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David John. Reeleder

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ASPECTS OF THE PHOTOPERIODIC RESPONSE OF THE
PEA APHID ACRYSIIPHON PISUM (HARRIS)

BY

DAVID JOHN REELEDER

A Thesis
Submitted to the Faculty of Graduate Studies through the
Department of Biology in Partial Fulfillment
of the Requirements for the Degree of
Master of Science at the
University of Windsor

Windsor, Ontario, Canada
1978
To my parents
ABSTRACT

The critical photoperiods for sexual females of the pea aphid, *Acythosiphon pisum* (Harris), were determined to be 13:06L and 11:49L at 15°C and 20°C, respectively. Experiments within the transition zone supported the notion of this being a variable response region. The ecological significance of this variability is discussed. As predicted from Danilevskii's hypothesis, the critical photoperiod at 20°C for a more southerly located Harrow clone (lat. 42° 02') is less than that of a more northerly located Markham clone (lat. 43° 25'). The sensitive period findings indicate that a different sensitive region exists for both sexual morphs; the sensitive region for the male morph beginning a few days prior to parental birth and ending near parental birth, while that for sexual females beginning about the same time but extending 4 or 5 days after parental birth. The physiological and ecological import of this is discussed. Unfortunately, the fluctuating temperature experiments were contradictory but suggest the possibility that the insect is sensitive to a complex, non-linear combination of night and day temperatures. However, reasonably good predictions of both sexual morphs were obtained through the computer simulation studies when minimum temperatures were assumed to be most important. The effects of differing light intensities on the sexual female morph response were simulated by including or excluding civil
twilight in daylength calculations. Including civil twilight over-estimated the empirically derived sampling date by less than a week and excluding it led to an under-estimation.

The photoperiodic response patterns of the alatae showed that this morph produces few or no male offspring when subjected to photoperiodic treatments capable of inducing male production in their apterous counterparts. However, differences in sexual female production in the two morphs was found to be minimal. This may be a mechanism for this presumed colonizer to maximize population growth within the host field.
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GENERAL INTRODUCTION

Most insects in the mid-latitudes live in an environment which would be lethal unless the insects possessed specific physiological adaptations for seasonal changes. To buffer themselves against harsh conditions such as extreme cold or aridness, insects have evolved a protective mechanism known as diapause, a state of arrested development occurring in a particular physiological stage (Beck, 1968). Environmental information is used by many insects to anticipate the arrival or departure of these harsh conditions. Daylength, interacting with a variety of other factors, has been shown to provide seasonal cues for a large number of insect species (Beck, 1968; Danilevskii, 1965; Tauber and Tauber, 1976). Insects probably rely on daylength because it is the single most reliable indicator of the changing seasons (Danilevskii, 1965).

The mid-point of daylengths at which an often sharp transition from low to high incidence of diapause occurs is referred to as the critical daylength. In the laboratory, the effects of daylength have usually been studied by exposing insects to a light and dark period with a sharp transition between them. The assumption has been that such a stimulus simplifies but retains the essential features of the natural diurnal cycle of night and day. However, to
recognize the difference between laboratory conditions and the slow natural change through dawn and dusk, the phrase, 'critical photoperiod', is usually used to refer to laboratory treatments. This concept has been used most frequently in studies of the initiation of diapause, but Lees (1959) has also used it in reference to a seasonal process, sexual morph determination, of the aphid, *Megoura viciae*. He defined critical photoperiod to be that photoperiod at which 50 per cent of the aphids produced sexuals and 50 per cent produced asexuals.

The photoperiodic responses of insects may be grouped into four categories (Beck, 1968). Type I refers to those species which enter into diapause in response to short daylengths. This type is characteristic of insects in northern latitudes responding to the shortening of autumn days. The Type I response that initiates an autumn diapause is analogous to one shown by pea aphids, the subject of the present study. The Type II response is exactly the converse of Type I. The insect is sensitized to longer photoperiods and enters an aestival or summer diapause which is usually an adaptation to unusually hot or arid conditions. Some examples include the commercial silkworm, *Bombyx mori* (Kogure, 1933); the geometrid *Abraxis miranda* (Masaki, 1956); and strains of the cabbage noctuid *Namestra brassicae* (Masaki and Sakai, 1965).

The other two types of response, the Type III and IV are rarer and characterized by two well-defined critical daylengths. For the Type III at very short daylengths no
diapause is observed. Complete diapause is observed at
daylengths from 10 to 14 hours. Daylengths of 16 or more
hours are also not diapause inducing. The mid-points of
the transition zones, representing the critical daylengths,
are approximately 9 and 15 hours respectively. Daylengths
shorter than about 8 hours represent conditions that are
never encountered by insects in their natural habitats
during the growing season hence it is difficult to give an
adaptive significance to them. The longer critical daylength,
however, is similar in effect to the Type I conditions with
the insect entering into diapause in response to the shortening
of the days, as observed in the autumn. An example of this
type would be the species, *Ostrinia nubilalis* (Beck, 1962a).
The Type IV response has a diapause induction curve exactly
the converse of Type III. It is characterized by an absence
of diapause over a restricted range of relatively long
daylengths, with critical daylengths at approximately 15 and
21 hours. All other photoperiodic conditions result in a
high incidence of diapause. Again, the adaptive significance
of this response is not clear. An example of this final
category is the species, *Carposina niponesis* (Toshima et al.,
1961). All four types of photoperiodic responses have been
studied using standardized experimental procedures at
stationary photoperiods and relatively constant temperatures.

Danilevskii (1965) pointed out the ecological
importance of the relationship between daylength and the
environment for an insect species. Observations by Danilevskii
on several species of moths in the Soviet Union, showed that
within a given species there were differences in critical photoperiods for populations from different latitudes. For example, populations of the noctuid *Acronycta rumicis* from the Leningrad area (60°N) have a critical photoperiod of 19 hours while Sukhumi populations (43°N) have critical photoperiods of only 15 hours. The differences are too large to be accounted for merely by the natural daylength differences within the diapause-induction period in late summer at the two locations. Rather, the explanation for the longer critical photoperiod in the northern population is that the northern races are subjected to a colder environment sooner than the southern and hence cued to longer photoperiod to avoid the risk of diapausing too late. Experimental evidence for this notion is widespread (Bradshaw, 1976; Danilevskii, 1965; Masaki, 1965; Tauber and Tauber, 1973).

The shift in critical photoperiod with latitude which presumably reflects genetic differences between populations is thought to represent adaptation to colder climates, and should not be confused with the more immediate effect of temperature on critical photoperiod. While working with the aphid, *Megoura viciae*, Lees (1963) noted that raising the temperature resulted in a shortened critical photoperiod for the production of the female morph. Since the shift was small, Lees argued that it merely reflected incomplete temperature compensation of the photoperiodic mechanism. He believed that temperature compensation is necessary in a poikilothermic organism and that the photoperiodic mechanism is basically
temperature independent. However, this generalization does not seem to be valid for the pea aphid, *Acyrthosiphon pisum*. Lamb and Pointing (1972) have shown a strong inverse relationship between critical photoperiod and temperature in this aphid. It appears that this insect responds to both photoperiod and temperature cues. The nature of the interaction suggests that the inclusion of a temperature response increases the precision of timing of the initiation of sexual morph production in the field during autumn. On this basis then, it may be hypothesized that *Acyrthosiphon pisum* is more evolutionarily advanced than *Megoura viciae*. This corroborates earlier speculation by Lamb and Pointing (1972).

The specific stage in the life history of a species in which the insect perceives diapause-inducing stimuli is termed the 'sensitive period' (Tauber and Tauber; 1976). In some species, the sensitive stages and the diapausing stage are widely separated within the same generation or may even be found in different generations (Danilevskii, 1965). For example, in the Chinese silkworm *Bombyx mori* the sensitive stage occurs in the embryo of the maternal generation, while it is the egg of the next generation that diapauses (Beck, 1968).

In nature, an insect is exposed to a diurnal regime of temperatures. Little attention has been directed towards the effect of such fluctuating temperatures on the response to photoperiod. One exception is Beck's (1962a, b) study of the European corn borer, *Ostrinia nubilalis*. His results
are clear showing that, for this insect at least, it is the length of the scotophase under the cooler night temperatures that determines the onset of diapause. Scotophase is a term widely used in the photoperiodic literature to refer to the dark portion of the photoperiod, where photoperiod represents the laboratory simulated sequence of day and night usually characterized by the length of the photophase, or light period.

While the aim of the research on photoperiodism and seasonality has been to understand seasonal processes in nature, studies of the effects of the photoperiod and temperature on the ecology of overwintering have nearly all been undertaken in the laboratory. Rarely has even the change in occurrence of diapausing forms over the seasons been precisely documented. Laboratory studies have been emphasized because of the difficulties in manipulating light regimes in nature. To simplify experiments, unrealistic light regimes and constant temperatures have been used in contrast to the complex light and temperature stimuli that insects actually experience in nature. There are two approaches that could be used to test whether hypotheses developed to account for laboratory results are adequate for understanding the complexities of the processes in nature. Experiments could be reproduced under field conditions by carefully selecting the times of year when the appropriate daylengths and temperatures occur. However, such experiments are risky since any particular daylength in mid-latitudes occurs only twice a year and then the experiments are subject
to the vagaries of the weather which may cause unsuitable temperature regimes to coincide with the pre-selected day-lengths. The second approach involves the use of computer simulation methods. A computer model can be constructed that links the various laboratory results and hypotheses in such a way that predictions concerning the timing of seasonal patterns in nature can be made. Even if the predictions prove inaccurate, this approach can be useful since it may point to deficiencies in the laboratory results and help reveal areas that have been overlooked in the laboratory studies.

The 'biological clock' upon which the environmental cues act in the pea aphid controls the timing of sexual morph determination, and has many similarities with the timing mechanisms responsible for diapause formation in other insects (Beck, 1968). The aphid reproduces parthenogenetically and viviparously all summer. In the fall, a single sexual generation produces overwintering eggs. Ease of rearing and fast generation time make aphids, more specifically A. pisum, excellent research models to study photoperiodic responses. Lamb and Pointing (1972) showed that this species has two distinct critical photoperiods, one for male production, and one controlling the production of sexual females. At 20°C the critical photoperiod for male production was found to be 90 minutes longer than that for the oviparous morph. Unlike the species Megoura viciae (Lees, 1963), the male morph always follows the sexual female morph within a given apterous family (See Appendices I, II).
Various physiological and ecological implications of the photoperiodic response of the pea aphid still remain to be studied. The sensitive period is not known for either male production or ovipara production. The effects of fluctuating temperatures on the photoperiodic response have not been studied. For example, there is no indication whether it is night or day temperatures or a combination which interact with photoperiod to induce sexual production.

The transition zone which contains the critical photoperiod of photoperiodic response curves also warrant further examination. The transition zone has rarely been partitioned into small increments of time, and one aim of the current photoperiod experiments is to attempt this. Most studies on other species have used increments of 30 minutes to identify this zone. Bradshaw (1976) reported an $R^2$ of 0.98 when regression critical photoperiod on latitude and altitude using an experimental photoperiod increment of 30 minutes. He had implicitly assumed that he had pinpointed the critical photoperiods. However, studies on the pea aphid (Lamb and Pointing, 1972) have suggested that at least in this species, a 'region of instability' exists within the transition zone. Smaller, more precise increments of photoperiod would permit the shape of this zone to be determined so that precise estimates of the critical photoperiod could be made.

Nearly all our knowledge on the photoperiodic response of *A. pismum* results from research on a single possibly atypical clone. Study of the photoperiodic response on another clone drawn from a different population was warranted to determine
the generality of previous studies.

The aim of the research outlined below was the description of sexual morph production, a seasonal process of the pea aphid, *Acyrthosiphon pismum* (Harris). The process was first studied by Kenton (1955) who emphasized the role of temperature. Further work was done by Sharma et al. (1972, 1973, 1974) and Lamb and Pointing (1972, 1975), particularly on the photoperiodic responses and the relation between male production and sexual female production. These studies were all conducted in the laboratory. The emphasis of the present work was to extend our knowledge through laboratory studies of the sensitive period and the effects of fluctuating temperature on the photoperiodic response. A second goal was to relate the laboratory findings to the process of sexual morph determination as it occurs in nature and clarify the importance of the interaction between daylength and temperature.

While the primary aim was to investigate the photoperiodic response of the apterous or non-winged morph of the pea aphid, the response of the alatae was also studied. It is generally conceded that the alatae of this aphid are produced to ensure survival of the species by allowing migration away from unsatisfactory local environments (Sutherland, 1969a, b). This morph is the colonist: presumably a population of these alatae carry genetic information from different geographical locations; offspring of the colonizers may then mate with local residents producing the latitudinal gradation of photoperiodic responses noted by Danilevskii (1965).
Alary morph determination is regulated at various times by several different environmental cues, including photoperiod, temperature, host plant and population density (Lees, 1966). Intrinsic factors have also been implicated. Besides clonal variability, MacKay and Wellington (1977) have discovered a maternal effect whereby the age of the asexual parent influences the proportion of alate progeny she can produce. A significant difference in the photoperiodic response between winged and non-winged morphs would provide yet another criteria for distinguishing their ecological roles. Physiologically, it has been concluded that the mechanism responsible for sexual morph determination in aphids is independent of that controlling the presence of wings (Lees; 1966), and there was no a priori physiological reason for assuming the morphs would respond differently to photoperiod. The photoperiodic responses of alatae are described separately in Chapter III.
CHAPTER I

Aspects of the Photoperiodic Response

of the Apterous Morph
CHAPTER I

METHODS AND MATERIALS

Field Temperature Studies

A Taylor maximum-minimum thermometer (accuracy, ±1°C) was calibrated in the laboratory with a YSI series 400 electronic thermometer (accuracy, ±0.5°C) and then placed in the canopy of an alfalfa field (Medicago sativa L.) 10 metres from a weather station operated on the property of the Agriculture Canada Research Station, (lat. 42°02'; long. 82°53'). On various days in early autumn from September 8 to September 15, 1977, readings from the thermometer were taken. As the field was ploughed under September 15, a new alfalfa field (137 m x 66 m) was selected for study. It was located only 250 m from the first one. Another maximum-minimum thermometer was calibrated and placed in the new field. Recordings were taken intermittently until October 2. Stevenson screen temperature data and daily hours of sunshine were kindly supplied by the Agriculture Canada Research Station.

To study spatial variation in temperature in the habitat of the aphid, temperatures were recorded at various locations in the field using a YSI series 400 thermometer. The maximum-minimum thermometer provided a fixed-location...
reference temperature. Temperatures were recorded on two cloudy and two sunny days between 0930 and 1500 hours when temperature variation was expected to be highest. Transects and points along each transect were selected using a table of random numbers. For several days in early September temperature readings at randomly selected locations were made at the bottom, middle and top of alfalfa stems. Three leads from the thermometer permitted almost simultaneous temperature records to be obtained at the three levels. Identical studies were also carried on in the new field with simultaneous temperature readings taken using a YSI series 400 thermometer and the maximum-minimum thermometer as a control.

Field Sampling

From September 23 to October 31, 1977, the alfalfa field was sampled 9 times with a cloth sweep-net. Sweeping was done in the cooler morning hours to minimize the loss of aphids which tend to drop from the plants when disturbed. Owing to the nature of the method, only upper portions of the alfalfa plants were sampled. Starting from arbitrary points at the ends of the field, rows were swept in a straight line taking care not to re-sweep a section. Between 15-20 sweeps were adequate to secure a sufficient sample to determine morph frequencies. Assuming aphids are distributed at random between plants according to a Poisson distribution, a mean count of \( N \) aphids/plant would give a standard error of \( \sqrt{N} \). Samples were
more than large enough to give a 10 per cent standard error as recommended by Southwood (1966).

Once collected, late instar and adult pea aphids were transferred into vials and brought to the laboratory where they were immersed in a 50 per cent ethanol-water solution to kill them for counting. The larvae were discarded and the adult asexual females and sexuals distinguished and counted using a Wild Model 8 zoom stereomicroscope. Adult males and females are readily distinguishable without the aid of a microscope. Males are distinctly smaller than the females with less rotund abdomens and darker pigmentation. Adult virginoparae contain a large number of embryos with red eyes which are often visible through the mothers' abdominal wall. Sexual females are more brightly coloured and contain bright green eggs that can often be seen through the aphid's abdomen. However, it was necessary to rupture this area with a dissecting instrument to distinguish eggs from embryos and identify morphs with certainty.

Early in the autumn of 1977, several aphids were collected from the Harrow alfalfa field and brought to the laboratory where clones were established and reared for experimental purposes.

**Experimental Studies**

**General**

From the Harrow clones, one was chosen which appeared to be the most fecund and produced the lowest number of winged offspring. From this clone the stock culture was
produced and maintained. The aphids were reared on individual, cut leaves of broad bean, *Vicia faba* L., in small petri dishes (15 x 60 mm.) lined with a double layer of paper towelling which was kept moist with a modified Hoagland's nutrient solution. Insects were transferred from petri dishes with a fine moistened paintbrush.

The broad bean plants were grown in a mixture (1:1) of perlite and vermiculite and watered with the same solution. Plants were grown at room temperature (≈20°C) under Agro Lite fluorescent tubes (Westinghouse) at a photoperiod of 16L: 8D. The aphid stock culture was kept at 20±5°C in environmental chambers under cool-white 20 watt fluorescent tubes (General Electric) at 16L: 8D. This assured asexual reproduction. The temperatures of the environmental chambers were monitored twice daily with a YSI series 400 thermometer and adjusted when any discrepancy from the desired temperature was noted. Maximum-minimum thermometers were placed in the control chamber and the two experimental environmental chambers to trace any possible extreme night temperatures. For the early part of the experiments the electric timers built into the chambers were used to control photoperiod. Exact photoperiod exposures were measured with the aid of an outside electronic clock calibrated against the Canadian National Research Council Official Time Signal. Owing to the added flexibility gained from doing more simultaneous photoperiod experiments cookie tins were used and placed in continuously lighted environmental chambers. A temperature probe was inserted into a petri dish
located inside an empty tin to monitor temperatures and
detect any 'green-house effect'. A petri dish in an open
tin was found to have \(\approx 1{\degree}C\) higher temperature than in a closed
one, hence the temperatures within the chambers were modified
to compensate for whether the lid was in the 'on' or off
position thus maintaining a constant temperature in the petri
dish.

Three lines were set up in the stock culture to provide
the number of insects required for the experimental regimes.
Care was taken in the formation of each line only to use the
first-born and discard the rest of the larvae. This
precaution reduced possible photoperiodic response variability
due to maternal age effects (MacKay and Wellington, 1977).
The birth of the first aphid in each line was dated to ensure
a fix on the ages of the offspring. Hence when insects were
transferred from control to experimental conditions the ages
of the offspring were known and the sensitive period could
be studied. To minimize alatae formation and ensure healthy
growing animals, leaves were replaced periodically and the
insects grown singly on a leaf.

**Sexual Female Critical Photoperiod Studies**

Three generations of aphids were involved in each
experiment. Following the nomenclature of Lees (1959), the
members of these generations will be termed, respectively
the 'grandparents', the 'parents', and the 'offspring'. The
term 'family' will be applied to the offspring of one parent.

Aphid larvae (grandparents) were taken from the stock
culture and placed under experimental photoperiod-temperature conditions when they were approximately 3 days of age (at $20^\circ C$). When the parents began giving birth the experimental regime was ended. The parents were then transferred to a new leaf in a petri dish and the batches of offspring followed daily for a variable time into the reproductive period. Between 14-20 parents were used in an experiment. Only apterous parents were used in the calculation of female critical photoperiods. Critical photoperiods were determined both at $15^\circ C$ and $20^\circ C$.

The duration of exposure to an experimental photoperiod at $15^\circ C$ averaged about 26 days for each experiment. However, the time from the start of an experiment to the identification and counting of offspring lasted about 32 days. The duration of each exposure at $20^\circ C$ was about 15 days while the actual time from initiation to termination of each experiment was approximately 20 days. The identification of the critical photoperiods at $15^\circ C$ and $20^\circ C$ could only be achieved after several photoperiod experiments had been carried out at the respective temperatures.

**Developmental Rates**

Developmental rates were determined for asexual females, sexual females and males at $15^\circ C$ and $20^\circ C$. Rates of development for asexuals were also measured at $23.5^\circ C$. Asexuals were produced from a line of the stock culture. Within hours after an aphid had begun parturition in one of
the selected lines it was transferred to a single leaf and allowed to reproduce for up to 12 hours. At this time, between 1-8 first-born larvae were on the leaf. The adult was then discarded and the progeny placed in the experimental chambers. Times of introduction into stated temperatures were noted. After a few days the larvae were then transferred to separate leaves. Care was taken to choose leaves of highest quality from near the tops of the broad bean plants. Leaves were changed at various times throughout each experiment to ensure leaf quality was not interfering with rates of growth. It was noted (± 12 hours) when the asexuals moulted to the adult stage. The time from the adult moult to the onset of reproduction was also measured. Temperature was monitored at various intervals in the day to ensure it was constant (± 0.5°C).

Similar data was collected for sexual females and males except measurement ended when the respective sexuals moulted into adults. The sexuals were generated in the photoperiod experiments. All females produced in short photoperiods (i.e. 11:00L at 20°C) are sexual. As soon as the parents from this regime began reproducing they were transferred on to separate leaves. Progeny produced within the first 12 hours were then placed into the desired temperature. At short photoperiods males are always produced following a group of female offspring and a pause in the reproductive period when no offspring are born (Lamb and Pointing, 1975). First-born male larvae were gathered under these conditions and developmental rates monitored as for
the females.

**Sensitive Period Studies**

Sensitive periods were investigated at photoperiods near the critical photoperiod where 50 per cent of the female offspring are sexual, and also at photoperiods which induced 100 per cent sexual female production if grandparents and parents were given a standard exposure. Studies were done simultaneously at 15°C and 20°C in the two experimental chambers.

For each experiment, the animals were taken from the stock culture at known ages and placed in labelled petri dishes for pre-determined exposure periods at the desired photoperiod and temperature. Leaves were replaced periodically to minimize alatae formation.

The birth of the parental generation provided a convenient fixed point in time for study of the sensitive period. Some exposures were terminated at this point, some started, and some overlapped the grandparent and parent generations. Lees (1963) has already noted that the aphid *Megoura viciae* is sensitive to photoperiod for a few days just prior to the birth of the parental generation. When the stimulus period was complete the insects were transferred back into the control chamber (16L at 20°C). Once parental reproduction began the offspring in several consecutive batches were identified and counted. Care was taken not to terminate an experiment until it was clear whether or not male production had started.
The duration of the exposure to a regime was variable depending on the particular photoperiod/temperature condition desired. For sensitive period studies performed entirely at 20°C the actual time from initiation of each experiment to termination was approximately 20 days. For similar studies performed with experimental regimes at 15°C, the actual time from initiation of each experiment to termination may have been as long as 32 days or as short as 20 days. As part of the sensitive period experiments required information about the photoperiodic responses within the transition zones at the two temperatures these studies could be performed only after the transition zones had been determined.

Fluctuating Temperature Studies

Once the critical photoperiod experiments at 15°C and 20°C were completed the necessary information to begin studies on fluctuating temperatures was available. Two types of experimental regime were used. The first was composed of a more natural diurnal cycle with a 20°C day and 15°C night. The second provided the reverse, a 15°C day and 20°C night. Both were repeated at the critical photoperiod for 15°C and 20°C. The environmental chambers were continuously lighted and cookie tins used to provide the night-day light sequence. Grandparents at 3 days of age were taken from the stock culture and placed in appropriately labelled tins. The tins were alternately shunted between the 2 chambers depending on the particular photoperiod regime desired. When the grandparents produced the first batch of young, they were discarded
and the parent generation raised until reproduction commenced, at which point the experimental stimulus ended. The offspring were identified as to morph and counted as the parent was serially transferred from leaf to leaf at regular intervals.

Some experiments were also attempted combining the effect of a diurnal temperature regime (20°C day; 15°C night) with a short photoperiod (much less than the critical photoperiod) and a reduced time of exposure as in the sensitive period studies. The short photoperiod was chosen because it clearly elicited 100-per cent sexual female production under standard exposure regimes. Hence, the response variability found to be associated with photoperiods near the critical photoperiod would be avoided and the effects of fluctuating temperatures and short exposures more easily detected.

Grandparents were taken from the stock culture at various ages (in days at 20°C) and placed inside petri dishes located in the tin cans. As noted above, the lid was closed in the 15°C experimental chamber simulating night-time, and then the lid was removed and the tin transferred to the 20°C chamber for the day. After a predetermined number of days of exposure to this treatment, the aphids were transferred into the control chamber until the parents began reproducing. As before, the offspring were counted and identified.

Field Experiments

To test the laboratory findings under field conditions,
several aphids serving as grandparents were taken from the stock culture at known ages and placed in a location near St. Clair College, Windsor, in early April. This particular time period was selected based on assumptions concerning the photoperiodic response of the aphids and data from Tables of Sunset and Sunrise at this latitude.

A small aquarium (18 x 8 x 10") was inverted and a wire screen placed underneath it. Some cardboard was attached to the glass to shield the insects from the direct rays of the sun. It was lifted above ground a few inches to allow rain water to drain and air to circulate, thus reducing any greenhouse effect. As before, aphids were placed in petri dishes with leaves changed periodically. A maximum-minimum thermometer was placed near the dishes and the temperature extremes recorded. At various times, insects were transferred from the field conditions into the control chamber in the laboratory. The parental generation was allowed to reproduce and the progeny identified.

**Statistical Analysis**

The statistical analyses were carried out using the Statistical Analysis System programs (Barr et al, 1976) on an IBM S/360 model 65 computer.
RESULTS

Field Temperature Studies

The relationship between the dependent variable field minimum temperature and the independent variables, Stevenson screen minimum temperature and hours of sunshine was determined (see Table I) using linear regression. The $R^2$ was not significantly increased by the addition of hours of sunshine. From data collected in the range from $5^\circ C$ to $18^\circ C$, the dependent variable field minimum and the independent variables Stevenson screen minimum and hours of sunshine was determined (Table 2; $Y_1 = 1.18026 X_1 -5.99$ where $Y_1 = \text{field min.}$, $X_1 = \text{Stevenson screen min.}$). Similarly, the relationship between the dependent variable field maximum, in the range from $15^\circ C$ to $28^\circ C$, and the independent variables Stevenson screen maximum and hours of sunshine was determined (Table 3; $Y_2 = 0.837 X_2 + 5.120$ where $Y_2 = \text{field max.}$, $X_2 = \text{Stevenson screen max.}$). Again, the $R^2$ was not improved by the addition of hours of sunshine.

When the control field temperatures, monitored with the stationary maximum-minimum thermometer, were regressed against temperature readings from the tips of the alfalfa plants selected at random from the field, a highly significant relationship was found ($P < 0.0001$; Table 4; $Y_3 = 2.33 + 0.981 X_3$ where $Y_3 = \text{tip temperature}$, $X_3 = \text{control temperature}$).
Table 1. Relationship between dependent variable, Harrow minimum temperature, and independent variable, Stevenson screen minimum.

Variable Sunshine Removed

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>Prob. &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>1</td>
<td>75.4846</td>
<td>75.4846</td>
<td>44.14</td>
<td>0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>17.1020</td>
<td>1.7102</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>92.5866</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B Value

Intercept        6.7154
Stev. screen min. 0.6907

$R^2 = 0.815$
Table 2. Regression equation allowing prediction of Harrow field minimum temperatures from Stevenson screen minimums.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>1</td>
<td>128.9735</td>
<td>128.9735</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>29.2207</td>
<td>2.9220</td>
<td>44.14</td>
<td>0.0001</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>158.1942</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B-Value

Intercept  -5.9958
Stev. screen min.  1.1803

$R^2 = 0.815$
Table 3. Regression equation allowing prediction of Harrow field maximum temperatures from Stevenson screen maximums.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>Prob. &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>1</td>
<td>88.2812</td>
<td>88.2812</td>
<td>33.23</td>
<td>0.0003</td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>23.9101</td>
<td>2.6566</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>112.1913</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B Value

Intercept       +5.1206
Stev. screen max. 0.8379

$R^2 = 0.787$
Table 4. Regression equation allowing prediction of alfalfa tip temperatures during the day from a fixed control thermometer located within the field.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>Prob.&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>1</td>
<td>287.7628</td>
<td>287.7628</td>
<td>246.33</td>
<td>0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>14</td>
<td>16.3547</td>
<td>1.1682</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>304.1175</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B Value

Intercept 2.3304
Control 0.9808

$R^2 = 0.946$
variable alfalfa height was not significant. In the field temperature studies it was assumed that differences between tip temperatures and control temperatures would be the result of differential radiant heating so comparisons were only made during the day.

With the three sets of equations \((Y_1, Y_2, Y_3)\) it is possible to estimate quite accurately the temperatures of the alfalfa tips from readily available Stevenson screen data. The linking equation (Table 5; equation 4) relating these estimates may be derived by assigning arbitrary temperature values from \(0^\circ C\) to \(24^\circ C\) (thus approximating those temperatures experienced by the aphid in late summer and autumn) to Stevenson screen maximums to obtain estimates of field maximums, terminal tip temperatures, and combinations of these two. Data from the estimated combination of temperatures, \(Y_{\text{cmax}}\), may then serve as dependent variable observations to regress against the same Stevenson screen maximums. The regression weights obtained in this linear regression are useful in predicting terminal maximum temperatures if the assumption is made that maximum temperatures are crucial in the photoperiodic response of the aphid.

Similarly, equation 5 built on equation 1 and 4 may be developed to aid in the prediction of daily average Stevenson screen temperatures in the field (Table 5). This equation would aid in designating daily linear averages of temperature as being most important in the prediction of
Table 5. Summary of linear regression equations allowing predictions of field temperatures from Stevensen screen data.

<table>
<thead>
<tr>
<th>Field Equations</th>
<th>Reference No.</th>
<th>Variables Defined</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Y_{\text{min.}} = 1.18X_{\text{min.}} - 5.99$</td>
<td>(1)</td>
<td>$Y_{\text{min.}}$: Max.–min. thermometer in crop; $Y_{\text{max.}}$: max. and min. temperature</td>
</tr>
<tr>
<td>$Y_{\text{max.}} = 0.837X_{\text{max.}} + 5.12$</td>
<td>(2)</td>
<td>$X_{\text{min.}}$: Stevensen screen temperature; $X_{\text{max.}}$:</td>
</tr>
<tr>
<td>$Y_{\text{tip.}} = 0.981X + 2.33$</td>
<td>(3)</td>
<td>$Y_{\text{tip}}$: temperatures recorded with probe in terminal</td>
</tr>
<tr>
<td>$Y_{\text{cmax.}} = 0.821X_{\text{max.}} + 7.35$</td>
<td>(4)</td>
<td>$Y_{\text{cmax.}}$: temperatures derived from combination $Y_{\text{max.}}$ and $Y_{\text{tip.}}$</td>
</tr>
<tr>
<td>$Y_{\text{avg.}} = X_{\text{avg.}} + 0.65$</td>
<td>(5)</td>
<td>$Y_{\text{avg.}}$: daily average of $Y_{\text{cmax.}}$ and $Y_{\text{tip.}}$; $X_{\text{avg.}}$: Stevensen screen temperatures</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stevensen Screen Temperatures</th>
<th>Min. Temp. (1)</th>
<th>Max. Temp. (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-5.99</td>
<td>7.3</td>
</tr>
<tr>
<td>2</td>
<td>-3.63</td>
<td>8.9</td>
</tr>
<tr>
<td>4</td>
<td>-1.27</td>
<td>10.6</td>
</tr>
<tr>
<td>6</td>
<td>1.09</td>
<td>12.2</td>
</tr>
<tr>
<td>8</td>
<td>3.45</td>
<td>13.9</td>
</tr>
<tr>
<td>10</td>
<td>5.81</td>
<td>15.5</td>
</tr>
<tr>
<td>12</td>
<td>8.17</td>
<td>17.1</td>
</tr>
<tr>
<td>14</td>
<td>10.53</td>
<td>18.9</td>
</tr>
<tr>
<td>16</td>
<td>12.89</td>
<td>20.4</td>
</tr>
<tr>
<td>18</td>
<td>15.25</td>
<td>22.1</td>
</tr>
<tr>
<td>20</td>
<td>17.60</td>
<td>23.7</td>
</tr>
<tr>
<td>22</td>
<td>20.00</td>
<td>25.4</td>
</tr>
<tr>
<td>24</td>
<td>22.30</td>
<td>27.1</td>
</tr>
</tbody>
</table>
field aphid responses.

Throughout the calculations of the linear regressions different ranges of temperatures were utilized, as within the autumn time span when temperatures were recorded, maximum upper and lower limits differed substantially from the minimum. The dangers of extrapolating beyond the range of data utilized are appreciated (Wesolowsky, 1976); however, it is probably safe in assuming that these temperature relationships hold at least for the late summer and autumn period in question because of the reliability of the measuring instruments.

Various simultaneous temperature readings of individual alfalfa plants at different heights from the ground were made. Table 6 is a summary of the data. A trend is evident with the terminal tip temperature < middle < ground temperatures. Although the days were partitioned into cloudy and sunny this partitioning yielded no consistent temperature differences between the two.

Field Sampling

Sexuals and asexuals were sampled at various times throughout the late summer and autumn (Table 7A). The mean and range of sample sizes were 827.4 and 362 - 1319 respectively.

Males and sexual females made their first appearance in the sweep-nets October 3 and October 11, respectively. Once sexuals were spotted there was a rapid increase in their numbers. When percentages of male were log-transformed their
Table 6. Temperature profiles of individual alfalfa plants read simultaneously with an electronic thermometer\(^a\) on 4 separate days.

<table>
<thead>
<tr>
<th>General Weather Conditions</th>
<th>Date</th>
<th>Time</th>
<th>Tip</th>
<th>Middle</th>
<th>Ground</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunny</td>
<td>Sept. 7</td>
<td>14:05 hrs</td>
<td>22.0 cm^0</td>
<td>11.00 cm</td>
<td>22.0^0 C</td>
</tr>
<tr>
<td>Sunny</td>
<td>Sept. 9</td>
<td>11:35 hrs</td>
<td>20.0 cm</td>
<td>11.50 cm</td>
<td>27.3^0 C</td>
</tr>
<tr>
<td>Sunny</td>
<td>Sept. 9</td>
<td>13:00 hrs</td>
<td>30.5 cm</td>
<td>14.00 cm</td>
<td>28.0^0 C</td>
</tr>
<tr>
<td>Sunny</td>
<td>Sept. 9</td>
<td>13:00 hrs</td>
<td>30.5 cm</td>
<td>14.00 cm</td>
<td>28.0^0 C</td>
</tr>
<tr>
<td>Cloudy</td>
<td>Sept. 10</td>
<td>12:00 hrs</td>
<td>30.5 cm</td>
<td>15.25 cm</td>
<td>18.5^0 C</td>
</tr>
<tr>
<td>Cloudy</td>
<td>Sept. 10</td>
<td>12:10 hrs</td>
<td>25.0 cm</td>
<td>20.00 cm</td>
<td>18.0^0 C</td>
</tr>
<tr>
<td>Cloudy</td>
<td>Sept. 10</td>
<td>12:15 hrs</td>
<td>29.0 cm</td>
<td>9.00 cm</td>
<td>18.0^0 C</td>
</tr>
<tr>
<td>Partially Cloudy</td>
<td>Sept. 11</td>
<td>11:30-12:00 hrs^d</td>
<td>34.0 cm</td>
<td>17.00 cm</td>
<td>22.0^0 C</td>
</tr>
<tr>
<td>Partially Cloudy</td>
<td>Sept. 11</td>
<td>11:30-12:00 hrs</td>
<td>30.0 cm</td>
<td>15.00 cm</td>
<td>21.0^0 C</td>
</tr>
<tr>
<td>Partially Cloudy</td>
<td>Sept. 11</td>
<td>11:30-12:00 hrs</td>
<td>29.0 cm</td>
<td>14.50 cm</td>
<td>21.0^0 C</td>
</tr>
<tr>
<td>Partially Cloudy</td>
<td>Sept. 11</td>
<td>11:30-12:00 hrs</td>
<td>29.0 cm</td>
<td>14.50 cm</td>
<td>21.0^0 C</td>
</tr>
<tr>
<td>Partially Cloudy</td>
<td>Sept. 11</td>
<td>11:30-12:00 hrs</td>
<td>32.0 cm</td>
<td>16.00 cm</td>
<td>21.2^0 C</td>
</tr>
<tr>
<td>Partially Cloudy</td>
<td>Sept. 11</td>
<td>11:30-12:00 hrs</td>
<td>29.0 cm</td>
<td>14.50 cm</td>
<td>21.0^0 C</td>
</tr>
</tbody>
</table>

\(^a\): read with a YSI tele-thermometer (± 0.5^0 C)  
\(^b\): height of alfalfa (cm.)  
\(^c\): temperature at respective height  
\(^d\): 6 temperature readings done within half-hour period
Table 7A. Data summary of sexual morph sampling at Harrow in 1977.

<table>
<thead>
<tr>
<th></th>
<th>% Asexual Female</th>
<th>% Sexual Female</th>
<th>% Male</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>100.0</td>
<td>0.0</td>
<td>0.0</td>
<td>1319</td>
</tr>
<tr>
<td>26</td>
<td>100.0</td>
<td>0.0</td>
<td>0.0</td>
<td>523</td>
</tr>
<tr>
<td>30</td>
<td>100.0</td>
<td>0.0</td>
<td>0.0</td>
<td>938</td>
</tr>
<tr>
<td>Oct.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>99.8</td>
<td>0.0</td>
<td>0.2</td>
<td>1013</td>
</tr>
<tr>
<td>6</td>
<td>99.8</td>
<td>0.0</td>
<td>0.2</td>
<td>945</td>
</tr>
<tr>
<td>11</td>
<td>99.6</td>
<td>0.1</td>
<td>0.3</td>
<td>996</td>
</tr>
<tr>
<td>17</td>
<td>92.7</td>
<td>5.5</td>
<td>1.8</td>
<td>712</td>
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<tr>
<td>24</td>
<td>70.2</td>
<td>22.8</td>
<td>7.0</td>
<td>639</td>
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<tr>
<td>31</td>
<td>41.1</td>
<td>42.0</td>
<td>16.9</td>
<td>362</td>
</tr>
</tbody>
</table>
Table 7B. Regression equation relating the occurrence of males with time in days. The date of first appearance was set equal to 1.

<table>
<thead>
<tr>
<th>df</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>Prob.&gt;F</th>
</tr>
</thead>
<tbody>
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<td>3.3906</td>
<td>113.67</td>
</tr>
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<td>Error</td>
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<td>0.1193</td>
<td>0.0298</td>
<td></td>
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<tr>
<td>Total</td>
<td>5</td>
<td>3.5099</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B Value

Intercept | -0.9504 |
Day | 0.07637 |

$R^2 = 0.966$
Table 8. Regression equation relating the occurrence of sexual females with time in days. The date of first appearance was set equal to 1.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>Prob. &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
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<td>3.4945</td>
<td>3.4944</td>
<td>10.27</td>
<td>0.0851</td>
</tr>
<tr>
<td>Error</td>
<td>2</td>
<td>0.6807</td>
<td>0.3404</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>4.1752</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B Value

Intercept  
-0.660  

Day  
0.125  

$R^2 = 0.837$
relationship with time was nearly linear ($R^2 = 0.966$) (Table 7E: $\log Y_6 = 0.076 X_6 - 0.950$ where $Y_6 =$ per cent males in sample, $X_6 =$ time in days). Similarly, when percentages of sexual females were log-transformed their relationship with time was approximately linear ($R^2 = 0.837$) (Table 8: $\log Y_7 = 0.125 X_7 - 0.660$ where $Y_7 =$ per cent sexual females in sample, $X_7 =$ time in days).

Experimental Studies

Developmental Rates

Developmental rates were measured, and corresponding standard errors calculated for asexuals at $15^\circ$C, $20^\circ$C and $23.5^\circ$C; and sexuals at $15^\circ$C and $20^\circ$C (Table 9). There was high mortality among the progeny of parents grown at $23.5^\circ$C. This temperature probably is near the upper lethal, where the curve of growth rate vs. temperature is non-linear (Gilbert et al, 1976).

The precise measurement of time-to-reproduction for the asexuals at $15^\circ$C and $20^\circ$C gave a fix on the ages at which the test grandparents entered the pre-selected photoperiodic regimes, and also the duration of exposure at selected temperatures necessary to test the effects of photoperiod at specific physiological ages within the life history of this aphid species.

Critical Photoperiod Studies

Photoperiod responses within the transition zone were determined experimentally for the Harrow clone at $15^\circ$C
Table 9. Mean developmental times of asexuals and experimentally produced sexuals with respective standard errors (in days).

<table>
<thead>
<tr>
<th>Morph and Developmental Rate Criteria</th>
<th>$15^\circ C$</th>
<th>$20^\circ C$</th>
<th>$23.5^\circ C$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Asexual females</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth-to-last Moult</td>
<td>$11.7 \pm 0.69$</td>
<td>$7.0 \pm 0.76$</td>
<td>$5.1 \pm 0.30$</td>
</tr>
<tr>
<td>Birth-to-onset reproduction</td>
<td>$14.2 \pm 0.61$</td>
<td>$8.2 \pm 0.44$</td>
<td>$7.4 \pm 0.51$</td>
</tr>
<tr>
<td><strong>Sexual Females</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth-to-last moult</td>
<td>$12.5 \pm 0.52$</td>
<td>$8.2 \pm 0.37$</td>
<td>-</td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth-to-last moult</td>
<td>$15.1 \pm 0.71$</td>
<td>$9.4 \pm 0.49$</td>
<td>-</td>
</tr>
</tbody>
</table>
and 20°C and the critical photoperiods estimated using linear regression at these two temperatures. As stated, the transition zone represents the interval of photoperiods between those which cause 100 per cent sexual females and 100 per cent asexual females and critical photoperiod to the photoperiod giving 50 per cent asexual and 50 per cent sexual females.

Using the data obtained from the photoperiodic response studies (see Appendix III) linear regressions were attempted. Photoperiod (in hours light) was regressed against the percentage of pooled sexual female morph. Table 11 illustrates the regression obtained at 15°C

\( Y_8 = -76.848 \times 8 + 1057.322 \) where \( Y_8 = \) per cent sexual morph, \( X_8 = \) photoperiod in hours light). As a predictive model it is not good \( (R^2 = 0.538) \), but it is significant at the 0.05 level. The equation obtained at 20°C \( Y_9 = -164.756 \times 9 + 1998.070 \) is only approximately linear \( (R^2 = 0.763) \) but not quite significant at the 0.05 level (see Table 12).

At 15°C, the critical photoperiod was estimated to be 13:06L. The end-points of the transition zone were also estimated to be 13:45L and 12:27L for photoperiodic responses of 0 per cent and 100 per cent, respectively. At 20°C, however, the critical photoperiod was estimated to be 11:49L with transitional photoperiod end-points at 12:07L and 11:31L, respectively. It is clear that for this clone a negative relationship exists between critical photoperiod and temperature (i.e. at 20°C the critical photoperiod is less than the critical photoperiod at 15°C).
Table 10. Variability in the percent oviparae produced by a Harrow clone in response to various photoperiods and temperatures. Replicate tests were at different times.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Photoperiod</th>
<th>Number of Parents</th>
<th>% Sexual Female Offspring</th>
<th>Number of Offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>12:39L</td>
<td>14</td>
<td>99</td>
<td>184</td>
</tr>
<tr>
<td>15</td>
<td>12:39L</td>
<td>17</td>
<td>90</td>
<td>238</td>
</tr>
<tr>
<td>15</td>
<td>12:39L</td>
<td>16</td>
<td>94</td>
<td>219</td>
</tr>
<tr>
<td>15</td>
<td>13:21L</td>
<td>17</td>
<td>85</td>
<td>221</td>
</tr>
<tr>
<td>15</td>
<td>13:21L</td>
<td>17</td>
<td>5</td>
<td>230</td>
</tr>
<tr>
<td>15</td>
<td>13:21L</td>
<td>17</td>
<td>-1</td>
<td>298</td>
</tr>
<tr>
<td>15</td>
<td>13:21L</td>
<td>19</td>
<td>0</td>
<td>285</td>
</tr>
<tr>
<td>20</td>
<td>11:42L</td>
<td>20</td>
<td>96</td>
<td>284</td>
</tr>
<tr>
<td>20</td>
<td>11:42L</td>
<td>18</td>
<td>77</td>
<td>244</td>
</tr>
</tbody>
</table>
Table 11. Relationship between photoperiod (hours of light) and sexual female morph response within the transition zone at 15°C.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>Prob. &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>1</td>
<td>11809.583</td>
<td>11809.583</td>
<td>11.66</td>
<td>0.0066</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>10131.514</td>
<td>1013.151</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>21941.097</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B Value

Intercept        | 1057.322
Photoperiod      | -76.848

$R^2 = 0.538$

Lower limit
100% oviparous morph
12:27L* C.P. = 13:06L*

Upper limit
0% oviparous morph
13:45L*

* Regression estimates
Table 12. Relationship between photoperiod (hours of light) and sexual female morph response within the critical photoperiod transition zone at 20°C.

<table>
<thead>
<tr>
<th>df</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>Prob. &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>1</td>
<td>4981.776</td>
<td>4981.776</td>
<td>9.67</td>
</tr>
<tr>
<td>Error</td>
<td>3</td>
<td>1545.944</td>
<td>515.315</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>6527.720</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B Value

Intercept 1998.070
Photoperiod -164.756

$R^2 = 0.763$

Lower limit
100% oviparous morph
11:31L*

Upper limit
0% oviparous morph
12:07L*

Transition Zone at 20°C

* Regression estimates
Experimentally, it was found that the upper and lower end-points of the response zone at 15°C were not clearly defined; 12:53L was the longest photoperiodic regime which gave 100 per cent sexuals. Replications at 12:39L, however, gave sexual morph percentages of 89.6 and 93.5. Near the estimated upper limit, 13:45L, there was variability in experimentally determined responses at 13:21L (Table 10). On one occasion, of replicates done independently, the photoperiodic response was 85 per cent; on another, a 0 per cent response was noted. Fewer experimental regimes were attempted at 20°C, but of replicates done independently of one another at 11:42L similar variability was noted.

The range of transitional photoperiods is an indication of the sensitivity of the aphid to differences in photoperiod. The estimated range at 20°C utilizing a linear regression is 36 minutes, while the range estimated similarly at 15°C is 78 minutes. The precise estimate of ranges at either temperature is complicated by the end-point transitional variability noted. If 12:53L is set as the lower limit at 15°C, and 13:21L as the upper limit at 15°C then the ranges at either temperature would become quite similar. Justification for selecting 12:53L as the lower limit rests on grounds as noted above that it is the longest experimental photoperiod which induces 100 per cent oviparae production; 13:21L, as the upper limit, on grounds that it is the shortest experimental photoperiod inducing 0 per cent oviparae production. A difference in day-length (including civil twilight) of 30 minutes as then would be the case for the
range of these experimental studies, would encompass a time period of about 3 weeks in early autumn (Beck, 1968).

**Sensitive Period**

In these investigations the period or stage during which the insect is sensitive to the experimental light stimulus was studied. When the parents were transferred to the control conditions after the stimulus, the morph of the offspring was identified and counted in two ways. As before, the number of oviparae and asexual females were counted and the per cent of sexual females tabulated. Pooling was necessary because the numbers of the two morphs was small and variable within each family. However, male aphids, were either present or absent within a particular parents' offspring. Therefore, to discriminate the response patterns of their parents it was more appropriate to count the number of parents which produce male offspring and determine a percentage responding. Initially, the sensitive period studies concentrated on photoperiods within the transition zones at the two temperatures. However, since the instability of the response in this zone complicates interpretation of the results, the effects of shorter photoperiods were also studied. At their respective temperatures, these light regimes gave 100 per cent sexual female offspring and 100 per cent male production when lines were exposed from early in the life of the grandparent through the life of the parents. Beginning on Day 1 (D1), birth of the grandparents, the aphids were exposed for various periods at
either 15\(^\circ\)C or 20\(^\circ\)C. From the developmental rate experiments
time-to-reproduction was known with some precision. Hence
a 'fix' on the ages at which the stimulus begins and ends
could be made.

Table 13A shows results obtained within the
transitional zones at 13:21L (15\(^\circ\)C), 13:07L (15\(^\circ\)C) and
11:57L (20\(^\circ\)C). Exposures within the grandparent generation
were sufficient to produce males. As can be seen at 11:57L,
just two days exposure after the grandparent has moulted to
the adult stage, was enough to produce parents, 78 per cent
of which give birth to males. When the stimulus was extended
to four days prior to birth of the parental generation, all
of the parents produced males. Only one shortened period of
exposure caused the production of any sexual female offspring,
and then only 1 per cent. This exposure period ended just
after the parents were born.

Tables 13B and 13C show results obtained with short
periods of exposure and short photoperiods at 11:15L (15\(^\circ\)C),
12:00L (15\(^\circ\)C) and 11:00 (20\(^\circ\)C). It is clear that exposures
early after the birth of the grandparent have no effect on
sexual morph determination (Table 13B: Experiments 1, 2, 11).
It is the later adult stage which is sensitive. A one day
exposure (at 15\(^\circ\)C) as soon as the aphid has moulted to the
adult morph does not appear to be sufficient to produce
males (Table 13B: Experiment 3). A three day exposure just
prior to the adult moult of the grandparents starting on D8
at 15\(^\circ\)C or D5 at 20\(^\circ\)C leads to a large proportion of male
offspring being produced suggesting that the grandparent
Table 13A. The effect of various periods of exposure to photoperiods in the transition zone on sexual morph production. The beginning and end of the exposure are indicated by a day number with the day of birth of the grandparent as day 1. Parents are born on day 15 at 15°C and day 9 at 20°C. The remainder of the developmental time was spend at 16L:20°C.

<table>
<thead>
<tr>
<th>Expt. #</th>
<th>Temperature (°C)</th>
<th>Photoperiod</th>
<th>Exposure Period</th>
<th>Number of Parents</th>
<th>% Producing Males</th>
<th>% Oviparae</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>13:21L</td>
<td>4</td>
<td>10</td>
<td>16</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>13:21L</td>
<td>4</td>
<td>13</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>13:21L</td>
<td>3</td>
<td>16</td>
<td>15</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>13:21L</td>
<td>1</td>
<td>14</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>13:21L</td>
<td>1(control)</td>
<td>30</td>
<td>17</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>13:21L</td>
<td>1(control)</td>
<td>30</td>
<td>17</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>15</td>
<td>13:07L</td>
<td>2</td>
<td>19</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>20</td>
<td>11:57L</td>
<td>4</td>
<td>8</td>
<td>17</td>
<td>100</td>
</tr>
<tr>
<td>9</td>
<td>20</td>
<td>11:57L</td>
<td>7</td>
<td>9</td>
<td>18</td>
<td>78</td>
</tr>
</tbody>
</table>
Table 13B. The effect of periods of exposure at 15°C to photoperiods capable of inducing 100 per cent sexual morph production under standard (control) conditions. The beginning and end of the exposure are indicated by a day number with the day of birth of the grandparent as day 1. Parents are born on day 15. The remainder of the developmental time was spent at 16L:20°C.

<table>
<thead>
<tr>
<th>Expt. #</th>
<th>Temperature (°C)</th>
<th>Photoperiod</th>
<th>Exposure Period Start</th>
<th>Exposure Period End</th>
<th>Number of Parents</th>
<th>% Producing Males</th>
<th>% Oviparae</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>11:15L</td>
<td>1</td>
<td>2</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>11:15L</td>
<td>5</td>
<td>8</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>11:15L</td>
<td>6</td>
<td>7</td>
<td>16</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>11:15L</td>
<td>8</td>
<td>11</td>
<td>15</td>
<td>85</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>11:15L</td>
<td>4</td>
<td>16</td>
<td>11</td>
<td>91</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>11:15L</td>
<td>3</td>
<td>13</td>
<td>14</td>
<td>86</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>15</td>
<td>11:15L</td>
<td>1</td>
<td>11</td>
<td>14</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>15</td>
<td>11:15L</td>
<td>6</td>
<td>15</td>
<td>17</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>15</td>
<td>11:15L</td>
<td>6</td>
<td>18</td>
<td>7</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>15</td>
<td>11:15L</td>
<td>1(control)</td>
<td>30</td>
<td>20</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>11</td>
<td>15</td>
<td>12:00L</td>
<td>1</td>
<td>2</td>
<td>18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>15</td>
<td>12:00L</td>
<td>1(control)</td>
<td>30</td>
<td>18</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 13C. The effect of periods of exposure at 20°C to photoperiods capable of inducing 100 per cent sexual morph production under standard (control) conditions. The beginning and end of the exposure are indicated by a day number with the day of birth of the grandparent as day 1. Parents are born on day 9. The remainder of the developmental time was spent at 16L:20°C.

<table>
<thead>
<tr>
<th>Expt. #</th>
<th>Temperature (°C)</th>
<th>Photoperiod</th>
<th>Exposure Period Start</th>
<th>End</th>
<th>Number of Parents</th>
<th>% Producing Males</th>
<th>% Oviparae</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>11L</td>
<td>5</td>
<td>8</td>
<td>18</td>
<td>88</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>11L</td>
<td>1</td>
<td>8</td>
<td>14</td>
<td>85</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>11L</td>
<td>12</td>
<td>8</td>
<td>14</td>
<td>100</td>
<td>86</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>11L</td>
<td>17</td>
<td>6</td>
<td>14</td>
<td>100</td>
<td>78</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>11L</td>
<td>13</td>
<td>6</td>
<td>14</td>
<td>100</td>
<td>74</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>11L</td>
<td>17</td>
<td>4</td>
<td>4</td>
<td>100</td>
<td>86</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td>11L</td>
<td>14</td>
<td>4</td>
<td>4</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>8</td>
<td>20</td>
<td>11L</td>
<td>11</td>
<td>15</td>
<td>4</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>20</td>
<td>11L</td>
<td>10</td>
<td>11</td>
<td>10</td>
<td>100</td>
<td>22</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>11L</td>
<td>14</td>
<td>12</td>
<td>10</td>
<td>100</td>
<td>94</td>
</tr>
<tr>
<td>11</td>
<td>20</td>
<td>11L</td>
<td>18</td>
<td>20</td>
<td>10</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 13D. The effect of duration of exposure to a short photoperiod on sexual morph production at two temperatures.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Male Production</th>
<th>Oviparae Production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exposure</td>
<td>Duration End Day</td>
</tr>
<tr>
<td>15</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>20</td>
<td>3</td>
<td>8</td>
</tr>
</tbody>
</table>
generation is sensitive at this time (Table 13B: Experiment 4). Clearly, the time of the exposure within the life history is important and for male production, a quite short exposure at the correct time is effective.

Many treatments that elicited male production did not elicit oviparae production. As can be seen from the results of experiments 1 and 2 in Table 13C short or long exposures during the grandparental generation resulted in no oviparae. However, exposures that lasted into the parental generation particularly to day 12 or longer at 20°C elicited strong responses. Day 12 is 3 days into the parental generation at 20°C. At 15°C the one experiment that continued to day 18, 3 days into the parental generation at that temperature also was the only experimental treatment to cause oviparae production. These results show that the sensitive period for oviparae production occurs later than for male production, perhaps ending 4 or 5 days later. No short periods of exposure were tested during the sensitive period for oviparae production and so comparison of the length of the sensitive periods for the two morphs is not possible.

While the data are limited they seem to support the hypothesis that the length of the sensitive period is not markedly affected by temperature. For both sexes a comparison of exposures that end near the end of the sensitive period indicates that exposures of similar durations cause similar levels of response irrespective of temperature (Table 13C). The insect may be responding to a fixed number of diurnal cycles that occur within the sensitive period.
In summary, two sensitive periods were found. The period for male production begins a few days prior to birth of the parental generation and ends near the birth of the parents. The sensitive period for sexual females occurs prior to the birth of the parents and extends a few days after their birth. Males could be produced through exposure of only the grandparents, while oviparae could only be produced when the exposures overlapped both generations.

**Fluctuating Temperature Experiments**

This experiment was designed to determine if it was night, day or a combination of the two temperatures which interacted to affect the critical photoperiod. Test photoperiods of 11:45L and 13:00L near the critical photoperiods for temperatures 20°C and 15°C, respectively, were chosen. The 4 designs are summarized diagrammatically in Table 14. Regimes B and C correspond to a more natural diurnal temperature pattern with maximum temperature in the photophase and minimum temperatures in the scotophase.

On the assumption of a negative linear relationship between daily mean temperature and critical photoperiod, the mean temperature 17.5°C should generate a critical photoperiod at 12:29L. Thus, regimes A and B should give nearly 100 per cent sexuals, while regimes C and D 0 per cent since the transition zone is about 30 minutes long, 15 minutes either side of the critical photoperiod. On the other hand, if it is night temperatures that are important, regimes A and C
Table 14. Predictions of hypotheses concerning the effects of fluctuating temperature experiments on the photoperiodic response of the Harrow clone.

<table>
<thead>
<tr>
<th>Regime A</th>
<th>Regime B</th>
<th>Regime C</th>
<th>Regime D</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:15D 20°C</td>
<td>12:15D 15°C</td>
<td>11:00D 15°C</td>
<td>11:00D 20°C</td>
</tr>
<tr>
<td>11:45L 15°C</td>
<td>11:45L 20°C</td>
<td>13:00L 20°C</td>
<td>13:00L 15°C</td>
</tr>
</tbody>
</table>

1) **Day temperatures important:**
   - Regime A: 100 per cent sexual females
   - Regime B: transitional response
   - Regime C: 0 per cent sexual females
   - Regime D: transitional response

2) **Night temperatures important:**
   - Regime A: transitional response
   - Regime B: 100 per cent sexual female response
   - Regime C: transitional response
   - Regime D: 0 per cent sexual females

3) **Linear combination night and day temperatures:**
   - Regime A: 100 per cent sexual females
   - Regime B: 100 per cent sexual females
   - Regime C: 0 per cent sexual females
   - Regime D: 0 per cent sexual females
should generate a transition zone response and regime D and B 100 per cent asexual and sexual offspring respectively. Conversely, if the aphid responds to the day temperatures, regimes B and D would produce transition-zone responses and regimes A and C 100 per cent sexual and asexual offspring respectively. These results could be compounded by the fact that within the transition zone, the response is variable.

Table 15 presents the results obtained in the fluctuating temperature experiments. On the basis of the data it is difficult to make a clear-cut case for any of the above mentioned hypotheses. If day temperatures were more important in the photoperiodic response it would have been expected that regime A would produce more oviparae than regime B but this was not the case.

On the other hand, as expected for this hypothesis, regime C produced fewer sexuals than regime D. Consistent results were also not obtained if it was assumed night temperatures were crucial. As expected under this hypothesis regime B produced more sexuals than regime A, but contrary to this expectation regime D produced more sexuals than regime C. Regime D also gave an unusual result if the hypothesis of a linear average of night and day temperatures was considered. Rather than give an expected morph response of 0 per cent sexual females this regime produced an 100 per cent oviparous response. The fluctuating temperature experiments suggest that rather than respond in a simple linear fashion to temperature the aphid responds in a more
Table 15. The effects of fluctuating temperatures on the photoperiodic response of the Harrow clone of A. pisum. Experimental regimes are outlined in Table 14. Experiments 1, 2, 4 and 5 represent replicates done independently.

<table>
<thead>
<tr>
<th>Regimes</th>
<th>Experiment</th>
<th>Number of Parents</th>
<th>Number of Offspring</th>
<th>Percent Oviparae</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>12</td>
<td>152</td>
<td>94</td>
</tr>
<tr>
<td>A</td>
<td>2</td>
<td>12</td>
<td>163</td>
<td>99</td>
</tr>
<tr>
<td>B</td>
<td>3</td>
<td>14</td>
<td>190</td>
<td>100</td>
</tr>
<tr>
<td>C</td>
<td>4</td>
<td>14</td>
<td>181</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>5</td>
<td>8</td>
<td>119</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>6</td>
<td>20</td>
<td>288</td>
<td>100</td>
</tr>
</tbody>
</table>
complex, non-linear manner. This response may be an artifact of the simplified light and temperature regimes in the laboratory but more study is needed to verify this.

Four experiments were attempted combining the effects of fluctuating temperatures with reduced exposure times. Sets of tests, grandparents of known ages were taken out of control conditions (20°C; 16L) and placed into experimental conditions with a scotophase of 13D·(15°C) and a photophase of 11L (at 20°C). Experiments were terminated at various times, and the test aphids placed back into control conditions until the birth of parental progeny occurred. Male offspring were then identified and counted as a percentage of the total number of parents in each experiment. Sexual females, or oviparae were identified and counted as a pooled percentage of the number of females produced by all the parents within a given experiment. The particular experimental photoperiod regime used in these studies was selected because it is much less than the photoperiods associated with the aphid critical photoperiodic response at either temperature. Thus, under standard exposure conditions where test grandparents and their parental progeny are exposed throughout, 100 per cent sexual offspring are generated.

Table 16 illustrates the aphid responses found. The experimental results may be analysed in terms of the differing sensitive periods for both sexes, and the effects of differing durations of exposure on the aphid response. The results from experiment 1 reinforce the earlier observation (see Tables 13A, B, C) that the stimulus exposure must at least
Table 16. The influence of fluctuating temperatures and different exposure periods on the photoperiodic response of *Acyrthosiphon pisum*.

<table>
<thead>
<tr>
<th>Expt. #</th>
<th>Exposure Period Start</th>
<th>Exposure Period Finish</th>
<th>No. of Parents</th>
<th>Number of Offspring</th>
<th>Male Production Oviparae</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>10</td>
<td>12</td>
<td>-*</td>
<td>75</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>12</td>
<td>13</td>
<td>215</td>
<td>100</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>12</td>
<td>11</td>
<td>164</td>
<td>100</td>
<td>42</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>12</td>
<td>5</td>
<td>76</td>
<td>100</td>
<td>52</td>
</tr>
</tbody>
</table>

* female offspring not counted but 100% asexual offspring produced.

All experiments at 1:1L with 20°C photophase and 15°C scotophase.
overlap the birth of parents before any oviparae are produced. Although exposed at the late, larval stages (beginning day 7) the test grandparents had not produced progeny by day 10. This finding that most parents produced males (75%) is not surprising given the exposure times. Experiments 2, 3 and 4 however, all had exposures overlapping the parental generation hence; as expected, oviparae were produced. Also, in all cases, 100 per cent males were produced confirming earlier observations that the male sensitive period ends near birth of the parents.

Experiments 2, 3 and 4 indicate either that lengthened stimulus exposure per se or that stimulation at an early age enhances the production of sexual females. Experiment 2 through 4 show rising oviparous morph frequencies with increasing stimulus duration and younger, starting aphid grandparents. These results are unlike earlier oviparous sensitive period findings (see Tables 13B, C) because they suggest that fluctuating temperatures may lead to grandparents being sensitive to light and dark stimuli at a younger age. Caution should be used when interpreting experiment 4 because of the limited parental sample size.

Field Studies

Several aphids of various ages and serving as grandparents were placed in the field and exposed to field conditions for different periods of time. After selected field exposure the test grandparents were placed back into controlled laboratory conditions (16L: 20°C), allowed to
reproduce, and the offspring morph identified and counted as before. From the developmental rate experiments the ages of the aphids were known with certainty at the time of the introduction into the field. Natural daylengths including civil twilight were estimated to be between 13:68L and 14:23L from April 1st to 15th at this location. Mean minimum and maximum temperatures were determined to be 2.17°C and 12.4°C. Assuming a negative relationship between temperature and photoperiod, this field regime would have been expected to produce sexual offspring if exposure(s) lasted through the sensitive periods. Thus other provision would, of course be, that aphid physiological growth was possible and the photoperiodic mechanism was not shut down at these low temperatures.

Table 17 shows the data obtained on aphids which survived the extremely low temperatures in the field during this time. In all cases, no sexuals were produced. It has been suggested in the sensitivity period studies, within the linear range of growth (see Table 13D) near the end of the male sensitive period, that exposures of similar durations caused similar levels of response irrespective of temperature. Hence, on the basis of length of exposure in the field, experimental conditions 1, 2 and 3 were expected to stimulate the production of males. Experiments 4 and 5 would have had the appropriate exposure lengths to produce males if field temperatures approximated those that occur in late summer and autumn.

A priori, aside from the effects of the low
Table 17. The influence of spring conditions on sexual morph production.

<table>
<thead>
<tr>
<th>Expt. #</th>
<th>Starting Dates</th>
<th>Age of grandparent at start</th>
<th>Date exposure ended</th>
<th>Number of parents</th>
<th>Number of Offspring (Male or Female)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>April 7</td>
<td>6</td>
<td>April 10</td>
<td>10</td>
<td>209</td>
</tr>
<tr>
<td>2</td>
<td>April 11</td>
<td>6</td>
<td>April 14</td>
<td>12</td>
<td>195</td>
</tr>
<tr>
<td>3</td>
<td>April 1</td>
<td>1</td>
<td>April 11</td>
<td>15</td>
<td>288</td>
</tr>
<tr>
<td>4</td>
<td>April 1</td>
<td>6</td>
<td>April 10</td>
<td>16</td>
<td>367</td>
</tr>
<tr>
<td>5</td>
<td>April 5</td>
<td>2</td>
<td>April 11</td>
<td>15</td>
<td>220</td>
</tr>
</tbody>
</table>


temperatures, it is not surprising that oviparae were not produced. Laboratory studies demonstrated that (see Tables 13A, B, C, D) experimental exposures had to at least overlap the parental generation before any sexual females were produced. Only experimental conditions in number 2 met these requirements through the production of parental progeny in the field.

Several other grandparents had been tested in the field and exposed at earlier date(s), and at dates coincident with Table 17, but aphid mortality had been high and hence could not be followed-up. Even of those aphids that survived the field conditions not all were capable of producing progeny in the laboratory. The grandparents that were selectively used in this set of experiments were stunted and showed little physiological growth in the field. On several occasions in the test period temperatures dropped below the thermal threshold for this aphid species. Physiological growth apparently must occur before the aphid is receptive to the sexually-inducing photoperiodic stimulus. The results from experiment 2 further suggests that physiological growth is not the only necessary criterion. Limited growth had taken place here but no sexuals were produced. Perhaps the photoperiodic mechanism is shut down at temperatures which normally allow some physiological growth to occur.
DISCUSSION

Field Temperature Studies

The influence of temperature on the photoperiodic response is basically two-fold. Temperature largely determines development times (as insects are poikilotherms) hence, it has the capability of producing variable age structures in the aphid population. This has a direct bearing on the insect's sensitive period and the proportion capable of responding to photoperiod. Temperature also interacts with photoperiod to directly influence the production of sexual forms.

Aphids generally feed on alfalfa tips at the top of the canopy. However, it is not known whether they move to a preferred or optimal temperature zone within the plant canopy, or if they ignore small temperature differences. If the former is the case, one might expect more homogenous photoperiod responses within the field ignoring possible clonal variability. However, if the aphid plays a more passive role with regards to temperature one might expect more variability in photoperiodic response patterns within the field.

The regression equations developed in this investigation relating alfalfa terminal temperatures to Stevenson screen data could easily be fitted into the simulation model. The utility of such a fit, however, would be whether or not it increased the predictability of the model (Gilbert et al., 1976). The
minimum and maximum temperatures in the field as estimated in Table 4 demonstrate that on average terminal temperatures approximate closely those observed in the Stevenson screen. In terms of physiological growth then it probably is safe to assume that aphids at the terminal tips in the field have the same temperatures as those observed in a nearby Stevenson screen. In the development of the equations relating terminal temperatures to Stevenson screen temperatures actual field measurements were only carried out in the daytime. It probably is a safe assumption that during the night terminal temperature variability throughout the field is minimal as the effects of differing atmospheric conditions in the penetration of sunlight can be ignored. Use of the equations can directly test the hypothesis that it is day temperatures, night temperatures, or a daily linear average which is most important in the photoperiodic response. As stated in the description of field growth rates sufficient approximations of each hypothesis are contained in data from the Stevenson screen.

Based on the temperature profiles of individual alfalfa plants read simultaneously (Table 5) it is apparent that only small temperature differences exist between the terminals and the ground. Regardless of the position of the aphids on the plants then it is reasonable to assume that the temperatures experienced by the insects within the vegetation canopy approximate those in the Stevenson screen.

Unlike the observations of Pinter et al (1975) field temperature measurements near the ground were higher than the tips of the vegetation during the daytime. In this
investigation temperatures were monitored when the alfalfa was young (≤34 cm. height) and less mature than the above mentioned study. A less dense growth of alfalfa would imply less evapotranspiration and the potential for more penetrating insolation. Unlike the older alfalfa, the effects of shading are not so important in the younger plants (Geiger, 1965). Measurements near the surface on bare ground show characteristically a temperature inversion effect under cloudy conditions. That is, temperatures on the surface become lower than immediately above it. This was not found when the cloudy and sunny days were partitioned in the alfalfa fields of this present study. Within a vegetation canopy exchanges of long wavelength radiation and turbulent mixing probably interfere with the inversion phenomena (Geiger, 1965).

Field Sampling

The sampling of aphids in the field showed that the sexual females appeared about one week after the first appearance of males. Sample sizes were larger and the sampling carried on for a longer time than previously had been done (Lamb, 1970) hence the data obtained are probably more reliable. The field data was consistent with the experimental laboratory results in that, as predicted from laboratory knowledge gained about the longer male critical photoperiod, male appearance began before sexual females. Further, the sensitivity studies performed for the two sexual morphs also suggested that male initiation would occur sooner as less stimulus exposure is needed to produce
this morph.

Some caution is advised in the interpretation of these field results. As can be seen from Table 7A the number of males found in the earlier samples is small indeed. The possibility always remains that there was some sampling error; sexual females being present, but not detected through the sweeping done. There is no easy mathematical way of placing confidence limits around these morph sample percentages. On the basis of the large sizes of the earlier samples it is probably not unrealistic to say, however, that small differences could be detected. Also, the field was swept twice in the earlier samples before sexual females were noted. This adds secondary support that these findings were real, i.e. males appear earlier than sexual females.

Critical Photoperiod Studies

The critical photoperiodic response of *A. pisum* clones at Harrow and Markham were compared to test and either lend positive or negative support to Danilevskii's hypothesis that since more northern insect populations encounter cold temperatures sooner, it makes sense for them to enter diapause earlier to minimize the risks of exposing themselves to lethal climatic conditions.

The possibility remains in this comparison that the variation observed may be due to interclonal variability or differing biotype adaptations. The distinction between these concepts is not clear and sometimes both terms are used
interchangeably (Eastop, 1973; Frazer, 1972). In any case, findings from Frazer (1972) and Cartier et al. (1965) suggest that only one or very few clones are involved in the infestation of a single field. The comparisons between fields may be limited with regards to measured fecundity estimates (Frazer, 1972) but no data is available suggesting that the photoperiodic mechanisms are similarly linked. The photoperiodic response patterns and the mechanisms associated with the development of these are simply not understood. There would be extreme difficulty in assessing clonal photoperidic response variability even if, a priori, it is suspected to occur. To obtain a sufficient sample of clones from a field, and experimentally determine precise critical photoperiod estimates at a variety of temperatures would take a great deal of work as already indicated earlier. Hence, in these studies it was assumed that such variability is minimal.

The critical photoperiod at 20°C for the Harrow clone is clearly less than the critical photoperiod for the Markham clone (Lamb, 1970) (see Appendix IV). This is as expected from Danilevskii's hypothesis. The upper limits of the photoperiodic thresholds separating the Markham and Harrow clones differ by 48 minutes. This appears to be a large difference considering the two locations are only separated by 1° 23' of latitude. This may represent adaptation as while the distance in latitude is small the Harrow average temperatures are significantly higher than those in Markham. At 15°C, the critical photoperiods of the two clones were
approximately the same. The estimated upper limit of the photoperiodic thresholds as determined at Harrow differed by 22 minutes from those observed at Markham. However, the arbitrarily selected upper limit at Harrow (13:21L) was quite similar to that of the compared clone. The estimated lower limit of the more southern clone differed by 27 minutes from the northern one. The photoperiodic response data are contrary to Danilevskii's hypothesis at 15°C, and suggest a better way of testing it. Rarely in the literature have critical photoperiod studies been reported for different temperatures. Most studies have been done at a single higher temperature. If as Lamb and Pointing (1972) suggest the mechanism for sexual morph production utilizes both photoperiod and temperature cues, Danilevskii's hypothesis would be better tested by comparing the slopes of lines relating temperature to critical photoperiod at the different geographical locations. It is unfortunate that most investigators have dealt only with time increments of half an hour or more within the diapause transition zone (Danilevskii, 1961; Tauber and Tauber, 1972; Bradshaw, 1976). Comparisons of lengths of transition zones between different species would otherwise have been possible and their evolutionary significance more easily interpreted.

Clearly high photoperiodic response variability occurred within the transition zones. Regression analyses were attempted to objectively estimate the critical photoperiods and the transitional response end-points. These
statistics have usually been calculated through visual interpolation. Increments of test photoperiods were generally large, and only rough estimates could be made. Although in these present studies a number of treatment exposures were utilized, the critical photoperiod was probably still not precisely determined. Many treatments, perhaps taking many months of tedious work would be needed to possibly account for all this variability. Even then, physiological and genetic factors assumed to be under control might interfere with interpretation of the findings. The problem of instability (Lamb and Pointing, 1972) near the end-points of the transition zones where non-linearities presumably exist might also directly affect the simulation model. The end-point data used to calculate the thresholds in the simulations gave fairly good predictions, as will be shown, so perhaps it can be assumed that more accurate estimates are unnecessary. While causing difficulties in statistical analysis the transition zone instability may have evolutionary significance and probably should receive more attention. Having a variable photoperiodic response zone probably serves as a form of risk minimization. If a deterministic zone existed within each clone it is possible that the sexual stimulus might be initiated too late and unfavourable conditions would kill all members of the clone before fertilization of eggs could take place. Aphid clones are probably largely selected for on the basis of the previous year's climatic conditions (Williams, 1975). However, the weather in the autumn of one year may be poorly
correlated with weather in the next. It is suggested here that deterministic reliance on the conditions a year before may well prove to be fatal for an aphid clone. Data is not available on how widespread this "zone of instability" is in other species.

Sensitive Period

The sensitive period findings are interesting both from a physiological and an ecological point of view. While working with the aphid *Megoura viciae*, Lees (1959, 1963, 1964) concluded that embryonic development was under the control of a maternal switching mechanism located in the head of the parent insect. He suggested that this mechanism involved a light receptor and a humoral component. In response to an interaction of photoperiod and temperature, the postulated 'hormone' produced was seen as having a target, the developing embryo, which in turn differed in sensitivity depending on its relative age. His results for the sexual female morph indicated that photo sensitivity developed some two days before birth. The response patterns for the oviparous morph are subject to photoperiodic manipulation in *M. viciae*, whereas males are not. This contrasts to *A. pisum* where both sexual morphs appear to be under temperature and photoperiodic control.

Lees (1964) also showed that embryos (parent generation) within the grandparents are sensitive to photoperiod and temperature cues transmitted through the semi-transparent body wall of the grandparent. Stimulation is
required at this stage to switch on the mechanisms responsible for the production of oviparae and males. My studies suggest that the cues perceived in the embryonic stage are sufficient to effect male production in A. pismum. However, it appears that stimulation overlapping two generations is required to switch on the mechanism responsible for sexual female production. In other words, for oviparae production the parent's sensitive period starts while the parent is still an embryo and continues into its early larval life.

**Fluctuating Temperatures**

The results obtained with the fluctuating temperature experiments do not clearly support any of the proposed hypotheses but suggest rather that the insect in the laboratory responds to the experimental regime(s) in a complex, non-linear manner. The photoperiodic response of the pea aphid may be peculiar because of the variability within the transition zones. However, as critical photoperiod estimates for other insects have been obtained at much longer time increments it may well be that these experimenters have missed similar 'zones of instability'. The effects of a simplified fluctuating temperature regime within this zone are not clear. In nature insects are not exposed to such constant minimum and maximum temperatures interrupted by abrupt transition zones of dark and light. It is also not known, for the pea aphid at least, what the effects of grossly abnormal temperature fluctuations are. It is possible that regimes A and D with maximum night temperatures and minimum day
temperatures disturb a circadian rhythm responsible for measuring the length of photoperiod. It may well be that a diurnal temperature regime, as found in nature, interacts with photoperiod to produce a response pattern different from that normally observed in the laboratory.

Few other investigators have dealt with the problems of fluctuating temperatures and constant photoperiods on the induction of a critical photoperiodic response. Beck (1962 a, b) at least however, has obtained evidence with the European corn borer suggesting that it is night temperatures which are most crucial in the diapause response. He utilized a different experimental design. When larvae were reared at a constant temperature of 26°C, the critical daylength was found to be approximately 15 hours. The effect of fluctuating temperatures on the induction of diapause was tested at that daylength. A relatively shallow temperature cycle was used, in which the high temperature was 31°C and the low temperature was 21°C, giving a daily mean of 26°C. He found the incidence of diapause was very high when the cool phase of the thermoperiod occurred during the scotophase, but was very low when the warm phase coincided with the scotophase. Beck's study (1962 a, b), however, also suffers from the above mentioned interpretation problems. His experimental design utilized simplified photoperiod/temperature conditions and thus there is little way of knowing whether or not his results were an artifact of the laboratory situation.

Determining the aphid response near the critical photoperiod at 17.5°C, as would be necessary if Beck's design was used
however, may have given direct experimental evidence as to
the supposed 'linearity' of the temperature vs. critical
photoperiodic response. Such studies clearly warrant
further investigation.

Field Experiment

The field experiments initially promised to produce
sexuals during a season they were not expected to occur.
They also appeared to offer a way of testing laboratory
determined sensitive period results. The negative findings,
as suggested earlier, were probably related to the low
temperatures experienced at this time. On several occasions,
the minimum temperatures dropped below the thermal threshold
for this species, the temperature at which growth is presumed
to terminate (Gilbert et al, 1976). The effects of
fluctuating temperatures on either developmental rates or
the photoperiodic mechanism at these low extremes has not
been adequately described in the literature. It may well
be that the photoperiodic mechanism is shut down at such
extremes.

Aside from the effects of temperature on the photoperiodic mechanism, it may also be possible that the direction
doing length changes interferred with the mechanism itself.
This would be the case if the aphid perceives and responds
to the direction of daylength changes, as well as to the
absolute length of daylength. The aphid may respond naturally
to a decrease in length of daylength as observed during the
autumn of mid-latitudes, but unnaturally to an increase.
of daylengths as observed during the springtime. This phenomena has rarely been recorded in the laboratory (Tauber and Tauber, 1970) utilizing stationary photoperiod regimes, and has only been inferred to actually occur in nature.

The only other attempt to produce sexual aphids under field conditions was tried by Sharma et al (1974). These investigators, however, transferred aphids at unknown ages to the field hence their procedure made it impossible to confirm that part in the aphid life history where it is sensitive to photoperiodic stimuli. At least, their autumn exposure times were sufficient to elicit some sexual production. Rather than using spring exposure conditions where temperatures may be precariously low and where the direction of daylength changes may be unnatural, the present studies indicate more information could be obtained by placing laboratory reared insects of known ages in an outside shelter in the autumn where the conditions may be more accommodating.
CHAPTER II

Computer Simulation Studies
CHAPTER II

COMPUTER SIMULATION STUDIES

Introduction

Studies of the effects of photoperiod on the ecology of overwintering have mostly been undertaken in the laboratory because of the difficulties in manipulating light regimes in nature. To simplify experiments, unrealistic light regimes and constant temperatures have been used in contrast to the complex light and temperature stimuli that insects actually experience in nature. The approach of computer simulation is one method that can be used to test whether hypotheses developed to account for laboratory data are adequate for understanding the complexities of the processes in nature. The model complements the laboratory and field studies and permits one in some objective and quantitative way to explore the relationship between the two. By objectively linking the various theories involved to explain the photoperiodic response, the model also provides a way of identifying problems that may arise in the interpretation of the response.

A computer simulation model of sexual morph initiation developed by R. J. Lamb (unpublished, see Appendix V) was modified for use in this study.
The code was altered so that temperatures in °C rather than °F could be input. Ninety-two days of minimum and maximum temperature readings obtained from Stevenson screen data at the Harrow, Agriculture Canada Research Station were fed into the model using as a starting date August 1, 1977. The model includes a linear regression equation relating daylength to date. The coefficients for the equation were calculated from data obtained from tables of sunrise and sunset (Beck, 1968) for a latitude of 42° corresponding to the latitude of Harrow. Computer runs were attempted with and without the inclusion of civil twilight in the estimate of daylength. Other parameters in the model were determined from the laboratory results on the responses of aphids to photoperiod and temperature. Various computer runs were made assuming that either night temperatures, day temperatures or an average daily temperature caused the temperature effect on the photoperiodic response.

Model Description

The model is composed of 2 sub-parts. Sub-model I attempts to predict the date of the first sexual stimulus in nature; and Sub-model II estimates the time between the date of this stimulus to the first appearance of sexuals.

An aphid clone from the location desired is sampled and tested in the laboratory to determine the threshold critical photoperiod necessary to invoke the production of sexual females or males. The threshold photoperiod is the
upper limit of the transitional photoperiods, the longest photoperiod at which the fall forms are produced. The threshold photoperiods for both sexual morphs at two temperatures were derived from the photoperiodic response curves for the species. The relationship between threshold photoperiod and temperature is assumed to be linear for the model. To use the photoperiod/threshold lines in predicting the onset of sexual morph determination in nature, estimates of natural daylength and temperature that might correspond to the laboratory conditions must be obtained. It was assumed for most of the simulation runs that the natural photoperiod for any day consisted of the time from sunrise to sunset plus twice the length of civil twilight. Civil twilight is defined as the time required for the upper limb of the sun to traverse an arc from the horizon to a point lying 6° below the horizon. Beck (1968) has indicated that many of the species so far studied are sensitive to light down to the intensities found near the end of this zone.

Sub-model I determines the natural photoperiod for each day, and then, using the photoperiod/temperature threshold equations, determines what temperature for the day would be just low enough to induce sexual morph production. This threshold temperature is compared with the actual maximum and minimum temperatures as recorded in a nearby Stevenson screen for late summer and early autumn, and the threshold temperature abstracted from the Stevenson screen temperature. Thus, the output of the model consists of
one number for each fall day, that may be positive, negative, or zero. If the number is negative, the field temperature dropped below the temperature threshold for that day, and the model predicts a sexual morph stimulus has occurred.

The starting date(s) generated from Sub-model I are input into Sub-model II. A development rate algorithm adapted from Stinner et al. (1974) is used. This group uses a developmental rate vs. temperature equation which is more accurate, particularly at low temperatures, than the linear relationship used in the classical degree-day technique. Essentially, this involves fitting the diurnal fluctuations of temperature in nature to a sine curve to obtain increments of growth each day. This algorithm requires data from developmental rate experiments.

Both values known as PER's and developmental rate parameters were required in Sub-model II to utilize the information generated in Sub-model I. PER's represent proportions of the developmental times (DT) of oviparae and males to the developmental time of virginoparae at 20°C, where:

\[
\begin{align*}
\text{DT (oviparae)} &= \text{pre-birth sensitive period} + \text{time to reproduction by the parent} + \text{time for oviparae to reach the adult moult.} \\
\text{DT (males)} &= \text{pre-birth sensitive period} + \text{time to reproduction by the parent} + \text{time to the onset of male production} + \text{time for males to reach the adult moult.}
\end{align*}
\]
Hence, these values represent the minimum intervals from the time when the temperature drops below the threshold to the time when adult oviparvae or males ought to appear in nature. They offer a means of incorporating the data on the timing of the sensitive periods. Various simulations were run with different PER values. The developmental rate parameters utilized in the model were calculated as in Stinner et al (1974).

A least-squares procedure was used to estimate the values C, B1 and B2. C, representing the asymptote, was selected to maximize the correlation coefficient. B1 and B2 represent the intercept and slope of the regression estimates. A value known as TOPT representing the temperature at which the maximum developmental rate occurs for the pea aphid was taken from Lamb (1970).

Model Input Parameters and Statistical Analysis

For the sexual female threshold equation the critical photoperiods utilized at 20°C and 15°C were 12:00L and 13:21L respectively. The male threshold regression equation was estimated from information determined experimentally by Lamb (1972). The simplest assumption was that the time differences between the female critical photoperiod upper limits and the male critical photoperiod upper limits at the Markham clone were the same as the difference at Harrow.

The regression relating time-to-natural daylength or photoperiod, including twice civil twilight, at the Harrow location is very nearly linear ($R^2 = 0.99; \hat{Y}_{10} = 15.590 - 0.047X_{10}$
where \( Y_{10} \) = photoperiod, \( X_{10} \) = days) within the late summer and early autumn time span. Excluding civil twilight, the relationship is \( Y_{11} = 14.551 - 0.045X_{11} \). The other equations needed as input information are those relating to the male and sexual female threshold photoperiods and temperature. The former may be represented by the equation \( Y_{12} = 65.113 - 3.759X_{12} \) (where \( Y_{12} \) = temperature, \( X_{12} \) = female photoperiodic threshold); and the latter assuming an identical relationship as Lamb and Pointing (1972), as \( Y_{13} = 51.861 - 2.479X_{13} \) (where \( Y_{13} \) = temperature, \( X_{13} \) = male photoperiodic threshold).

The statistical analyses were carried out using the Statistical Analysis System programs (Barr et al., 1976) on an IBM S/360 model 65 computer. For the simulation studies a WATFIV compiler on an IBM computer was utilized.

**Simulation Results**

Tables 18A and 18B show the various PER values and developmental rate parameters utilized in the simulations. PER values were calculated directly from Table 9. Four temperatures were used in the developmental rate equations. The estimated rate at 12°C for the Harrow clone was determined to be 0.057 units.

Table 19 illustrates the results of simulations predicting the appearance of the male morph using various male threshold critical photoperiod estimates and sensitive regimes. Varying the critical photoperiod threshold estimates meant that the regression coefficients entered into sub-model I were adjusted. Essentially, these adjustments
Table 18A. PER correlates of parental pre-birth sensitive periods utilized in simulation runs.

<table>
<thead>
<tr>
<th>Pre-birth Sensitive Period (days)</th>
<th>Adult Morph</th>
<th>PER Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sexual Female</td>
<td>2.49</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>2.63</td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>2.77</td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
<td>2.91</td>
</tr>
<tr>
<td>1</td>
<td>Male</td>
<td>3.23</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>3.37</td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>3.51</td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
<td>3.66</td>
</tr>
</tbody>
</table>

Table 18B. Developmental rate parameters for the Harrow clones utilized in simulation runs.

<table>
<thead>
<tr>
<th>C</th>
<th>TOPT</th>
<th>BI</th>
<th>E2</th>
<th>Clone Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.50</td>
<td>27</td>
<td>3.231</td>
<td>-0.098</td>
<td>Harrow, Ontario</td>
</tr>
</tbody>
</table>
Table 19. Simulation results predicting appearance adult male morph in Harrow field assuming parental pre-birth sensitive period of 4 days and utilizing Harrow developmental rate parameters.

<table>
<thead>
<tr>
<th>Male Critical Photoperiod Estimate</th>
<th>Sensitive Regime</th>
<th>Prediction(s) dates of Appearance Harrow, 1977</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular threshold critical photoperiod estimate&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Min.</td>
<td>Sept. 28</td>
</tr>
<tr>
<td></td>
<td>Max.</td>
<td>&gt; Oct. 31</td>
</tr>
<tr>
<td></td>
<td>Linear&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Sept. 28</td>
</tr>
<tr>
<td>Add 10 minutes to threshold critical photoperiod estimate at 15°C and 20°C</td>
<td>Min.</td>
<td>Oct. 1</td>
</tr>
<tr>
<td></td>
<td>Max.</td>
<td>&gt; Oct. 31</td>
</tr>
<tr>
<td></td>
<td>Linear&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Sept. 24</td>
</tr>
<tr>
<td>Deduct 10 minutes from threshold critical photoperiod estimate at 15°C and 20°C</td>
<td>Min.</td>
<td>Sept. 24</td>
</tr>
<tr>
<td></td>
<td>Max.</td>
<td>&gt; Oct. 31</td>
</tr>
<tr>
<td></td>
<td>Linear&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Oct. 27</td>
</tr>
<tr>
<td>Add 15 minutes to threshold critical photoperiod estimate at 15°C and 20°C</td>
<td>Min.</td>
<td>Sept. 24</td>
</tr>
<tr>
<td></td>
<td>Max.</td>
<td>&gt; Oct. 31</td>
</tr>
<tr>
<td></td>
<td>Linear&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Oct. 1</td>
</tr>
<tr>
<td>Add 15 minutes to threshold critical photoperiod estimate at 15°C</td>
<td>Min.</td>
<td>Sept. 24</td>
</tr>
<tr>
<td></td>
<td>Max.</td>
<td>&gt; Oct. 31</td>
</tr>
<tr>
<td></td>
<td>Linear&lt;sup&gt;e&lt;/sup&gt;</td>
<td>&gt; Oct. 31</td>
</tr>
<tr>
<td>Deduct 10 minutes from threshold critical photoperiod estimate at 15°C</td>
<td>Min.</td>
<td>Sept. 23</td>
</tr>
<tr>
<td></td>
<td>Max.</td>
<td>&gt; Oct. 31</td>
</tr>
<tr>
<td></td>
<td>Linear&lt;sup&gt;e&lt;/sup&gt;</td>
<td>&gt; Oct. 31</td>
</tr>
</tbody>
</table>

<sup>a</sup>: Simulation run assuming difference between male and sexual female C.P. in minutes from Lamb (1972) identical to relationship found in Harrow clone.

<sup>b</sup>: Represents sensitivity check: 10 minutes added to difference found by Lamb (1972) to Harrow clone.

<sup>c</sup>: Assuming minimum night temperatures critical (scotophase).

<sup>d</sup>: Assuming maximum day temperatures critical (photophase).

<sup>e</sup>: Assuming linear combination of night and day temperatures critical.
amounted to a sensitivity check.

Bearing in mind October 3 was the initial date of appearance of the male morph, Table 19 indicates that the assumption of minimum temperatures being most important gives the best over-all predictions. The assumption that maximum (day) temperatures are crucial leads to the worst predictions. At the regular male threshold it apparently does not matter whether a minimum or linear combination of temperatures is utilized. When it is assumed that night temperatures are most important the model consistently underestimates the field appearance of males; whereas, if it is assumed that maximum day temperatures are most important the model continually over-estimates in its prediction.

As Table 19 indicates the simulation where 10 minutes is added to threshold critical photoperiod estimates at both temperatures gives better predictions than when the regular threshold critical photoperiod estimate is assumed. This may indicate a real difference in critical photoperiodic response, or differences due to the variable nature of the critical photoperiod zone of 'instability'. Threshold endpoint instability may well alter the predicted dates of appearance of the male morph. The sensitivity check indicates that a mere 15 minutes difference in threshold measurement may alter dates of appearance by a week.

Table 20 illustrates the results of simulations predicting the appearance of the male morph while utilizing Harrow development rate parameters and minimum temperatures to test the predictability of various PER values. Bearing

* Note comment on page 61
Table 20. Simulation results predicting appearance of adult male morph in Harrow field assuming night temperatures critical and utilizing Harrow developmental rate parameters.

<table>
<thead>
<tr>
<th>Male Critical Photoperiod Estimate</th>
<th>PER&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Prediction(s) dates of Appearance Harrow, 1977</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular threshold critical photoperiod estimate</td>
<td>3.23</td>
<td>Sept. 24</td>
</tr>
<tr>
<td></td>
<td>3.37</td>
<td>Sept. 25</td>
</tr>
<tr>
<td></td>
<td>3.51</td>
<td>Sept. 27</td>
</tr>
<tr>
<td></td>
<td>3.66</td>
<td>Sept. 28</td>
</tr>
<tr>
<td>Add 10 minutes to threshold critical photoperiod estimate at 15°C and 20°C</td>
<td>3.23</td>
<td>Sept. 20</td>
</tr>
<tr>
<td></td>
<td>3.37</td>
<td>Sept. 21</td>
</tr>
<tr>
<td></td>
<td>3.51</td>
<td>Sept. 23</td>
</tr>
<tr>
<td></td>
<td>3.66</td>
<td>Sept. 24</td>
</tr>
<tr>
<td>Deduct 10 minutes from threshold critical photoperiod estimate at 15°C and 20°C</td>
<td>3.23</td>
<td>Sept. 19</td>
</tr>
<tr>
<td></td>
<td>3.37</td>
<td>Sept. 20</td>
</tr>
<tr>
<td></td>
<td>3.51</td>
<td>Sept. 22</td>
</tr>
<tr>
<td></td>
<td>3.66</td>
<td>Sept. 24</td>
</tr>
<tr>
<td>Add 10 minutes to threshold critical photoperiod estimate at 15°C and 20°C</td>
<td>3.23</td>
<td>Sept. 19</td>
</tr>
<tr>
<td></td>
<td>3.37</td>
<td>Sept. 20</td>
</tr>
<tr>
<td></td>
<td>3.51</td>
<td>Sept. 21</td>
</tr>
<tr>
<td></td>
<td>3.66</td>
<td>Sept. 24</td>
</tr>
</tbody>
</table>

<sup>a</sup> See Table 18A for sensitive period correlates of PER values.
in mind October 3rd was the date field sampling showed the first appearance of males, it is clear a pre-parental birth sensitive period of four days is the best predictor. Developmental rates vary with the mean temperature regime experienced by the aphid. With this in mind Table 20 suggests that for this autumn time interval, about four days difference in predictability exists between the assumptions of a pre-birth sensitive period of one day and of four days.

Tables 21 and 22 illustrate the simulations attempted for the sexual female morph in the field. Field sampling earlier had shown that the sexual female morph made its first appearance October 11. Given the Harrow developmental rate parameters and assuming a pre-birth sensitive period of four days, Table 21 again indicates that minimum temperature assumptions generate the best simulation predictions; whereas, maximum temperatures elicit the worst.

When civil twilight is ignored a reasonable but underestimated prediction of sexual females is made, assuming night temperatures are most important. Including civil twilight leads to an overestimation of timing of appearance of this morph. At this light intensity the prediction is improved when it is assumed the pre-birth sensitive period is one day. Here only one week over-estimation of the actual sampling date October 11 is found. However, when simulations were run ignoring civil twilight, it appears that a pre-birth sensitive period of four days gives the best predictions (see Table 22).

In the simulation studies, starting days from

*Note comment on page 61.
Table 21: Simulation results predicting appearance of sexual adult female morph in Harrow field utilizing Harrow developmental rate parameters and PER of 2.91.

<table>
<thead>
<tr>
<th>Light Intensity Simulation</th>
<th>Sensitive Regime</th>
<th>Predicting dates of appearance Harrow, 1977</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ignore Civil&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Min.</td>
<td>Sept. 29</td>
</tr>
<tr>
<td>Twilight</td>
<td>Max.</td>
<td>&gt; Oct. 31</td>
</tr>
<tr>
<td></td>
<td>Linear</td>
<td>Oct. 26</td>
</tr>
<tr>
<td>Include Civil&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Min.</td>
<td>Oct. 26</td>
</tr>
<tr>
<td>Twilight</td>
<td>Max.</td>
<td>&gt; Oct. 31</td>
</tr>
<tr>
<td></td>
<td>Linear</td>
<td>&gt; Oct. 31</td>
</tr>
</tbody>
</table>

<sup>a</sup>: regular photophase ignoring civil twilight at Harrow location (from Beck, 1968)

<sup>b</sup>: regular photophase including twice civil twilight
Table 22. Simulation results predicting appearance sexual adult female morph in Harrow field assuming night temperatures critical and utilizing Harrow developmental rate parameters.

<table>
<thead>
<tr>
<th>Light Intensity</th>
<th>PER Values</th>
<th>Prediction(s) dates of appearance Harrow, 1977</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ignore Civil Twilight</td>
<td>2.49</td>
<td>Sept. 25</td>
</tr>
<tr>
<td></td>
<td>2.63</td>
<td>Sept. 26</td>
</tr>
<tr>
<td></td>
<td>2.77</td>
<td>Sept. 28</td>
</tr>
<tr>
<td></td>
<td>2.91</td>
<td>Sept. 29</td>
</tr>
<tr>
<td>Civil Twilight</td>
<td>2.49</td>
<td>Oct. 18</td>
</tr>
<tr>
<td>Included</td>
<td>2.63</td>
<td>Oct. 20</td>
</tr>
<tr>
<td></td>
<td>2.77</td>
<td>Oct. 23</td>
</tr>
<tr>
<td></td>
<td>2.91</td>
<td>Oct. 26</td>
</tr>
</tbody>
</table>
sub-model I were only selected when a string of negative values followed each other (as explained previously each negative value was interpreted to mean a sexual stimulus had occurred). Although the field sampling procedures undertaken were extensive (Table 7A) the statistical chances of missing a few sexuals earlier in the autumn probably were high. The use of a string of negative values rather than a single value to indicate a sexual stimulus has occurred would mean in effect then, that a rapid rise of the sexual morph in the population could be predicted. Once it is obvious that the simulation produces such a string, the sexual starting date preceding a rise could be fixed and empirically derived equations $Y_6$ and $Y_7$ used to predict the extent of the sexual morph rises. Equations $Y_6$ and $Y_7$, however, could not be used on data from which they were generated (Wesolowsky, 1976). They might be suitable in predicting the rise in sexual morph frequencies in other populations.

Comments

The performance of the modified model in the prediction of timing of either morph within the field is clearly not perfect; however, few such models are. At least, consistent with the laboratory determined expectations and field sampling investigations the simulations predicted males to appear earlier than sexual females. Further, the predicted morph occurrences coincided closely with the actually sampled field appearances. This gives indirect
evidence that clonal photoperiodic response variability within the field is minimal.

Of special interest in the simulations is the conclusion that minimum temperatures should be most important in the determination of sexual production. This is in contradiction to the physiological experiments which suggest a more complex, non-linear combination of night and day temperatures are crucial. Clearly, the realism of the minimum temperature assumption remains to be tested in different geographical locations before a firm answer can be given. The differences between the physiological and computer simulation results may imply that the insect's physiological mechanism for interpreting fluctuating temperatures in the laboratory is different from that in the field. More study is needed to verify this.

The results of the simulation runs for the Harrow clone suggest that better predictions of the timing of the sexual female morph in the field can be made if civil twilight is ignored, and a pre-birth sensitive period of four days is utilized. However, when sensitive periods are chosen which increase the predictability of timing of this morph, the converse conclusion is reached. The actual effects of differing light intensities on the photoperiodic response is not known. Differential cueing to light intensity by either morph may offer yet another physiological means of achieving a closer synchrony of sexual appearance. Aside from the simulation studies, no evidence has been obtained to support this hypothesis. Physiological experiments may be in order here.
CHAPTER III

Alatae Photoperiodic Response Studies
CHAPTER III

METHODS AND MATERIALS

Photoperiodic Responses of Alatae

Periodically, alatae were produced in the parental generation by grandparents which had been exposed to various photoperiodic regimes. Rather than discard them the alatae were treated as a separate group and examined. Photoperiodic response patterns were studied at various photoperiods and exposure periods and the effects of constant or fluctuating temperatures examined. As for the apterous (or non-winged morph), when the experimental stimulus was complete, the insects were transferred into control conditions. Consecutive batches of offspring were then followed for various times until the parents died. Care had to be taken in the transferring procedure because the alatae were especially sensitive to handling, and mortality was high through drowning when nutrient solution was added.

The number of male offspring produced by the two morphs (alatae and apterae) were compared. To this end the numbers of males and females within each alate family were counted and the counts pooled to obtain percentages for each. As a control, 10 apterous parents from a 13:2L (at 15°C) regime were selected and allowed to reproduce until the end of their

85
reproductive sequences and the number of males and females counted.
RESULTS

Alate Photoperiodic Response Studies

Qualitatively it was noted that the alatae were less fecund than the non-winged morphs on individual Vicia faba leaves. This observation is in agreement with earlier investigations of aphid fecundity on their host plant (MacKay, 1975). Within the range of photoperiods studied, alatae produced few or no males. Instead, they continued to produce female offspring from the beginning of reproduction to the end. Unlike their apterous counterparts, there was no reproductive pause signalling the end of female production and the beginning of male.

For this study the assumption was that similar male/female ratios could be found in any photoperiodic regime which produced males. This assumption is not strictly true, near the critical photoperiod (Lamb, 1975) but the difference in responses of the two morphs is so great as to make the general findings still valid. For these comparisons the alatae were followed until death or the end of reproduction. Only 3 out of 9 pooled experimental regimes, or 10 out of 46 alate parents produced males. By contrast, 100 per cent of apterous parents under identical conditions gave birth to this morph. Clearly, (Table 23) the percentages of male/female offspring were markedly different in the alatae vs. apterous morphs. There
Table 23. The influence of photoperiod on the production of males by apterae and alatae.

<table>
<thead>
<tr>
<th>Alatae</th>
<th>Temperature (°C)</th>
<th>Photoperiod</th>
<th>Exposure Period Start</th>
<th>Finish</th>
<th>Number of Offspring</th>
<th>Number of Parents</th>
<th>Offspring % Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>13:07L</td>
<td>Entire period</td>
<td></td>
<td></td>
<td>36</td>
<td>2</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>15</td>
<td>13:21L</td>
<td>4</td>
<td>13</td>
<td></td>
<td>402</td>
<td>16</td>
<td>7</td>
<td>93</td>
</tr>
<tr>
<td>15</td>
<td>12:53L</td>
<td>Entire period</td>
<td></td>
<td></td>
<td>283</td>
<td>15</td>
<td>1</td>
<td>99</td>
</tr>
<tr>
<td>15</td>
<td>12:30L</td>
<td>Entire period</td>
<td></td>
<td></td>
<td>81</td>
<td>2</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>15</td>
<td>11:15L</td>
<td>6</td>
<td>15</td>
<td></td>
<td>56</td>
<td>2</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>20</td>
<td>11:00L</td>
<td>3</td>
<td>13</td>
<td></td>
<td>24</td>
<td>1</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>20</td>
<td>11:45L</td>
<td>Entire period</td>
<td></td>
<td></td>
<td>144</td>
<td>4</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>15 (night) and 20 (day)</td>
<td>11:45L</td>
<td>Entire period</td>
<td></td>
<td></td>
<td>147</td>
<td>2</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>15 (night) and 20 (night)</td>
<td>13:00L</td>
<td>Entire period</td>
<td></td>
<td></td>
<td>84</td>
<td>4</td>
<td>2</td>
<td>98</td>
</tr>
</tbody>
</table>

Apterae Control
13:21L; 15°C
10 apterous parents

<table>
<thead>
<tr>
<th>Percent</th>
<th>Number of Offspring</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>69</td>
<td>338</td>
<td>Male</td>
</tr>
<tr>
<td>31</td>
<td>153</td>
<td>Female</td>
</tr>
</tbody>
</table>
may be a trend towards slightly more males being produced at higher photoperiods.

Comparisons were also made of female sexual morph production (Table 24). Counts of the numbers and kinds of females were pooled for each and the percentages of oviparae and viriginoparae recorded. On the basis of these 2 comparisons little difference in proportion of sexual females produced is evident.
Table 24. The influence of photoperiod on the production of females by apterae and alatae.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Morph</th>
<th>Photoperiod</th>
<th>Exposure Period</th>
<th>Number of Offspring</th>
<th>Number of Parents</th>
<th>Percent Oviparae Virginoparae</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Alatae</td>
<td>11:15L</td>
<td>6</td>
<td>15</td>
<td>56</td>
<td>2</td>
</tr>
<tr>
<td>15</td>
<td>Apterous</td>
<td>11:15L</td>
<td>6</td>
<td>15</td>
<td>323</td>
<td>17</td>
</tr>
<tr>
<td>15</td>
<td>Alatae</td>
<td>13:21L</td>
<td>4</td>
<td>13</td>
<td>374</td>
<td>16</td>
</tr>
<tr>
<td>15</td>
<td>Apterous</td>
<td>13:21L</td>
<td>4</td>
<td>13</td>
<td>64</td>
<td>3</td>
</tr>
<tr>
<td>15</td>
<td>Alatae</td>
<td>12:53L</td>
<td>Entire Exposure</td>
<td>280</td>
<td>13</td>
<td>100</td>
</tr>
<tr>
<td>15</td>
<td>Apterous</td>
<td>12:53L</td>
<td>Entire Exposure</td>
<td>49</td>
<td>3</td>
<td>100</td>
</tr>
</tbody>
</table>
DISCUSSION

Alatae Photoperiodic Response Studies

The experiments on alatae demonstrated that the photoperiodic response of the winged forms differs from that of the non-winged morph. Production of sexual female offspring appeared to be equivalent in the two morphs; whereas few, if any, males were produced by the alate morph at photoperiod and temperature regimes, which clearly gave 100 per cent male production in their apterous counter-parts.

The physiological basis of alata-production is probably quite complex (MacKay, 1977). In certain species of aphids, changes in the activity of the corpus allata and in the concentration of juvenile hormone have been correlated with the production of alatae (Lees, 1961, 1966; Johnson, 1959). It has been suggested that an apterous aphid represents a neotenic (juvenilized) adult. Lees (1961) reported that wing development could be partially suppressed by topical applications of JH concentrates to the early instars of alate-determined vetch aphid nymphs. Through decapitation experiments this latter investigator also demonstrated that the aphid embryos do not become competent to respond to alata-promoting influences until a few hours before birth. It is the parent that determines whether the aphid is to be winged or non-winged. Crowding and host plant stimuli are
presumed to be the main environmental cues which trigger this physiological mechanism in the parent (Lees, 1967; Sutherland, 1969b).

Little direct evidence is available linking the alate and sexual morphs. In certain parthenogenetic and polyphagous species, photoperiod has been suggested as an environmental cue in alata-production. In the former case, such as the species Macrosiphoniella sanborni, this cue may be utilized (White, 1946), however, this is not really pertinent as sexual morphs are not produced by this species. Where host switching is evident, as in Macrosiphum euphorbiae, MacGillivray and Anderson (1964) found that apterous parents tended to produce more alate viviparous females and males; while alate parents gave a higher proportion of opterous asexuals and oviparae. The difficulty in assessing the latter finding is that no indication is given of the sensitive period in this species. Further, unlike the pea aphid, male production does not seem to follow sexual female formation within a single apterous family. The pea aphid appears to be the only species of aphid discovered so far in which the sensitive periods for male and sexual female production, and the photoperiodic responses of the alate and apterous morph differ.

The physiological findings on the photoperiodic response of the parental alatae complicate, and contradict (Lees, 1966) previous hypotheses concerning the timing of the effector mechanisms involved in aphid polymorphisms. It had been assumed previously that the sex of the offspring is
determined first, followed by the determination of the female sexual morph, and finally the determination of wing polymorphism. The alate response patterns studied here indicate otherwise. The sensitivity studies performed (see Tables 23: 13A, B, C) suggest the parental, embryonic apterous morph is sensitive to the stimuli controlling male morph production and that this sensitivity ends very near parental birth. The fact that the parental alate morph produced few, if any male progeny suggests that either the ovarioles within the alatae are rendered incompetent to receive the male stimuli, or that the supposed endocrinological stimuli (Lees, 1961, 1963) initiating male production did not occur. For these sequence of events to have occurred the implication is that the alate or apterous morphs are determined prior to the determination of the sex of the offspring.

The sexual female response patterns (see Tables 24: 13A, B, C) suggest that the sensitive period for this morph within the life histories of the alate and apterous morphs are similar. This finding alone is consistent with the previously mentioned hypotheses (Lees, 1966), but when combined with the male data suggest real photoperiodic response differences. There may be ecological reasons for the development of these differences in the two morphs.

If it is assumed that alatae land in a field previously uncolonized or one in which the aphids are patchily distributed through their habitats (Way and Cammell, 1970) it would be a good evolutionary strategy for them to increase their numbers as quickly as possible before the onset of harsh
conditions. Assuming random mortality over the winter, maximizing the numbers of individuals in a clone would increase the probability of the survival of that clone. By producing asexual offspring and oviparae, but not males, such a mechanism is provided. Asexual multiplication would have the effect of greatly increasing the numbers of aphids to serve as grandparents for the sexual stimulus. Many more fertilized eggs capable of entering diapause could then be formed. Diverting alate reproductive effort to males production would decrease the number.

This general scheme rests on the assumption that the original alate colonist is able to produce asexual females. This only occurs in the autumn when the sexual cues have not specified 100 per cent sexual female production. Later in the autumn, however, if a disperser landed in a new field she would have to depend on resident males to fertilize her sexual female offspring. On the other hand, if the field had yet to be colonized or the population was 'patchy' such that local males were isolated on individual plants this might make fertilization difficult and on this basis, late migrations of alatae would not be expected to occur.
SUMMARY

Field and laboratory studies on the pea aphid, *Acyrthosiphon pisum* (Harris), were carried out to test and compare various hypotheses concerning the photoperiodic response of this aphid. Laboratory studies using simplified photoperiod and temperature treatments were utilized to determine the critical photoperiod and transition zone for this insect at 15°C and 20°C. Developmental rates were measured at these same temperatures to obtain a more, precise fix on the ages at which the test aphids entered the experimental regimes. Utilizing a variety of exposures at both short photoperiods and critical photoperiods the periods of sensitivity in the life history of the insect were investigated. The effects of fluctuating temperatures near critical photoperiods on sexual morph production were also studied. Independent investigations of the photoperiodic response of the alate morph were further undertaken. The field work included various temperature measurements in an alfalfa field; sampling of the sexuals in the fall; and a field experiment designed to test the laboratory sensitive period findings by transferring aphids at known ages to an outdoor shelter in early April. A computer simulation model was utilized to provide a conceptual link between the field and laboratory studies.
**APPENDIX I**

Life History terminology utilized to describe the pea aphid, *Acrystosiphon pisum* (Harris), and aphids in general.

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Technical Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asexual females</td>
<td>virginoparae</td>
</tr>
<tr>
<td>Sexual females</td>
<td>Oviparae</td>
</tr>
<tr>
<td>Non-winged aphids</td>
<td>Apteræ</td>
</tr>
<tr>
<td>Winged aphids</td>
<td>Alatae</td>
</tr>
</tbody>
</table>

**Parental Offspring Options**

- Grandparent generation (asexual aphid) → Parent generation (asexual aphid)

**Offspring**
- Males
- Oviparae
- Virginoparae
APPENDIX II
Photoperiodic response strategies for the pea aphid, *Acyrthosiphon pisum* (Harris).

- **Parent**
  - **Offspring**

(a) **Parent aphid**

- Asexual females
  - Males

(b) **Parent aphid**

- Asexual females
  - Pause
  - Males

(c) **Parent aphid**

- Asexual and Sexual females
  - Pause
  - Males

(d) **Parent aphid**

- Sexual females
  - Pause
  - Males
APPENDIX III

Photoperiodic Response Data at 15°C and 20°C.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Photoperiod (X Variable)</th>
<th>% Sexual Females (with replications done independently) (Y Variable)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15°</td>
<td>12:00L</td>
<td>100</td>
</tr>
<tr>
<td>15</td>
<td>12:39L</td>
<td>99, 90, 94</td>
</tr>
<tr>
<td>15</td>
<td>12:53L</td>
<td>100</td>
</tr>
<tr>
<td>15</td>
<td>13:00L</td>
<td>93</td>
</tr>
<tr>
<td>15</td>
<td>13:07L</td>
<td>52</td>
</tr>
<tr>
<td>15</td>
<td>13:21L</td>
<td>85, 5, 1, 0, 3</td>
</tr>
<tr>
<td>20</td>
<td>11:25L</td>
<td>98</td>
</tr>
<tr>
<td>20</td>
<td>11:42L</td>
<td>77, 96</td>
</tr>
<tr>
<td>20</td>
<td>11:50L</td>
<td>56</td>
</tr>
<tr>
<td>20</td>
<td>12:00L</td>
<td>0</td>
</tr>
</tbody>
</table>

Each experimental response represents the pooling of 180 to 320 female offspring produced from between 14-20 parents.
APPENDIX IV.

Comparison of photoperiodic response data of Markham and Harrow clones of the pea aphid, *Acyrthosiphon pisum* (Harris).

Latitude: 43° 25'  
Markham Clone

Latitude: 42° 02'  
Harrow Clone

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Lower Limit</th>
<th>C.P.</th>
<th>Upper Limit</th>
<th>Lower Limit</th>
<th>C.P.</th>
<th>Upper Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>20°C</td>
<td>&lt;12:01L</td>
<td>12:30L</td>
<td>12:55L</td>
<td>11:31L</td>
<td>11:49L</td>
<td>12:07L</td>
</tr>
<tr>
<td>15°C</td>
<td>12:00L</td>
<td>13:00L</td>
<td>13:23L</td>
<td>12:27L</td>
<td>13:06L</td>
<td>13:45L</td>
</tr>
</tbody>
</table>

* Photoperiodic response parameters estimated from regression analysis.

** Critical photoperiod.
Appendix V (Sub-Model I)

A. *pisum* phenology program

Calculates the number of degrees below the threshold temperature for each day, August 1st - October 15th.

**Read Statements**

Read, year (read weather data in °C)

Read (5,25)(Tmax(I),I=1,92)

Read, year

Read (5,25)(Tmin(I),I=1,94)

25 Format (26F3.1)

Read, AP,Bp (photoperiod parameters for a specific location used to calculate daily photoperiod)

Read, AT,BT (temperature parameters for a specific relationship between temperature and photoperiod thresholds).
JOB WATFIV XXXXXXXXXXXX REELEDER

REAL TMAX(122), TMIN(122), THMAX(76), THMIN(76), THSH
INTERGER DAY(100)
INTERGER DMON(100)

DO 4 I=1,31
DMON(I)=1
CONTINUE
READ,YEAR
READ(5,25)(TMAX(I),I=1,92)
READ,YEAR
READ(5,25)(TMIN(I),I=1,94)
READ,AP,BP
READ,AT,BT
DO 40 I=1,76
DAY(I)=I
X=AP+BP*I
THSH=AT+BT*X
THMAX(I)=TMAX(I)-THSH
THMIN(I)=TMIN(I)-THSH
CONTINUE
WRITE (6,55)
WRITE (6,70)
WRITE (6,75) AP,BP,AT,BT
WRITE(6,85)(DMON(I),I=1,31)
WRITE (6,90)(DAY(I),I=1,76)
WRITE(6,100)(THMIN(I),I=1,31), (TMAX(I),I=1,31)
WRITE (6,110)(DAY(I),I=32,61)
WRITE (6,115)(THMIN(I),I=32,61), (TMAX(I),I=32,61)
WRITE(6,120)(DAY(I),I=62,76)
WRITE(6,125)(THMIN(I),I=62,76), (TMAX(I),I=62,76)
WRITE(6,55)
FORMAT ('1')
FORMAT('0', 'THE NUMBER OF DEGREES BELOW THRESHOLD FOR EACH
1DAY')
FORMAT('0','DAILY PHOTO = ',1F9.3, '+' ,1F9.3, ' *DAY,
2THRESHOLD= ',21F9.3,' +',1F9.3,' *PHOTOPERIOD')
FORMAT(0', 'DAYS OF THE MONTH', 6X, 11I3)

FORMAT(0', 'AUGUST', 'DAY', 31I3)

FORMAT(0', 'SEPTEMBER', 'DAY', 31I3)

FORMAT(0', 'OCTOBER', 'DAY', 31I3)

FORMAT(0', 'NOVEMBER', 'DAY', 30I3)

FORMAT(0', 'DECEMBER', 'DAY', 31I3)

FORMAT(0', 'TMIN', 'TMX', 31F4.1, 'TMAX', 30F4.1)

FORMAT(26F4.1, 'TMIN', 15F4.1, 'TMAX', 25)

ENTRY
Appendix V (Sub-Model II)

A. pisum phenology program

Calculates the date(s) of appearance of either sexual morph (male or oviparae) given date(s) sexual stimuli presumed to begin.

Read Statements

Read, Number of fall days
Read (5,25) (Tmax (J), J=1,N)
25 Format (26F3.1)
Read (5,25) (Tmin(J), J=1,N
Read, Parameters for temperature/developmental rate curve
Read, C, TOPT, B1, B2
Read, Titles
Read (5,5, End = 53)
Read proportion of development (PER)
Read, PER
Read, Number of starting days
Read, NSD, (SDAY(I), I=1, NSD)
$JOB WATPIV XXXXXXXXXX REELEDER

REAL QUIP(20), TEMP(1224), TMAX(200), TMIN(200)
INTEGER YEAR, SDAY(20), SI
C READ NUMBER OF FALL DAYS
READ, N
C READ YEAR AND MAX. AND MIN. DAILY TEMPERATURES FOR FALL MONTHS
READ, YEAR
READ(5, 25) (TMAX(J), J=1, N)
READ, YEAR
READ(5, 25) (TMIN(J), J=1, N)
C READ PARAMETERS FOR TEMPERATURE/DEVELOPMENTAL RATE CURVE
READ, C, TOPT, B1, B2
C DO LOOP CALCULATES 8 DAILY TEMPERATURES FROM MAX. AND MIN.
K=1
DO 35 I=1, N
DO 30 J=1, 8
X=3*FLOAT(J)
TM=TMIN(I)
IT=I-1
IF(IT.LT.1) IT=1
IT1=I+1
IF(IT1.GT.N) IT1=N
TX=TMAX(IT)
IF(X.GT.5.) TX=TMAX(I)
IF(X.GT.14) TM=TMIN(IT1)
TEMP(K)=TCALG(X, TM, TX)
K=K+1
C 30 CONTINUE
C 35 CONTINUE
C READ TITLES
C READ (5, 5, END=53)
C READ PROPORTION OF DEVELOPMENT (PER)
READ, PER
C READ NUMBER OF STARTING DAYS
READ, NSD, (SDAY(I)), I=1, NSD
C DO-LOOP CALCULATES TIME FOR DEVELOPMENT STARTING FROM SDAY
C XINC IS LENGTH OF TIME INTERVAL BETWEEN ESTIMATED TEMPS
XINC=.125
N1=N*8
DO 50 I=1,NSD
SUM=0
X=0.
SI=SDAY(I)*8.7
DO 40 K=SI,N1
T=Temp(K)
X=X+XINC
IF(T,GT,TOPT)T=2,*TOPT-T
SUM=SUM+SHAPE (C,B1,B2,T)*XINC
IF(SUM,GE,PER)GO TO 45
CONTINUE
QUIP(I)=1,
GO TO 50
C ESTIMATE FIRST APPEARANCE OF MÖRPH
C ASSIGN FIRST APPEARANCE TO QUIP
QUIP(I)=SDAY(I)+X
CONTINUE
WRITE (6,60)
WRITE (6,65)
WRITE(6,70)
WRITE(6,75)
WRITE(6,5)
WRITE(6,8)
WRITE(6,9)
WRITE(6,10) N,PER,C, TOPT,B1,B2
WRITE(6,80) YEAR
WRITE (6,85)(SDAY(I),I=1,NSD)
WRITE (6,90)
WRITE(9,95)(QUIP(I),I=1,NSD)
GO TO 4
WRITE(6,60)
FORMAT('15X,' NUMBER OF DAYS PROP. OF DEVELOPMENT PARAMETERS FOR
2R DEVELOPMENTAL RATE FUNCTION')
61 9 FORMAT(12X,'N',1BX,'PER',18X,'C TOPT BI B2')
62 10 FORMAT(11X,1I3,16X,1F5.2,16X,1F5.2,1F5.0,1F6.3,1F9.3)
63 25 FORMAT(26F3.1)
64 60 FORMAT('1')
65 65 FORMAT(5X,'A.PISUM PHENOLOGY, PROGRAM 4')
66 70 FORMAT(5X,'ESTIMATES DAY OF FIRST APPEARANCE OF MORP')
67 75 FORMAT(5X,'Day 1 IS AUGUST ISt')
68 80 FORMAT(5X,'YEAR',12X,1I6)
69 85 FORMAT(5X,'START DAY',7X,15I6)
70 90 FORMAT(5X,'FIRST')
71 95 FORMAT(5X,'APPEARANCE',6X,15F6.1)
72 STOP
73 END

74 FUNCTION SHAPE(AK1,AK2,AK3,X)
75 C FUNCTION CALCULATES PROPORTION OF DEVELOPMENT FOR INTERVAL
76 A=AK2+AK3*X
77 IF(A.LT.-50.) GO TO 20
78 IF(A.GT.50.) GO TO 10
79 SHAPE=AK1/(1.+EXP(A))
80 RETURN
81 20 SHAPE=AK1
82 RETURN
83 10 SHAPE=0
84 RETURN
85 END
FUNCTION TCALC(T,TM,TX)
FUNCTION ESTIMATES TEMPERATURES FOR INTERVALS FROM MAX. AND MIN.

C
AK=.3490658504.
IF(T.LT.6.) GO TO 10
IF (T.LT.15.) GO To 20
T1=T-15
AK=.2094395103
GO TO 30
10 T1=T+9
GO TO 30
20 T1=T+3
30 ALP=(TX-TM)/2.
TCAL=TM+ALP*(1.+COS(AL*T1))
RETURN
END
LITERATURE CITED


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