The question to be answered is whether or not the short arrest

of development that occurred under some conditions is due to the

diapause mechanism (Kisimoto, 1958). Are there qualitative differ-

ences in the physiological changes accompanying long or short arrests

of development, or are there only quantitative differences? The

diapause mechanism that has evolved in an insect is one with the

proper arrest of development so that insect can survive in its

climatic area. The experiments in this section indicate that an

arrest of development of just about any duration can be obtained by

manipulation of the environmental conditions. It is reasonable to

infer that any degree of arrested development may have evolved in

nature, and therefore there is reason to expect that all dormancies

will not easily fit into any classification.

The abrupt photoperiodic changes given in the laboratory do

not occur in the field and it is possible that insects in the natural

environment react "spontaneously" to short days. However, the

circadian clock could detect changes, particularly as the critical

photoperiod was crossed (Adkisson, 1964, 1966; Saunders, 1970).

Tauber and Tauber (1970a) have shown that Chrysopa carnea reacted

to abrupt changes in daylength that did not cross the critical photo-

period. Kamm (1972) has shown that Crambus tutillus reacted to

changes of two minutes per day that did not cross the critical photo-

period. Norris (1962, 1965) found that diapause in the Red Locust,

Nomadacris septemfasciata was longer when a change from 13 to 12

hours of light per day occurred and was shorter when a change from

12 to 13 hours occurred, than was the case under constant 12 or 13-

hour daylengths. Diapause was only slightly longer under constant

12-hour days than under constant 13-hour days. Norris indicated

that the effect of one-hour changes occurred only under days of about

12-13 hours of photoperiod, which was also the daylength received

by the developing grasshopper nymphs in the field.

Bünning hypothesized (Bünning, 1936; 1958) that reaction to

photoperiod is based on circadian rhythms of sensitivity to light.

The phase relationships of these rhythms are set by light-on and

light-off signals (dawn and dusk). Bünning's theory provides a

theoretical explanation for photoperiodic control of diapause (Beck,

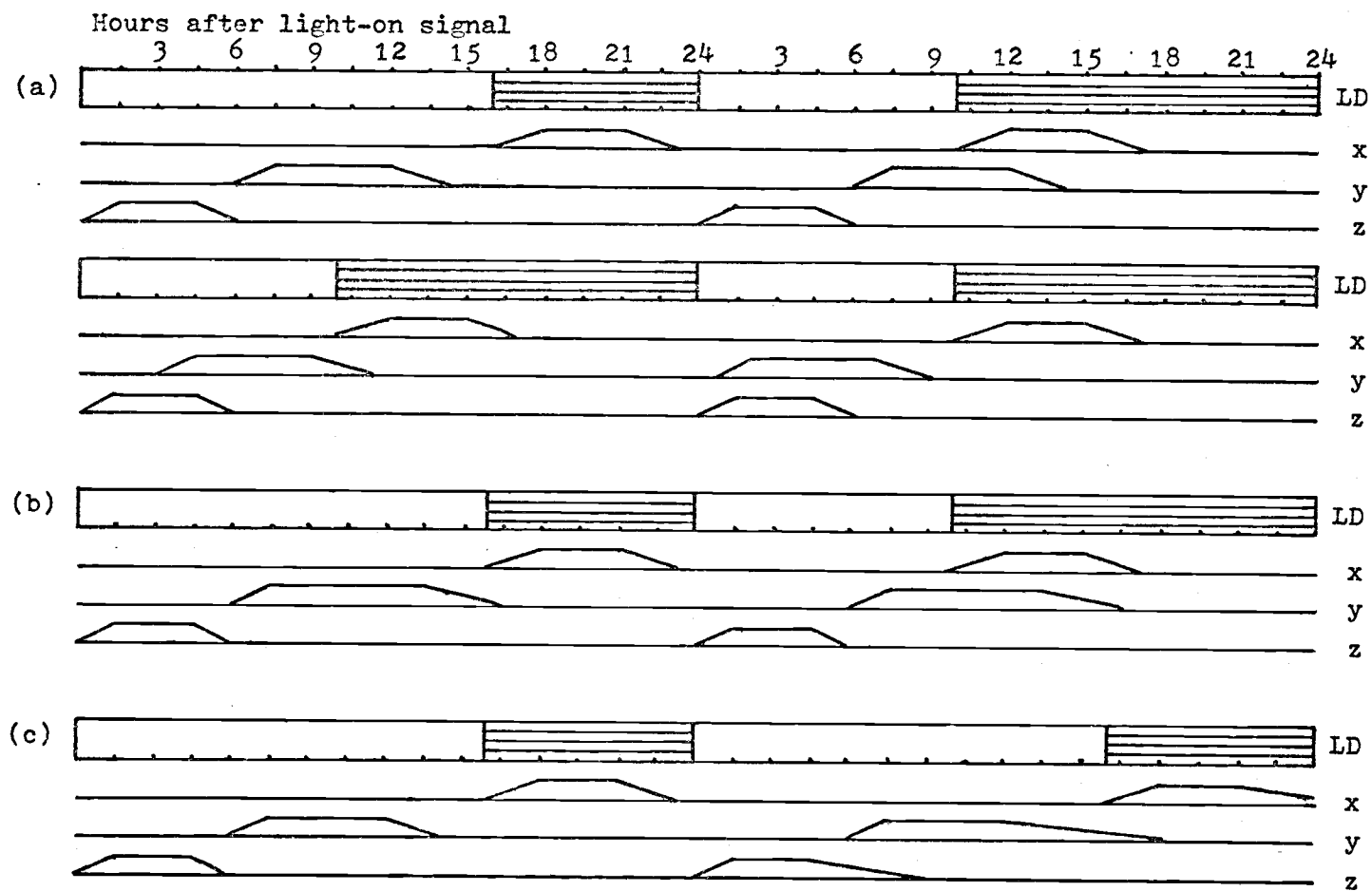1968:181; see also Pittendrigh and Minis, 1964, 1971). It is

Figure 8. Theoretical circadian basis for photoperiodic control of development. Horizontal lines on LD bars indicate darkness. The outlines on lines x, y, and z indicate titers of rhythmic physiological products. Rhythms x and y are set by light-off; rhythm z by light-on. Part (c) shows the hypothesized mode-of-action of chilling.

for small changes in photoperiodic conditions to have a (much)

greater effect than either photoperiodic regimen. Such an exact

balance is illustrated in Figure 8 (b). A small overlap in such

closely balanced rhythms could cause "spontaneous" termination

of diapause after a long period, and might occur in only some

insects.

In Agalliopsis abietaria a short days then long days sequence

is needed for continuous development. Short days, especially the

change to short days, may promote "diapause development" in the

same way that chilling of the neurosecretory cells of the brain of

some insects does (Highnam and Hill, 1969). Chilling may act by

slowing the rapid breakdown of some product necessary for develop-

ment. If this product is produced rhythmically, the slowing of its

decomposition may permit interaction with the product of an out-of-

phase rhythm. This hypothesized effect of chilling is diagrammed

in Figure 8 (c). Chilling and photoperiod may act in the same insect

(Wellso and Adkisson, 1966; Mansingh and Smallman, 1970; Saunders,

1967), so the mechanism must interact or be the same at some point.

Chilling frequently takes long periods of time to effect diapause

development (Morris and Fulton, 1970). Perhaps this is because,

while the product of the physiological rhythm is slow to decompose,

it is also slow to interact at low temperatures. A. abietaria was

chilled in the fourth stadium in the experiments reported here,

instead of in the fifth. This may have had an effect even in the fourth if it had been continued longer.

The termination of diapause is brought about by physiological changes in which the procoracotropic hormone and ecdysone play important roles. In an insect such as this leafhopper, which needs a short days then long days photoperiodic sequence, it may be that short days promote development by affecting a center involved chronologically early in the developmental cycle. Short days may promote activity of the neurosecretory cells of the brain, while long days may be more closely tied to the secretion or release of ecdysone. The answer, of course, is more complicated than this (for the photoperiodic effects of short days, at least) because there may be intervening molts between the reception of short days and their developmental effect.

It is possible that, in the absence of a signal in the form of a photoperiodic change, the insects would take a cue from some other environmental fluctuation. No irregularity in temperature or photoperiod occurred in the long-day cabinet during the time that set 4 of the constant long-day groups (Figure 5: K) was being run which might explain the relatively short and uniform fifth stadia in this group.

In Agalliopsis abietaria it is long days rather than (or in addition to) the rise in temperature in the spring that causes the termination of diapause and the resumption of active development. It is

likely that the photoperiodic response occurs only within a limited temperature range, but the photoperiod is the main environmental signal for the resumption of development. Photoperiodic control of development in this leafhopper appears to be of the same sort as in Dasychira pudibunda and Crambus tutillus mentioned in the introduction: there is a mechanism to insure a monocyclic life cycle. If the fourth molting cannot occur until the short days of autumn, then the fifth instar will not encounter long (warm) days until the coming of spring. If the fourth instar was completed under long days, the termination of the fifth stadium could not be controlled by photoperiod--and the insect would be multivoltine.

SUMMARY AND CONCLUSIONS

1. No effect of temperature on diapause was shown in the experiments reported here.

2. The leafhopper exhibited an arrest of development in the fourth stadium under long days.

3. Under short days the fourth stadium was short, averaging less than 16 days. The fourth-instar arrest of development was broken by short days.

4. The fourth stadium could not last much more than a month in the field because it occurs during the shortening days of the autumn. It was suggested that the fourth-instar arrest of development is a mechanism to ensure univoltinism.

5. The fifth instar is the overwintering stage. Fifth-instar diapause was broken by long days; the adults appeared an average of 18 days after the change from short days to long days under laboratory conditions.

6. All larval instars were sensitive to photoperiod. Of the first three instars, the third was most sensitive to photoperiod.

7. The first instar was least sensitive to photoperiod. It was suggested that this result occurred because the first instar did not perceive a <u>change</u> from short days to long days.

8. The data showed that the <u>change</u> from long days to short days

had an important effect. Insects which were moved to short days exhibited a duration of the fifth stadium that was related to the timing of the change to short days. Insects kept in short days from hatching did not show this effect.

9. The fourth stadium was minimized (to 12 days) by a light regimen of short days during the second and third instars (or the first three) and long days after that.

10. The fifth stadium was minimized by a regimen of short days for the first instar only. The fifth stadium was not significantly longer when short days were for the first two instars. The fifth stadium was significantly longer when short days began at the time of the molt to the second instar rather than at hatching.

11. A circadian-rhythm basis for the photoperiodic control of development in this leafhopper is hypothesized.

BIBLIOGRAPHY

Adkisson, P. L. 1964. Action of photoperiod in controlling insect diapause. The American Naturalist 98:357-374.

Adkisson, P. L. 1966. Internal clocks and insect diapause. Science 154:234-241.

Adkisson, P. L., R. A. Bell and S. G. Wellso. 1963. Environmental factors controlling the induction of diapause in the pink bollworm Pectinophora gossypiella (Saunders). Journal of Insect Physiology 9:299-310.

Andrewartha, H. G. 1952. Diapause in relation to the ecology of insects. Biological Review 27:50-107.

Barker, R. J., A. Mayer and C. F. Cohen. 1963. Photoperiod effects in Pieris rapae. Annals of the Entomological Society of America 56:292-294.

Bates, E. M. and W. Calhoun, Jr. 1970 Local climatological data for Oregon State University 1968, with normals, means and extremes. Special Report 277, revised July 1970.

Beck, S. D. 1964. Time-measurement in insect photoperiodism. The American Naturalist 98:329-346.

Beck, S. D. 1968. Insect photoperiodism. New York, Academic Press. 288 p.

Beck, S. D. and N. Alexander. 1964. Proctodone: an insect developmental hormone. Biological Bulletin 126:185-198.

Beck, S. D. and J. W. Apple. 1961. Effect of temperature and photoperiod on voltinism of geographical populations of the European corn borer Pyrausta nubilalis (Hbn.). Journal of Economic Entomology 54:550-558.

Beck, S. D., E. J. Cloutier and D. G. R. McLeod. 1963. Photoperiod and insect development. In: Insect physiology: Proceedings of the Twenty-third Annual Biology Colloquium, Corvallis, Oregon, 1962, ed. by V. J. Brookes. Corvallis, Oregon State University Press. p. 43-64.

Black, L. M. 1944. Some viruses transmitted by Agallian leaf-hoppers. Proceedings of the American Philosophical Society 88:132-144.

Bünning, E. 1936. Die endogene Tagesrhythmik als Grundlage der photoperiodischen Reaktion. Berichte der Deutsche Botanische Gesellschaft 54:590-607.

Bünning, E. 1958. The Physiological Clock. 1st English ed., 1964. 167 p.

Chapman, R. F. 1969. The insects, structure and function. New York, American Elsevier. 819 p.

Clark, L. R., P. W. Geier, R. D. Hughes and R. F. Morris. 1967. The ecology of insect populations in theory and practice. London, Methuen. 227 p.

Corbet, P. S. 1956. Environmental factors influencing the induction and termination of diapause in the emperor dragonfly, Anax imperator Leach. Journal of Experimental Biology 33: 1-14.

Danilevskii, A. S. 1961. Photoperiod and seasonal development of insects, 1965 tr. by J. Johnston. Edinburgh, Oliver and Boyd. 283 p.

de Loof, A. 1972. Diapause phenomena in non-diapausing last instar larvae, pupae, and pharate adults of the Colorado beetle. Journal of Insect Physiology 18:1039-1047.

Dickson, R. C. 1949. Factors governing the induction of diapause in the oriental fruit moth. Annals of the Entomological Society of America 42:511-537.

Geyspitz, K. F. 1965. Photoperiodic and temperature reactions affecting the seasonal development of the pine moths Dendrolimus pini and D. sibricus Tschetw. Entomological Review 44:316-325.

Highnam, K. C. and L. Hill. 1969. The comparative endocrinology of the invertebrates. New York, American Elsevier. 270 p.

Kamm, J. A. 1971. Environmental biology of a sod webworm Crambus tutillus (Lepidoptera, crambidae). Entomologia Experimentalis et Applicata 14:30-38.

Kamm, J. A. 1972. Photoperiodic regulation of growth in an insect: response to progressive changes in daylength. Journal of Insect Physiology 18:1745-1749.

Kamm, J. A. and K. G. Swenson. 1972. Termination of diapause in Draeculacephala crassicornis with synthetic juvenile hormone. Journal of Economic Entomology 65:364-367.

Kisimoto, R. 1958. Studies of the diapause in the planthoppers: effects of photoperiod on the induction and the completion of diapause in the fourth larval stage of the small brown plant-hopper, Delphacodes striatella Fallen. Japanese Journal of Applied Entomology and Zoology 2:128-134.

Kisimoto, R. 1959. Studies on the diapause in the planthoppers and leafhoppers (homoptera). II. arrest of development in the fourth and fifth larval stage induced by short photoperiod in the green rice leafhopper, Nephotettix bipunctatus cincticeps Uhler. Japanese Journal of Applied Entomology and Zoology 3:49-55.

Krunic, M. D. and C. F. Hinks. 1972. The effect of temperature and of temperature pretreatment on diapause and on the synchronization of adult emergence in Megachile rotundata (hymenoptera: megachilidae). Canadian Entomologist 104:889-893.

Mansingh, A. 1971. Physiological classifications of dormancies in insects. Canadian Entomologist 103:983-1009.

Mansingh, A. and B. N. Smallman. 1967. Effect of photoperiod on the incidence and physiology of diapause in two saturnids. Journal of Insect Physiology 13:1147-1162.

Mansingh, A. and B. N. Smallman. 1971. The influence of temperature on the photoperiodic regulation of diapause in saturniids. Journal of Insect Physiology 17:1735-1739.

Maramorosch, K. 1970. Insect infection caused by a plant tumor virus. World Review of Pest Control 9:29-41.

McClanahan, R. J. and J. Founk. 1972. Control of the European corn borer (lepidoptera: pyralidae) on sweet corn and peppers in southwestern Ontario. Canadian Entomologist 104:1573-1579.

McLeod, D. G. R. and S. D. Beck. 1963. Photoperiodic termination of diapause in an insect. Biological Bulletin 124:84-96.

Morris, R. F. and W. C. Fulton. 1970. Heritability of diapause intensity in Hyphantria cunea and correlated fitness responses. Canadian Entomologist 102:927-938.

Müller, H. J. 1958. Uber den einfluss der photoperiod auf diapause und körpergrosse der Delphacide Stenocranus minutus Fabr. Zoologischer Anzeiger 160:294-312.

Norris, M. J. 1962. Diapause induced by photoperiod in a tropical locust, Nomadacris septemfasciata (Serv.). Annals of Applied Biology 50:600-603.

Norris, M. J. 1965. The influence of constant and changing photoperiods on imaginal diapause in the red locust (Nomadacris septemfasciata Serv.).

Ohtaki, T., R. D. Milkman and C. M. Williams. 1968. Dynamics of ecdysone secretion and action in the flesh fly Sarcophaga peregrina. Biological Bulletin 135:322-334.

Oman, P. 1970. Leafhoppers of the Agalliopsis novella complex (homoptera: cicadellidae). Proceedings of the Entomological Society of Washington 72:1-29.

Peterson, D. M. and W. M. Hamner. 1968. Photoperiodic control of diapause in the codling moth. Journal of Insect Physiology 14:519-528.

Pittendrigh, C. S. and D. H. Minis. 1964. The entrainment of circadian oscillations by light and their role as photoperiodic clocks. The American Naturalist 98:261-294.

Pittendrigh, C. S. and D. H. Minis. 1971. The photoperiodic time measurement in Pectinophora gossypiella, and its relation to the circadian system in that species. In: Biochronometry, ed. by Michael Menaker, Washington, D. C., National Academy of Sciences. p. 212-250.

Saunders, D. S. 1966. Larval diapause of maternal origin--II. The effect of photoperiod and temperature on Nasonia vitripennis. Journal of Insect Physiology 12:569-581.

Saunders, D. S. 1967. Time measurement in insect photoperiodism: reversal of a photoperiodic effect by chilling. Science 156:1126-1127.

Saunders, D. S. 1970. Circadian clock in insect photoperiodism. Science 168:601-603.

Saunders, D. S. 1971. The temperature-compensated photoperiodic clock 'programming' development and pupal diapause in the flesh fly, Sarcophaga argyrostoma. Journal of Insect Physiology 17:801-812.

Sekeris, C. E., P. Karlson and L. F. Congote. 1971. Molecular action of ecdysone. In: The action of hormones, ed. by P. P. Foà, Springfield, Ill., Charles C. Thomas. p. 7-19.

Snedecor, G. W. and W. G. Cochran. 1967. Statistical Methods. 6th ed. Ames, Iowa, Iowa State University Press. 593 p.

Tauber, M. J. and C. A. Tauber. 1970a. Photoperiodic induction and termination of diapause in an insect: response to changing day lengths. Science 167:170.

Tauber, M. J. and C. A. Tauber. 1970b. Adult diapause in Chrysopa carnea: stages sensitive to photoperiodic induction. Journal of Insect Physiology 16:2075-2080.

Tauber, M. J. and C. A. Tauber. 1972. Larval diapause in Chrysopa nigricornis: sensitive stages, critical photoperiod, and termination (Neuroptera: chrysopidae). Entomologia Experimentalis et Applicata 15:105-111.

Tauber, W. J., C. A. Tauber and C. J. Denys. 1970. Diapause in Chrysopa carnea (Neuroptera: chrysopidae) II. Maintenance by photoperiod. Canadian Entomologist 102:474-478.

Wellso, S. G. and P. L. Adkisson. 1966. A long-day short-day effect in the photoperiodic control of the pupal diapause of the bollworm Heliothis zea (Boddie). Journal of Insect Physiology 12:1455-1465.

Zdarek, J. and G. Fraenkel. 1970. Overt and covert effects of endogenous and exogenous ecdysone in puparium formation of flies. Proceedings of the National Academy of Science 67: 331-337.