1979

Aspects of the photoperiodic response of the pea aphid Acyrthosiphon pisum (Harris).

David John. Reeleder
University of Windsor

ASPECTS OF THE PHOTOPERIODIC RESPONSE OF THE

PEA APHID ACYRTHOSIPHON 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
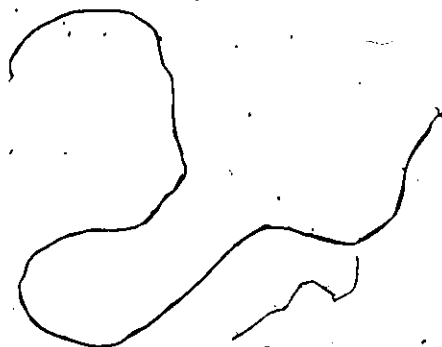
ABSTRACT

The critical photoperiods for sexual females of the pea aphid, Acyrthosiphon pisum (Harris), were determined to be 13:06L and 11:49L at $15^{o}C$ and $20^{o}C$, 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 $20^{o}C$ 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.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF APPENDICES

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

1

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 _Mamestra 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 <u>Acronycta</u> <u>rumicis</u> 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, <u>Megoura</u> <u>viciae</u>, 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 dirunal 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 $20^{o}C$ 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. pisum 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 pisum (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 photo-periodic 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, $\pm 1^{\circ}C$)
was calibrated in the laboratory with a YSI series 400
electronic thermometer (accuracy, $\pm$ .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

11

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 thermimeter and the maximum-minimum thermimeter 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 stereo-
microscope. 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, <u>Vicia</u> <u>faba</u> 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 ($\approx 20^\circ C$) under Agro Lite fluorescent tubes (Westinghouse) at a photoperiod of 16L: 8D. The aphid stock culture was kept at $20 \pm .5^\circ C$ 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 $\approx 1^{\circ}C$ 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°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°C and 20°C.

The duration of exposure to an experimental photo-
period at 15°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°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°C and 20°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°C and 20°C. Rates
of development for asexuals were also measured at 23.5°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 ($\pm$ 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 ($\pm$ 0.5^{o}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 $15^{\circ}C$ and $20^{\circ}C$ 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 $20^{\circ}C$). 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 grand-
parents 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 ($20^{o}C$ day; $15^{o}C$ 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 $20^{o}C$) and placed inside petri dishes located in the tin cans. As noted above, the lid was closed in the $15^{o}C$ experimental chamber simulating night-time, and then the lid was removed and the tin transferred to the $20^{o}C$ 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; $Y_1 = 1.18026 \, X_1 \quad -5.99$ where Y_1 = field min., X_1 = 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 = field max., X_2 = 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 = tip temperature, X_3 = control temperature). The

Table 1. Relationship between dependent
variable, Harrow minimum temperature,
and independent variable, Stevenson
screen minimum.

Variable Sunshine Removed

	df	Sum of Squares	Mean Square	F	Prob.$>$F
Regression	1	75.4846	75.4846	44.14	0.0001
Error	10	17.1020	1.7102		
Total	11	92.5866			

	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.

	df	Sum of Squares	Mean Square	F	Prob.>F
Regression	1	128.9735	128.9735		
Error	10	29.2207	2.9220	44.14	0.0001
Total	11	158.1942			

	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.

	df	Sum of Squares	Mean Square	F	Prob.>F
Regression	1	88.2812	88.2812	33.23	0.0003
Error	9	23.9101	2.6566		
Total	10	112.1913			

	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.

	df	Sum of Squares	Mean Square	F	Prob.$>$F
Regression	1	287.7628	287.7628	246.33	0.0001
Error	14	16.3547	1.1682		
Total	15	304.1175			

	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_{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.

Field Equations	Reference No.	Variables Defined	
$Y_{min.} = 1.18 X_{min.} - 5.99$	(1)	$Y_{min.}$ Y_{max}	Max.-min thermometer in crop
$Y_{max.} = 0.837 X_{max.} + 5.12$	(2)	$X_{min.}$ $X_{max.}$	Stevensen screen max. and min. temperature
$Y_{tip} = 0.981X + 2.33$	(3)	Y_{tip} -	temperatures recorded with probe in terminal
		X -	mercury thermometer in canopy
$Y_{cmax.} = 0.821 X_{max.} + 7.35$	(4)	$Y_{cmax.}$	temperatures derived from combination $Y_{max.}$ and $Y_{tip.}$
$Y_{avg.} = X_{avg.} + 0.65$	(5)	$Y_{avg.}$	-daily average of $Y_{cmax.}$ and $Y_{min.}$
		$X_{avg.}$	daily average Stevensen screen temperatures

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

field aphid responses.

Throughout the calculations of the linear regressioms 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.

General Weather Conditions	Date	Time	Tip	Middle	Ground
Sunny	Sept. 7	14:05 hrs	22.0 cm[b] 20.5°C[c]	11.00 cm 22.0°C	0,23.5°C
Sunny	Sept. 9	11:35 hrs	20.0 cm 27.3°C	11.50 cm 27.3°C	0,30.5°C
Sunny	Sept. 9	13:00 hrs	30.5 cm 28.0°C	14.00 cm 28.2°C	0,28.5°C
Sunny	Sept. 9	13:00 hrs	30.5 cm 28.5°C	14.00 cm 28.0°C	0,28.8°C
Cloudy	Sept. 10	12:00 hrs	30.5 cm 18.5°C	15.25 cm 19.2°C	0,19.9°C
Cloudy	Sept. 10	12:10 hrs	25.0 cm 18.0°C	20.00 cm 21.5°C	0,20.5°C
Cloudy	Sept. 10	12:15 hrs	29.0 cm 18.0°C	9.00 cm 18.0°C	0,19.1°C
Partially Cloudy	Sept. 11	11:30-12:00 hrs[d]	34.0 cm 22.0°C	17.00 cm 21.0°C	0,22.5°C
Partially Cloudy	Sept. 11	11:30-12:00 hrs	30.0 cm 21.0°C	15.00 cm 21.0°C	0,21.0°C
Partially Cloudy	Sept. 11	11:30-12:00 hrs	29.0 cm 21.0°C	14.50 cm 22.5°C	0,23.0°C
Partially Cloudy	Sept. 11	11:30-12:00 hrs	29.0 cm 21.5°C	14.50 cm 21.0°C	0,24.0°C
Partially Cloudy	Sept. 11	11:30-12:00 hrs	32.0 cm 21.2°C	16.00 cm 22.0°C	0,22.0°C
Partially Cloudy	Sept. 11	13:30-12:00 hrs	29.0 cm 21.0°C	14.50 cm 21.0°C	0,23.0°C

a: read with a YSI tele-thermometer ($\pm$ 0.5°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.

	% Asexual Female	% Sexual Female	% Male	N
Sept. 23	100.0	0.0	0.0	1319
26	100.0	0.0	0.0	523
30	100.0	0.0	0.0	938
Oct. 3	99.8	0.0	0.2	1013
6	99.8	0.0	0.2	945
11	99.6	0.1	0.3	996
17	92.7	5.5	1.8	712
24	70.2	22.8	7.0	639
31	41.1	42.0	16.9	362

Table 7B. Regression equation relating the occurrence
of males with time in days. The date of
first appearance was set equal to 1.

	df	Sum of Squares	Mean Square	F	Prob.>F
Regression	1	3.3906	3.3906	113.67	0.0004
Error	4	0.1193	0.0298		
Total	5	3.5099			

	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.

	df	Sum of Squares	Mean Square	F	Prob.>F
Regression	1	3.4945	3.4944	10.27	0.0851
Error	2	0.6807	0.3404		
Total	3	4.1752			

	B Value
Intercept	-0.660
Day	0.125

$R^2 = 0.837$

relationship with time was nearly linear (R^2 = 0.966)

(Table 7B; 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°C, 20°C and

23.5°C; and sexuals at 15°C and 20°C (Table 9). There was

high mortality among the progeny of parents grown at 23.5°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°C and 20°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°C

Table 9. Mean developmental times of asexuals and experimentally produced sexuals with respective standard errors (in days).

Morph and Developmental Rate Criteria	$(\bar{X} \pm S.E.)$ 15°C	20°C	23.5°C
Asexual females			
Birth-to-last Moult	11.7 ± 0.69 (n = 30)	7.0 ± 0.76 (n = 36)	5.1 ± 0.30 (n = 11)
Birth-to-onset reproduction	14.2 ± 0.61 (n = 30)	8.2 ± 0.44 (n = 29)	7.4 ± 0.51 (n = 11)
Sexual Females			
Birth-to-last moult	12.5 ± 0.52 (n = 14)	8.2 ± 0.37 (n = 39)	—
Males			
Birth-to-last moult	15.1 ± 0.71 (n = 14)	9.4 ± 0.49 (n = 21)	—

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 X_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 X_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.

Temperature (^{o}C)	Photoperiod	Number of Parents	% Sexual Female Offspring	Number of Offspring
15	12:39L	14	99	184
15	12:39L	17	90	238
15	12:39L	16	94	219
15	13:21L	17	85	221
15	13:21L	17	5	230
15	13:21L	17	-1	298
15	13:21L	19	0	285
20	11:42L	20	96	284
20	11:42L	18	77	244

Table 11.   Relationship between photoperiod (hours of light) and sexual female morph response within the transition zone at $15^{\circ}C$.

	df	Sum of Squares	Mean Square	F	Prob. > F
Regression	1	11809.583	11809.583	11.66	0.0066
Error	10	10131.514	1013.151		
Total	11	21941.097			

	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*

Transition Zone at $15^{\circ}C$
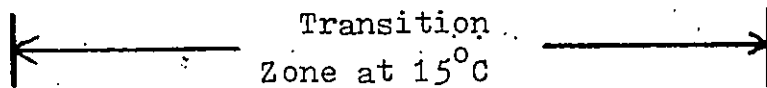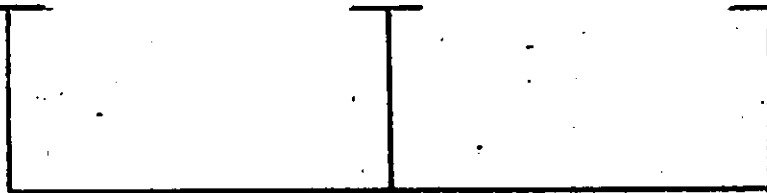
* Regression estimates

Table 12. Relationship between photoperiod (hours of

light) and sexual female morph response within

the critical photoperiod transition zone at $20^{o}C$.

	df	Sum of Squares	Mean Square	F	Prob. $>$ F
Regression	1	4981.776	4981.776	9.67	0.0529
Error	3	1545.944	515.315		
Total	4	6527.720			

	B Value
Intercept	1998.070
Photoperiod	-164.756

$R^2 = 0.763$

Lower limit
100% oviparous morph
11:31L*

C.P. = 11:49L*

Upper limit
0% oviparous morph
12:07L*

Transition
Zone at $20^{o}C$

* 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 rest
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 grand-
parents, the aphids were exposed for various periods at

either 15°C or 20°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°C), 13:07L (15°C) and 11:57L (20°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°C), 12:00L (15°C) and 11:00 (20°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°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°C or D5 at 20°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.

Expt. #	Temperature (°C)	Photoperiod	Exposure Period Start	Finish	Number of Parents	% Producing Males	% Oviparae
1	15	13:21L	4	10	16	100	0
2	15	13:21L	4	13	12	100	0
3	15	13:21L	3	16	15	100	*1
4	15	13:21L	1	14	4	100	0
5	15	13:21L	1(control)	30	17	100	3
6	15	13:21L	1(control)	30	17	100	85
7	15	13:07L	2	19	12	100	0
8	20	11:57L	4	8	17	100	0
9	20	11:57L	7	9	18	78	0

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.

Expt. #	Temperature (°C)	Photoperiod	Exposure Period Start	End	Number of Parents	% Producing Males	% Oviparae
1	15	11:15L	1	2	12	0	0
2	15	11:15L	5	8	12	0	0
3	15	11:15L	6	7	16	0	0
4	15	11:15L	8	11	15	85	0
5	15	11:15L	4	16	11	91	0
6	15	11:15L	3	13	14	86	0
7	15	11:15L	1	11	14	14	0
8	15	11:15L	6	15	17	100	0
9	15	11:15L	6	18	7	100	100
10	15	11:15L	1(control)	30	20	100	100
11	15	12:00L	1	2	18	0	0
12	15	12:00L	1(control)	30	18	100	100

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.

Expt. #	Temperature (°C)	Photoperiod	Exposure Period Start	End	Number of Parents	% Producing Males	% Oviparae
1	20	11L	5	8	18	88	0
2	20	11L	1	8	14	85	0
3	20	11L	1	12	12	100	86
4	20	11L	6	17	6	100	78
5	20	11L	6	13	18	100	74
6	20	11L	6	17	14	100	86
7	20	11L	6	14	8	100	80
8	20	11L	4	11	15	100	0
9	20	11L	4	11	10	100	22
10	20	11L	4	14	12	100	94
11	20	11L	1	18	20	100	100

Table 13D.   The effect of duration of exposure
to a short photoperiod on sexual morph
production at two temperatures.

Temperature	Male Production			Oviparae Production		
	Exposure Duration	End Day	% Male	Exposure Duration	End Day	% Oviparae
15	3	11	85	12	18	100
20	3	8	88	11	12	86

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 diagramatically 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.

Regime A		Regime B		Regime C		Regime D	
12:15D	20°C	12:15D	15°C	11:00D	15°C	11:00D	20°C
11:45L	15°C	11:45L	20°C	13:00L	20°C	13:00L	15°C

1)   Day temperatures important:

    Regime A   100 per cent sexual females

    Regime B   transitional response

    Regime C   0 per cent sexual females[6]

    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 confounded 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.

Regimes	Experiment	Number of Parents	Number of Offspring	Percent Oviparae
A	1	12	152	94
A	2	12	163	99
B	3	14	190	100
C	4	14	181	0
C	5	8	119	0
D	6	20	288	100

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 <u>Acyrthosiphon</u>
<u>pisum</u>.

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

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

All experiments at 11L with $20^{o}C$ photophase and
$15^{o}C$ 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. Ther 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.

Expt. #	Starting Dates	Age of grandparent at start	Date exposure ended	Number of parents	Number of Offspring	Percent Sexuals (Male or Female)
1	April 7	6	April 10	10	209	0
2	April 11	6	April 14	12	195	0
3	April 1	1	April 11	15	288	0
4	April 1	6	April 10	16	367	0
5	April 5	2	April 11	15	220	0

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 Stevensen 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

58

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 ($\leq$ 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 photoperiodic 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 $20^{o}C$ 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 $15^{o}C$, 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 $15^{\circ}C$, 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 single higher temperatures. If as Lamb and Pointing (1972) suggest the mechanism for sexual morph production utilizes both photo-period 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 photo-periods 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 <u>Megoura viciae</u>, 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 <u>M</u>. <u>viciae</u>, whereas
males are not. This contrasts to <u>A</u>. <u>pisum</u> 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 photo-
period 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. pisum. 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 rythm responsible for
measuring the length of photoperiod. It may well be that
a dirunal 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 $26^{o}C$, 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 $31^{o}C$ and the low temperature
was $21^{o}C$, giving a daily mean of $26^{o}C$. He found the incidence
of diapause was very high when the cool phase of the thermo-
period 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 $17.5^{o}C$, 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 photo-
periodic mechanism, it may also be possible that the direction
of daylength 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 ^{o}C rather than ^{o}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 $20^{o}C$, where:

DT (oviparae) = pre-birth sensitive period + time to reproduction by the parent + time for oviparae to reach the adult moult.

DT (males) = pre-birth sensitive period + time to reproduction by the parent + time to the onset of male production + time for males to reach the adult moult.

Hence, these values represent the minimum intervals from the time when the temperature drops below the threshold to the time when adult oviparae 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 $20^{o}C$ and $15^{o}C$ 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 ($R^2 = 0.99$; $Y_{10}=15.590 -0.047X_{10}$

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 $12^{\circ}C$ 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.

Pre-birth Sensitive Period (days)	Adult Morph	PER Value
1	Sexual Female	2.49
2	" "	2.63
3	" "	2.77
4	" "	2.91
1	Male	3.23
2	"	3.37
3	"	3.51
4	"	3.66

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

C	TOPT	BI	B2	Clone Location
0.50	27	3.231	-0.098	Harrow, Ontario

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.

Male Critical Photoperiod Estimate	Sensitive Regime	Prediction(s) dates of Appearance Harrow, 1977
Regular threshold critical photoperiod estimate[a]	Min.[c]	Sept. 28
	Max.[d]	>Oct. 31
	Linear[e]	Sept. 28
Add 10 minutes to threshold critical photoperiod estimate at 15°C and 20°C	Min.	Oct. 1
	Max.	>Oct. 31
	Linear	Sept. 24
Deduct 10 minutes from threshold critical photoperiod estimate at 15°C and 20°C	Min.	Sept. 24
	Max.	>Oct. 31
	Linear	Oct. 27
Add 15 minutes to threshold critical photoperiod estimate at 15°C and 20°C	Min.	Sept. 24
	Max.	>Oct. 31
	Linear	Oct. 1
Add 15 minutes to threshold critical photoperiod estimate at 15°C	Min.	Sept. 24
	Max.	>Oct. 31
	Linear	>Oct. 31
Deduct 10 minutes from threshold critical photoperiod estimate at 15°C	Min.	Sept. 23
	Max.	>Oct. 31
	Linear	>Oct. 31

a: simulation run assuming difference between male and sexual
   female C.P. in minutes from Lamb (1972) identical to
   relationship found in Harrow clone.
b: represents sensitivity check; 10 minutes added to difference
   found by Lamb (1972) to Harrow clone.
c: assuming minimim night temperatures critical (scotophase)
d: assuming maximum day temperatures critical (photophase)
e: 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 dur to the variable nature of the critical photoperiod zone of 'instability'. Threshold end-point 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 adult male morph in Harrow field assuming night temperatures critical and utilizing Harrow developmental rate parameters.

Male Critical Photoperiod Estimate	PER[a]	Prediction(s) dates of Appearance Harrow, 1977
Regular threshold critical photoperiod estimate	3.23	Sept. 24
	3.37	Sept. 25
	3.51	Sept. 27
	3.66	Sept. 28
Add 10 minutes to threshold critical photoperiod estimate at 15°C and 20°C	3.23	Sept. 20
	3.37	Sept. 21
	3.51	Sept. 23
	3.66	Sept. 24
Deduct 10 minutes from threshold critical photoperiod estimate at 15°C and 20°C	3.23	Sept. 19
	3.37	Sept. 20
	3.51	Sept. 22
	3.66	Sept. 24
Add 10 minutes to threshold critical photoperiod estimate at 15°C and 20°C	3.23	Sept. 19
	3.37	Sept. 20
	3.51	Sept. 21
	3.66	Sept. 24

a: 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 assunptions
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
sexual adult female morph in Harrow field
utilizing Harrow developmental rate
parameters and PER of 2.91.

Light Intensity Simulation	Sensitive Regime	Predicting dates of appearance Harrow, 1977
Ignore Civil[a] Twilight	Min.	Sept. 29
	Max.	> Oct. 31
	Linear	Oct. 26
Include Civil[b] Twilight	Min.	Oct. 26
	Max.	> Oct. 31
	Linear	> Oct. 31

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

b: 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.

Light Intensity Simulation	PER Values	Prediction(s) dates of appearance Harrow, 1977
Ignore Civil Twilight	2.49	Sept. 25
	2.63	Sept. 26
	2.77	Sept. 28
	2.91	Sept. 29
Civil Twilight Included	2.49	Oct. 18
	2.63	Oct. 20
	2.77	Oct. 23
	2.91	Oct. 26

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 13:21L (at 15°C) regime were selected and allowed to reproduce until the end of their

reproductive sequences and the number of males and females counted.

RESULTS

Alatae 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.

Alatae

Temperature (°C)	Photoperiod	Exposure Period Start	Finish	Number of Offspring	Number of Parents	Offspring % Male	Female
15	13:07L	Entire period		36	2	0	100
15	13:21L	4	13	402	16	7	93
15	12:53L	Entire period		283	19	1	99
15	12:30L	Entire period		81	2	0	100
15	11:15L	6	15	56	2	0	100
20	11:00L	3	13	24	1	0	100
20	11:45L	Entire period		144	4	0	100
15 (night) and 20 (day)	11:45L	Entire period		47	2	0	100
15 (night) and 20 (night)	13:00L	Entire period		84	4	2	98

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

Sex	Number of Offspring	Percent
Male	338	69
Female	153	31

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 virginoparae 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 aptarae and alatae.

Temperature (°C)	Morph	Photoperiod	Exposure Period Start	End	Number of Offspring	Number of Parents	Percent Oviparae	Percent Virginoparae
15	Alatae	11:15L	6	15	56	2	84	16
15	Apterous	11:15L	6	15	323	17	94	6
15	Alatae	13:21L	4	13	374	16	1	99
15	Apterous	13:21L	4	13	64	3	0	100
15	Alatae	12:53L	Entire Exposure		280	13	100	0
15	Apterous	12:53L	Entire Exposure		49	3	100	0

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 physiqlogical 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

91

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 $15^{\circ}C$ and $20^{\circ}C$. 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.

95

APPENDIX I

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

Common Name		Technical Name
Asexual females	-	virginoparae
Sexual females	-	Oviparae
Non-winged aphids	-	Apterae
Winged aphids	-	Alatae

Parental Offspring Options   Offspring

```
┌─────────────┐      ┌─────────────┐
│Grandparent  │      │  Parent     │
│generation   │─────▶│generation   │─────▶
│(asexual aphid)│    │(asexual aphid)│
└─────────────┘      └─────────────┘
```
                                                Males

                                                oviparae

                                                virginoparae
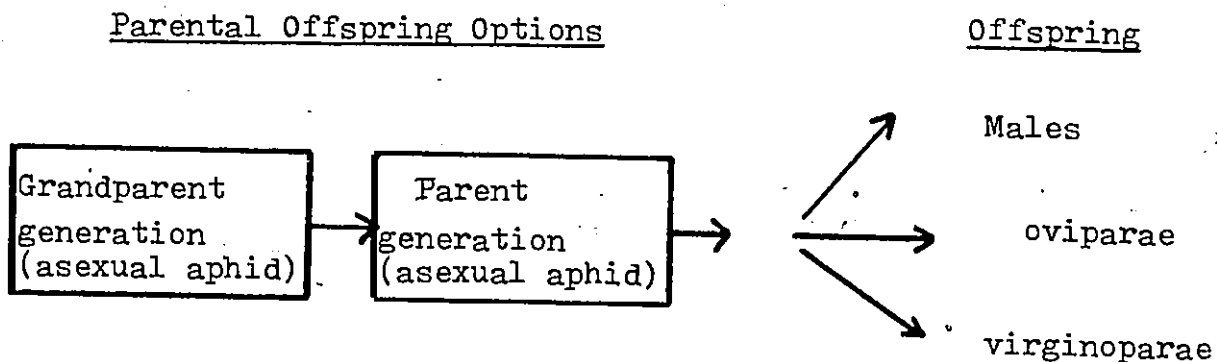
APPENDIX II

Photoperiodic response strategies for the pea aphid,
Acyrthosiphon pisum (Harris).

Parent   Offspring

(a)   Parent aphid   Asexual females

(b)   Parent aphid   Asexual females   Pause   Males

(c)   Parent aphid   Asexual and Sexual females   Pause   Males

(d)   Parent aphid   Sexual females   Pause   Males
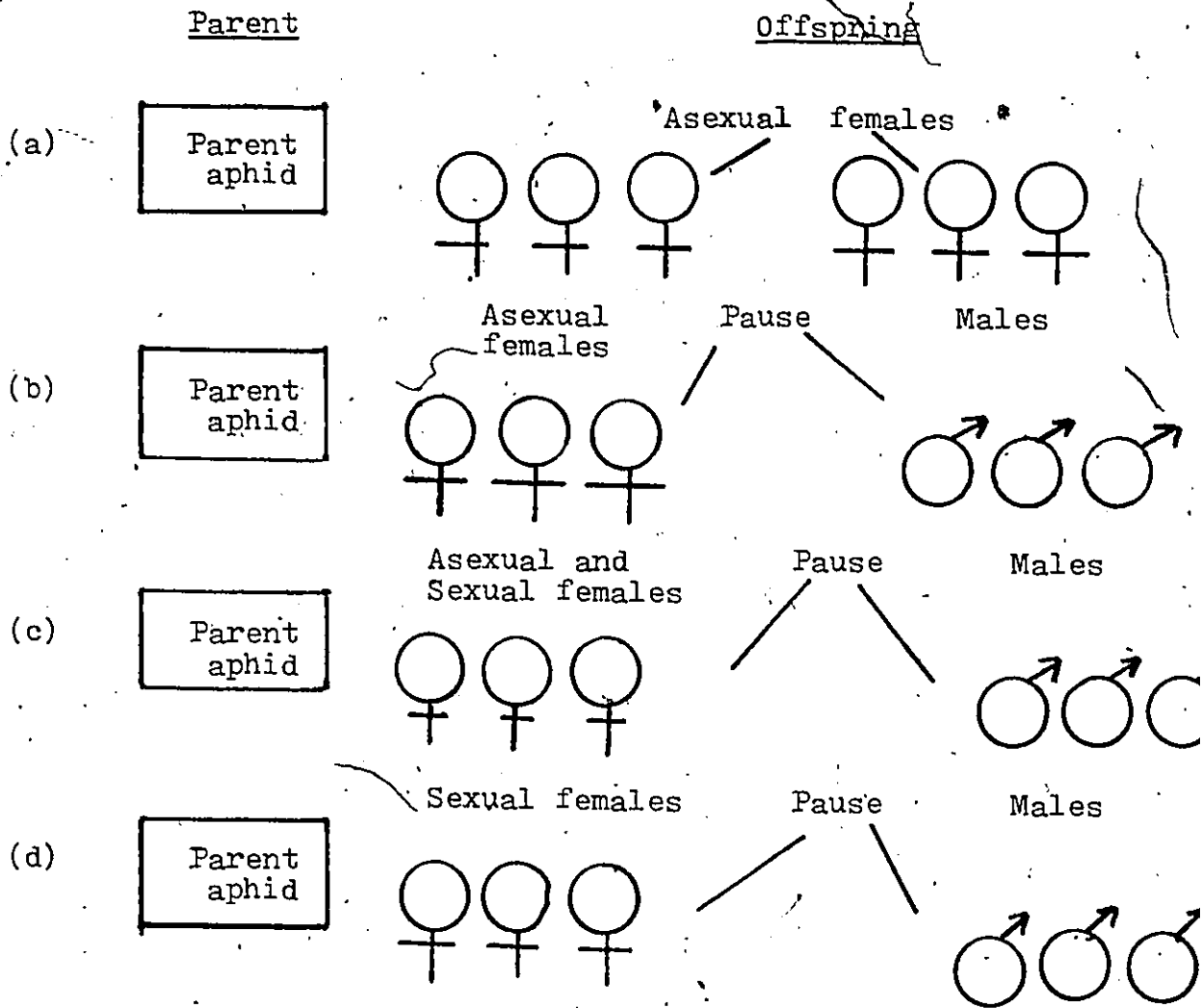
APPENDIX III

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

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

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*

lower limit	C.P.**	Upper limit	lower limit	C.P.	Upper l:
≤12:01L	12:30L	12:55L	11:31L	11:49L	12:0?

20°C

lower limit	C.P.	Upper limit	lower limit	C.P.	Upper 1:
12:00L	13:00L	13:23L	12:27L	13:06L	13:45

15°C
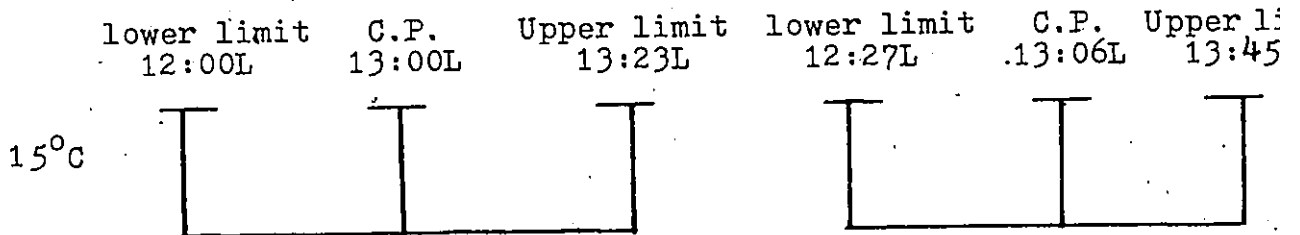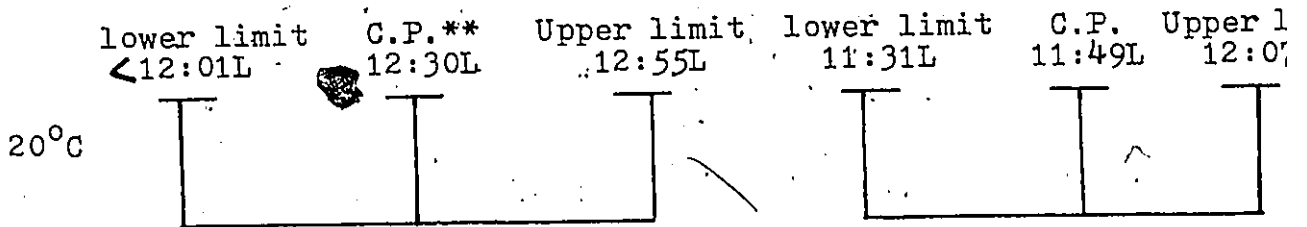
* Photoperiodic response parameters estimated from regression analysis.

** Critical photoperiod.

Appendix V (Sub-Model I)

<u>A</u>. <u>pisum</u> 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 ^{o}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 relation-
ship between temperature and photoperiod
thresholds).

```
JOB    WATFIV XXXXXXXXX  REELEDER

 1        REAL TMAX(122),TMIN(122),THMAX(76),THMIN(76),THSH
 2        INTERGER DAY(100)
 3        INTEGER  DMON(100)
 4        DO 4 I=1,31
 5        DMON(I)=1
 6      4 CONTINUE
 7        READ,YEAR
 8        READ(5,25)(TMAX(I),I=1,92)
 9        READ,YEAR
10        READ(5,25)(TMIN(I),I=1,94)
11        READ,AP,BP
12        READ,AT,BT
13        DO 40 I=1,76
14        DAY(I)=I
15        X=AP +BP*I
16        THSH=AT +BT*X
17        THMAX(I)=TMAX(I)-THSH
18        THMIN(I)=TMIN(I)-THSH
19     40 CONTINUE
20        WRITE (6,55)
21        WRITE (6,70)    AP,BP,AT,BT
22        WRITE (6,75)
23        WRITE(6,85)(DMON(I),I=1,31)
24        WRITE (6,90)(DAY(I),I=1,31)
25        WRITE(6,100)(THMIN(I),I=1,31),(THMAX(I),I=1,31)
26        WRITE (6,110)(DAY(I),I=32,61)
27        WRITE (6,115)(THMIN(I),I=32,61),(THMAX(I),I=32,61)
28        WRITE(6,120)(DAY(I),I=62,75)
29        WRITE(6,125)(THMIN(I),I=62,76),(THMAX(I),I=62,76)
30        WRITE{6,55)
31     55 FORMAT('1',)
32     70 FORMAT('0','THE NUMBER OF DEGREES BELOW THRESHOLD FOR EACH
          1DAY')
33     75 FORMAT('0','DAILY PHOTO.= ',1F9.3,'+', 1F9.3, '*DAY,
          2THRESHOLD= ',2lF9.3,'+',1F9.3,'*PHOTOPERIOD')
```

```
34   85   FORMAT('0','DAYS OF THE MONTH'//6X,31I3)
35   90   FORMAT('0','AUGUST'//' DAY',31I3)
36   110  FORMAT('0','SEPTEMBER'//' DAY',31I3)
37   100  FORMAT('0',' TMIN',31F4.1//' TMAX',31F4.1)
38   115  FORMAT('0','TMIN',30F4.1//' TMAX',30F4.1)
39   120  FORMAT('0','OCTOBER'//' DAY',31I3)
40   125  FORMAT('0','TMIN',15F4.1//' TMAX', 15F4.1)
41   25   FORMAT(26F3.1)
42        STOP
43        END
     $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   WATFIV XXXXXXXX  REELEDER
  1         REAL QUIP(20),TEMP(1224),TMAX(200),TMIN(200),SI
  2         INTEGER YEAR,SDAY(20),SI
   C        READ NUMBER OF FALL DAYS
  3         READ,N
   C        READ YEAR AND MAX. AND MIN. DAILY TEMPERATURES FOR FALL MONTHS
  4         READ,YEAR
  5         READ(5,25)(TMAX(J),J=1,N)
  6         READ,YEAR
  7         READ(5,25)(TMIN(J),J=1,N)
   C        READ PARAMETERS FOR TEMPERATURE/DEVELOPMENTAL RATE CURVE
  8         READ,C,TOPT,B1,B2
   C        DO LOOPCALCULATES 8 DAILY TEMPERATURES FROM MAX. AND MIN.
            K=1
  9         DO 35 I=1,N
 10         DO 30 J=1,8
 11         X=3*FLOAT(J)
 12         TM=TMIN(I)
 13         IT=I-1
 14         IF(IT.LT.1)IT=I
 15         IT1=I+1
 16         IF(IT1.GT.N)IT1=N
 17         TX=TMAX(IT)
 18         IF(X.GT.5.)TX=TMAX(I)
 19         IF(X.GT.14)TM=TMIN(IT1)
 20         TEMP(K)=TCALC(X,TM,TX)
 21         K=K+1
 22  30     CONTINUE
 23  35     CONTINUE
   C        READ TITLES
 24  4      READ (5,5,END=53)
   C        READ PROPORTION OF DEVELOPMENT (PER)
 25         READ,PER
   C        READ NUMBER OF STARTING DAYS
 26         READ,NSD,(SDAY(I),I=1,NSD)
   C        DO-LOOP CALCULATES TIME FOR DEVELOPMENT STARTING FROM SDAY
 27  C      XINC IS LENGTH OF TIME INTERVAL BETWEEN ESTIMATED TEMPS
```

```
28          XINC=.125
29          N1=N*8-
30          DO 50 I=1,NSD
31          SUM=0
32          X=0.
33          SI=SDAY(I)*8-7
34          DO 40 K=SI,N1
35          T=Temp(K)
36          X=X+XINC
37          IF(T.GT.TOPT)T=2.*TOPT-T
38          SUM=SUM+SHAPE (C,B1,B2,T)*XINC
39          IF(SUM.GE.PER)GO TO 45
40    40    CONTINUE
41          QUIP(I)=-1.
42          GO TO 50
      C     ESTIMATE FIRST APPEARANCE OF MORPH
      C     ASSIGN FIRST APPEARANCE TO QUIP
43    45    QUIP(I)=SDAY(I)+X
44    50    CONTINUE
45          WRITE (6,60)
46          WRITE (6,65)
47          WRITE(6,70)
48          WRITE(6,75)
49          WRITE(6,5)
50          WRITE(6,8)
51          WRITE(6,9)
52          WRITE(6,10) N,PER,C, TOPT,B1,B2
53          WRITE(6,80) YEAR
54          WRITE (6,85)(SDAY(I),I=1,NSD)
55          WRITE (6,90)
56          WRITE(9,95)(QUIP(I),I=1,NSD)
57          GO TO 4
58    53    WRITE(6,60)
59    5     FORMAT(',
60    8     FORMAT(/5X,'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 MORPH')
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)
    C   FUNCTION CALCULATES PROPORTION OF DEVELOPMENT FOR INTERVAL
75      A=AK2+AK3*X
76      IF(A.LT.-50.) GO TO 20
77      IF(A.GT.50.) GO TO 10
78      SHAPE=AK1/(1.+EXP(A))
79      RETURN
80  20  SHAPE=AK1
81      RETURN
82  10  SHAPE=0
83      RETURN
84      END
```
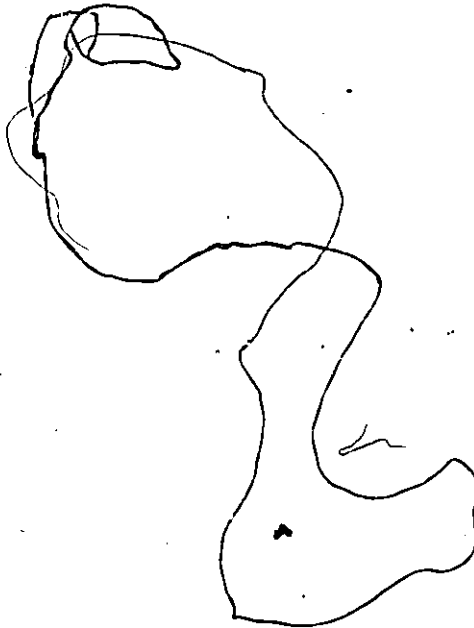
```
85        FUNCTION TCALC(T,TM,TX)
      C       FUNCTION ESTIMATES TEMPERATURES FOR INTERVALS FROM MAX. AND MIN.
86        AK=.3490658504
87        IF(T.LT.6.) GO TO 10
88        IF (T.LT.15.) GO TO 20
89        T1=T-15
90        AK=.2094395103
91        GO TO 30
92   10   T1=T+9
93        GO TO 30
94   20   T1=T+3
95   30   ALP=(TX-TM)/2.
96        TCAL=TM+ALP*(1.+COS(AL*T1))
97        RETURN
98        END
```

LITERATURE CITED

Barr, A. J., J. H. Goodnight, J. P. Sall and J. T. Helwig. 1976. A user's guide to SAS 76. SAS Institute, Raleigh.

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

Beck, S. D. 1962a. Photoperiodic induction of diapause in an insect. Biol. Bull. 122: 1-12.

Beck, S. D. 1962b. Temperature effects on insects: relation to periodism. Proc. N. Central Branch, Entomol. Soc. Am. 17: 18-19.

Bradshaw, W. E. 1976. Geography of photoperiodic response in diapausing mosquito. Nature 262: 384-386.

Campbell, A., B. D. Frazer, N. Gilbert, A. P. Gutierrez and M. Mackauer. 1974. Temperature requirement of some aphids and their parasites. J. Appl. Ecol. 11: 431-438.

Cartier, J. J., A. Isaak, R. H. Pointer and E. L. Sorensen. 1965. Biotypes of pea aphid in Acyrthosiphon pisum (Harris) in relation to alfalfa clones. Can. Ent. 97: 754-760.

Clarke, C. T. and L. L. Schkade. 1974. Statistical analysis for administrative decisions. (Second edition). Cincinnati: South-Western Pub. Co.

Danilevskii, A. S. 1965. Photoperiodism and seasonal development of insects. Edinburgh: Oliver and Boyd.

Eastop, V. F. 1973. Biotypes of aphids, in Perspectives in aphid biology. Ent. Soc. of New Zealand (Inc.) Carton Press.

Frazer, B. D. 1972. Population dynamics and recognition of biotypes in the pea aphid (Homoptera: Aphididae). Can. Ent. 104: 1729-33.

Geiger, R. 1965. The climate near the ground. (Fourth edition). Cambridge: Harvard University Press.

Gilbert, N., A. P. Gutierrez, B. D. Frazer and R. E. Jones. 1976. Ecological relationships. Reading and San Francisco: Freeman and Company.

Johnson, B. 1959. Effect of parasitization by _Aphidius platensis_ Brethes on the developmental physiology of its host _Aphis craccivora_ Koch. Entomol. exp. appl. 2: 82-99.

Kenten. J. (1955). The effect of photoperiod and temperature on reproduction in _Acyrthosiphon pisum_ (Harris) and on the forms produced. Bull. ent. Res. 46, 599-624.

Kogure, M. 1933. The influence of light and temperature on certain characters of the silkworm, _Bombyx mori_. J. Dept. Agr. Kyushu Univ. 4: 1-93.

Lamb, R. J. 1970. The effects of photoperiod and temperature on sexual morph determination in an aphid. M.Sc. Thesis The University of Toronto, Toronto, Canada.

Lamb, R. J. and R. J. Pointing. 1972. Sexual morph determination in the aphid, _Acyrthosiphon pisum_. J. Insect. Physio. 18: 2029-2042.

Lamb, R. J. and R. J. Pointing. 1975. Reproductive sequences in the aphid, _Acyrthosiphon pisum_. J. Insect Physio. 21: 1443-1446.

Lamb, R. J. 1978. Unpublished. A model of the role of photoperiod and temperature in the production of sexual morphs of the pea aphid, _Acyrthosiphon pisum_.

Lees, A. D. 1959. The role of photoperiod and temperature in the determination of parthenogenetic and sexual forms in the aphid _Megoura viciae_ Buckton - I. The influence of these factors on apterous virginoparae and their progeny. J. Insect. Physiol. 3: 92-117.

Lees, A. D. 1961. Clonal polymorphism in aphids. Symp. roy. entomol. Soc. London. 1: 69-79.

Lees, A. D. 1963. The role of photoperiod and temperature in the determination of parthenogenetic and sexual forms in the aphid _Megoura viciae_ Buckton-III. Further properties of the maternal switching mechanism in apterous aphids. J. Insect Physiol. 9: 153-164.

Lees, A. D. 1964. The location of the photoperiodic receptors in the aphid _Megoura viciae_ Buckton. J. exp. Biol. 41: 119-133.

Lees, A. 1966. The control of polymorphism in aphids. Adv. Insect Physiol. 3: 207-277.

Lees, A. D. 1967. The production of the apterous and alate forms in the aphid _Megoura viciae_ Buckton, with special reference to the role of crowding. J. Insect Physiol. 13: 289-318.

MacGillvary, M. E. and G. B. Anderson. 1964. The effect of photoperiod and temperature on the production of gamic and agamic forms in _Macrosiphum euphorbiae_ (Thomas). Can. J. Zool. 42: 491-510.

MacKay, P. A. and W. G. Wellington. 1975. A comparison of the reproductive patterns of apterous and alate virginoparous <u>Acyrthosiphon</u> <u>pisum</u>. Can. Ent. 107: 1161-1165.

MacKay, P. A. and W. G. Wellington. 1977. Maternal age as a source of variation in the ability of an aphid to produce diapausing dispersing forms. Res. Popul. Ecol. 18: 195-209.

Masaki, S. 1956. The local variation in the diapause pattern of the cabbage moth <u>Barathra</u> <u>brassicae</u> Linne, with particular reference to the aestival diapause. Bull. Fac. Agr. Mic. Univ. 13: 29-46.

Masaki, S. and T. Sakai. 1965. Summer diapause in the seasonal cycle of <u>Mamestra</u> <u>brassicae</u> Japan. J. Appl. Entomol. Zool. 9: 191-205.

Morris, R. F. and W. C. Fulton. 1970. Models for the development and survival of <u>Hyphantria</u> <u>cunea</u> in relation to temperature and humidity. Mem. Ent. Soc. Can. 70: 1-60.

Pinter, P. J., N. F. Hadley and J. H. Lindsay. 1975. Alfalfa crop micrometerology and its relation to insect pest biology and control. Envir. Ent. 4: 153-162.

Sharma et al., 1972. Etude des conditions particulières régissant la formation des mâles chez le puceron du pois <u>Acyrthosiphon</u> <u>pisum</u> (Harris). Ann. Soc. ent. Québ. 17: 133-137.

Sharma et al., 1973. Effects de la photopériode et des températures moyennes de 15°C sur la fêcondité et la production des sexués chez le puceron du pois, <u>Acyrthosiphon</u> <u>pisum</u> (Aphididae: Homoptera). Can. Ent. 105: 947-956.

Sharma et al., 1974. Production des formes sexués chez <u>Acyrthosiphon</u> <u>pisum</u> sur les conditions expérimentales de l'extérieur. Can. Ent. 106: 307-313.

Southwood, T. 1966. Ecological methods with particular reference to the study of insect populations. London: Methuen.

Stinner, R. E., A. P. Gutierrez and G. D. Butler. 1974. An algorithm for temperature-dependent growth rate simulation. Can. Ent. 106: 519-524.

Sutherland, O. WL R. 1969a. The role of crowding in the production of winged forms by two strains of the pea aphid <u>Acyrthosiphon</u> <u>pisum</u>. J. Insect. Physiol. 15: 1385-1410.

Sutherland, O. W. R. 1969b. The role of the host plant in the production of winged forms by two strains of the pea aphid <u>Acyrthosiphon</u> <u>pisum</u>. J. Insect. Physiol. 15: 2179-2201.

Tauber, M. J., and Tauber, C. A., 1970. Photoperiodic Induction and termination of diapause in an insect: Response to changing daylengths. Science 167: 170.

Tauber, M. J., and Tauber, C. A., 1972. Geographic variation in critical photoperiod and in diapause intensity of <u>Chrysopa</u> <u>carnea</u>. J. Insec. Physiol. 18: 25-29.

Tauber, M. J., and Tauber, C. A., 1973. Insect phenology; criteria for analyzing dormancy and for forecasting post-diapause development and reproduction in the field. Search (Agric.) Cornell Univ. Agric. Exp. Sta., Ithaca, NY3: 1-16.

Tauber, M. J. and Tauber, C. A., 1976. Insect seasonality: diapause maintenance, termination and post diapause development. A. Rev. Ent. 21: 81-107.

Toshima, A. K. Honma, and S. Masaki. 1961. Factors influencing the seasonal incidence of diapause in <u>Carposina</u> <u>niponensis</u>, Walshingham. Japan. J. Appl. Entomol. Zool. 5.: 260-269.

Way, M. J. and M. E. Cammell. 1970. Self-regulation in aphid populations. Proc. Adv. Study Inst. Dynamics Number Popul. (Oosterbeek, 1970), 232-242.

Wesolowsky, G. C. 1976. Multiple regression and analysis of variance. New York: Wiley-Interscience.

White, W. S. 1946. The environmental conditions affecting the genetic mechanisms of wing production in the chrysanthemum aphid. Am. Nat. 80: 245-270.

Williams, G. C. 1975. Sex and evolution. New Jersey: Princeton University Press.

VITA AUCTORIS

Born — September 26, 1951.
Truro, Nova Scotia.

1975 — B.Sc. (Hons.), McMaster University,
Hamilton, Ontario.

1978 — M.Sc. (Biology), University of Windsor,
Windsor, Ontario.

1979 — (Expected), M.B.A., McMaster University,
Hamilton, Ontario.

1976 — Teaching Assistant, Faculty of Business,
McMaster University, Hamilton, Ontario.

1977 — Teaching Assistant, Department of Biology,
University of Windsor, Windsor, Ontario.

Major Field(s) — Insect ecology; Industrial organization.