THE UNIVERSITY OF MANITOBA

COLONIZATION AND DIAPAUSE STUDIES OF *AEDES DORSALIS* (MEIGEN) (DIPTERA:CULICIDAE)

by

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ABSTRACT

COLONIZATION AND DIAPAUSE STUDIES OF AEDES DORSALIS (MEIGEN) (DIPTERA:CULICIDAE)

The optimum temperature for development and survival of Aedes dorsalis (Meigen) in the laboratory is 23°C. Larval development is completed in 7-8 days and pupal metamorphosis is concluded within an additional 1½-2 days. At the optimum temperature, photoperiod has no effect on development or survival of A. dorsalis, nor does photoperiod appear to effect fecundity of A. dorsalis female imagos.

Stenogamy is apparent in the laboratory colony; successful copulation being particularly evident between day 7 and 14 of adult life. Photoperiod appears not to influence mating. While the presence of another mosquito population, Aedes vexans (Meigen), generally produces no effect on the incidence of insemination in A. dorsalis, it is evident that A. vexans adults mate more readily in the presence of A. dorsalis. This is the first report of successful colonization of A. vexans in the literature (see Taylor and Brust, in press).

Experiments conducted under controlled temperature and photoperiod verify that embryonic diapause in A. dorsalis is determined by daylength. The diapause condition in the eggs is not a cumulative effect of photoperiodic influence on the preceding developmental stages. Photoperiods of 8L:16D through 14L:10D at 23°C induce diapause in

A. dorsalis eggs after 14 days. Embryonic diapause is averted at photoperiods of 15L:9D through 24L:0D at 23°C. The critical daylength for a population of A. dorsalis from Winnipeg, Manitoba (49°55'N lat.) is very near 14½L:9½D per diem.

Diapause is sustained in eggs, maintained at a short photoperiod (12L:12D) and 23°C, for a period of 3 months or longer. Exposure to high temperature (30°C) or long photoperiod (16L:8D) for 14 days or more terminates the diapause condition in a high percentage of eggs. The influence of photoperiod is masked at high temperature, i.e., photoperiodic influence on diapause is subordinate to the effect of high temperature.

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CHAPTER I

INTRODUCTION

This thesis is, primarily, a report on the investigations undertaken to establish a colonization technique for the maintenance of a continuous colony of the mosquito Aedes dorsalis (Meigen). Further investigations were undertaken to determine the influence of photoperiod and temperature on diapausing and non-diapausing eggs of A. dorsalis. The results of these investigations are reported herein.

Aedes dorsalis is a multivoltine (many generations per year) floodwater mosquito common to southern regions of Manitoba. Populations are reported to occur throughout southern Saskatchewan and Alberta, as well as in many areas of the United States, Europe and Asia. The species has economic importance as a major pest of man and animals and it is numbered among those species of importance in the transmission of western encephalitis. As is common in most multivoltine species, A. dorsalis exhibits a condition of facultative diapause. As the day-lengths in autumn decrease to a critical value, specific for the species at a given latitude, the eggs of A. dorsalis are induced to enter a period of diapause (not quiescence); a suppressed state of development and growth which ensures survival of the insect through adverse winter conditions. Diapause is terminated upon the return of favorable environmental stimuli in the spring; egg hatch coincides with conditions suitable for growth and development of the immature insect.

Those eggs laid in the summer by A. dorsalis complete embryogenesis within a few days after oviposition. The eggs will hatch at this time provided the oviposition sites are inundated and other hatching conditions are favorable. A temporary deviation of one or more environmental factors from the optimum produces the simplest type of dormancy in eggs, called quiescence. Reactivation from the quiescent state coincides with the return of favorable hatching conditions, which may be simply evaporation of the water in the pools and reflooding by rainfall.

Photoperiod is the most reliable environmental influence for relating seasonal changes and is utilized by insects for diapause control. Although it has little influence on the immediate well-being of the insect, photoperiod serves to synchronize the life cycle with the seasons.

There is a paucity of literature pertaining to the physiological and biochemical response mechanisms of insects as related to environmental stimuli and the over-wintering condition of diapause. This is particularly evident in studies of Aedes (Ochlerotatus) mosquitoes inhabiting the northern temperate regions where climatic conditions are varied and not uncommonly severe. This lack of information may be, in part, due to the absence of stenogamy (mating in a confined space) in most northern mosquitoes, which has been a very significant factor in discouraging studies of these species (Brust, 1971). Due to the difficulties in attaining conditions conducive to mating of these species in the laboratory, research material must be repeatedly collected in the field to replenish laboratory stocks.

Since it is imperative to understand the physiological, biochemical, and endocrinological relationships of diapause in order to interpret the distribution and the very existence of an insect species, there is a need for further investigation into the circumstances surrounding induction, maintenance, and termination of diapause. An investigation must first be undertaken, however, to discover a continuous supply of research material in the field or to develop a feasible colonization technique for a continuous and standard supply of the required stages of the insect concerned. This was the primary objective of the present study.

CHAPTER II

LITERATURE REVIEW

Laboratory Colonization

Since the literature pertaining to laboratory colonization of mosquitoes is now voluminous I shall only review briefly pertinent examples relative to aedine mosquitoes, particularly of the subgenus Ochlerotatus.

The presence of stenogamy in different species of Aedes (Ochlerotatus) mosquitoes has been described by Beckel (1958b), Haeger (1958), Garay and Hagmann (1964), Chapman and Barr (1969), Blakeslee et al. (1970), Grimstad et al. (1970), Smith and Brust (1970), and Brust (1971). Due to the difficulties of obtaining conditions in the laboratory which are conducive to copulation of mosquitoes, few species mate in cages.

Beckel (1958) reported adult mating and the oviposition of fertile eggs by autogenous Aedes communis (Degeer). Unfortunately, insufficient numbers of viable eggs were obtained to maintain a continuous colony. Haeger (1958) described cage colonization of Aedes taeniorhynchus (Wiedemann), in which the laboratory colony was found to be more narrowly selective than the outdoor colony, i.e., a lower percentage of the adults mated successfully under laboratory conditions. Brust (1971) reported stenogamy in autogenous A. communis and Aedes diantaeus Howard, Dyar, & Knab. While the degree of successful mating of autogenous A. communis varied between populations from different geographical locations, there was no significant difference in the number of eggs laid by females from

different populations. No mating occurred in the anautogenous 'strain' of A. communis. A. diantaeus mated more readily in cages and provided adequate numbers of eggs for continuous colonization. The readiness with which Aedes rempeli Vockeroth mated in small cages and the relatively high fecundity for autogenous females (Smith and Brust, 1970) suggests possibilities for further laboratory research. A brief account of laboratory colonization of Aedes tormentor Dyar and Knab is given by Chapman and Barr (1969). It is not clear whether A. tormentor is stenogamous by itself or whether it is induced to mate by the presence of another species. Garay and Hagmann (1964) stated that Aedes sollicitans (Walker) mated under extremely crowded conditions in a small cage. Unless a high incidence of female insemination occurred, the number of adults required to produce stenogamy would seem impractical for laboratory work. Although Gjullin et al. (1950) reported matings of both Aedes sticticus (Meigen) and A. vexans when the two species were confined in a small cage (34x34x36 in.), only the A. sticticus species was considered suitable for colonization. Of the few eggs that were laid by A. vexans, only a small percentage were viable. Gjullin et al. found, however, that A. vexans females which had mated in nature would readily lay two to four batches of eggs in the laboratory cages if given blood meals subsequent to the laying of each lot of eggs.

Colonies of Aedes nigromaculis (Ludlow), Aedes stimulans (Walker), and A. sollicitans have been successfully maintained using the forced-copulation technique of McDaniel and Horsfall (1957) (see Miura, 1967;

McDaniel and Horsfall, 1957; Anderson, 1970). Horsfall has maintained numerous Ochlerotatus species using this technique (Horsfall, unpublished).

Laboratory maintenance of a California 'strain' of Aedes dorsalis (Meigen), using the forced-copulation technique, was first described by Blakeslee et al. (1966). Attempts to colonize this species, using voluntary cage mating, met with limited success. Parents mated in the cage but F_1 adults did not; only non-viable eggs were obtained. With continued effort, self-sustaining laboratory colonies of A. dorsalis were later obtained by Blakeslee et al. (1970) and Grimstad et al. (1970). The results of their colonization techniques indicated that egg-hatch rates increased with successive generations. Through natural selection with successive generations, a high degree of adult mating was obtained in relatively small cages (2x2x2 ft.).

Diapause

The term "diapause" was originally coined by Wheeler (1893) to describe a stage during blastokinesis when growth of an insect embryo was arrested. Henneguy (1904) suggested use of the term to describe the arrest of growth for all stages during the life of an insect. Rouband in 1919 (see Wigglesworth, 1965) separated the higher Diptera into two categories: "homodynamic" insects which are temporarily dormant due to the direct action of unfavorable environment, and "heterodynamic" insects which are subjected to a prolonged dormant period independent of the

environment. Shelford (1929) later described growth retardation due to the effect of unfavorable environment as "quiescence". In cases where insect activity or development was arrested "spontaneously", Shelford applied the term dormancy or "diapause". Lees (1955) suggested that a distinction between "quiescence" and "diapause" could be made by considering the immediacy of response to environmental factors. However, since the immediacy of diapause termination is dependent upon ecological factors and inherent characteristics or species, there remains much confusion as to the distinction between the two phenomena.

Mansingh (1971) reviewed the literature pertaining to dormancy, investigated the physiological differences in dormancies and proposed further and more concise classifications. The generally accepted definition of diapause is that state of retarded or suppressed growth, resulting from the environmental triggering of neuroendocrine mechanisms, which is induced well before adverse environmental conditions set in and which is maintained for some time irrespective of environment (Harvey, 1962; McHaffey and Harwood, 1970; Mansingh, 1971). Quiescence, the simplest type of dormancy, is produced by a sudden, unanticipated, noncyclic, and usually short-duration deviation of one or more environmental factors from the optimum (Mansingh, 1971).

Diapause may be either obligatory or facultative. Univoltine* insect species exhibit an obligatory diapause that occurs automatically

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* Voltinism derives from Italian sericulture: volta=turn, time. Zool. The frequency or number of annual broods or generations.

High temperatures tend to avert or eliminate the diapause response (Lees, 1955; Danilevskii, 1965; de Wilde, 1962; Beck, 1968; Anderson, 1968, 1970; Kalpage, 1970; McHaffey and Harwood, 1970; Mansingh, 1971). In many instances, both high and low temperature extremes tend to mask the photoperiodic responses or shift the critical photoperiod to a point further along the response curve. Generally, when high temperatures coincide with scotophase (dark) the intensity of the diapause response is lessened and when low temperatures coincide with scotophase the intensity of the diapause response is increased (Danilevskii, 1965; Beck, 1968).

Way (1962) stated that diapause was an important factor in determining the distribution of an insect species. The intensity of the photoperiodic response, the effect of temperature on the response and the critical photoperiod may differ according to the geographical latitude of local populations of an insect species. Photoperiod-induced diapause tends to be more intense in populations inhabiting high latitudes (Vinogradova, 1960; de Wilde, 1962; Depner and Harwood, 1966).

Diapause studies with Aedes dorsalis

There are several accounts in the literature that report the influence of photoperiod and temperature on diapause in A. dorsalis. There are opposing views on the influence of these environmental factors. The opposing results may, in part, be attributed to problems in species classifications or to geographical variation. A. caspius dorsalis in

the USSR is probably a different species than A. dorsalis from North America.

Telford (1963) concluded that a decreasing temperature gradient induced facultative diapause in the eggs of A. dorsalis from California during the fall. Conversely, an increasing temperature gradient in the spring preceeded termination of egg diapause. Telford deemed photoperiodic changes insignificant as effectors of diapause and hatching response because, in the laboratory, A. dorsalis and Aedes nigromaculis (Ludlow) carried out all activities in a uniform manner regardless of photoperiod.

Khelevin (1958, 1959) working in the USSR, suggested that temperature was the principal environmental factor that stimulated or inhibited diapause induction in eggs of A. caspius dorsalis (Meigen). Although it is not certain whether or not Khelevin considers A. dorsalis to be a subspecies of Aedes caspius (Pallas), it should be noted that Stone et al. (1959) lists both as distinct species within the subgenus (Ochlerotatus). Khelevin suggested that successive generations of A. caspius dorsalis were different qualitatively with respect to diapause induction because different generations developed under different environmental conditions. Eggs oviposited by first-generation females could not be induced to diapause either by action of low temperatures on the females or by exposing the eggs to reduced temperatures. Eggs of second- and third-generation females, however, could be induced to diapause under similar conditions. The action of high temperatures ($30^{\circ}C$) prevented

diapause in eggs of autumn generations, while the action of low temperatures during the winter months promoted the termination of egg diapause and allowed further development of the insect during favorable environmental conditions. Khelevin did not rule out the possible effect of photoperiod on embryonic diapause in A. caspius dorsalis, stating that, "there is a possibility that the action of photoperiodism on autumn females of A. caspius dorsalis determines the development of eggs capable of entering a state of diapause" (Khelevin, 1958).

In contrast, McHaffey and Harwood (1970) concluded that photoperiod was the primary factor in determining whether or not diapause was to be induced or averted in the eggs of A. dorsalis from central Washington (46°N. lat.). In a fairly intensive study they determined that short photoperiods produced significantly more diapause eggs than did long photoperiods. However, at temperature extremes photoperiod appeared to be subordinate to temperature as the primary influence on egg diapause. Prolonged exposure to low temperatures terminated diapause in eggs, even under conditions of short photoperiod. Although no critical photoperiod was determined by McHaffey and Harwood, their results did show that 14½- and 13½-hr. photoperiods both produced significantly more egg hatch than either the 11- or 8-hr. photoperiods, but significantly less hatching than the 16-hr. photoperiod. McHaffey and Harwood noted a maternal influence on egg diapause, particularly at short photoperiods.

In a recent study of aedine mosquito eggs (Kalpage and Brust, 1968), two types of A. dorsalis eggs were found to be significantly different

in size and shape. The investigators suggested that in nature there are two distinct subspecies (races) of A. dorsalis. In addition to this, it is known that one brood of A. dorsalis hatches in snow-melt pools and another brood hatches in rain pools, often only a few weeks apart. These findings give rise to speculation that univoltine and multivoltine populations may occur in Manitoba. It could also mean that A. dorsalis is a species complex.

CHAPTER III

METHODS & MATERIALS

1. GENERAL METHODS

(a) Collection and storage of study material

The original material used for this study was collected from a drainage ditch, adjoining a livestock feeding pen, in July, 1971. Several thousand pupae were dipped from the shallow pool and returned to the laboratory. Subsequent collections of larvae, adults, and eggs have been taken from sites within the immediate Winnipeg area (Fig. 2), for the purpose of replenishing the laboratory colony. Details of the colonization of A. dorsalis are outlined in Chapter III, part 2.

All experiments were conducted in BOD incubators (Fig. 1a) which were equipped with an incandescent light source (40W bulb), a time clock for photoperiod control, and dual temperature controls. Temperature was controlled separately, during the dark and the lighted part of the cycle, at $\pm 1^{\circ}C$ of the set temperature. Relative humidity was maintained at $75 \pm 5\%$. All stages of the colony, exclusive of adult mating, were maintained under these conditions.

(b) Determination of diapause

Eggs that failed to hatch, after 24 hours in the nutrient broth solution, were bleached and examined to determine viability. The 24 hour time period was sufficient for maximum egg hatch. Little or no

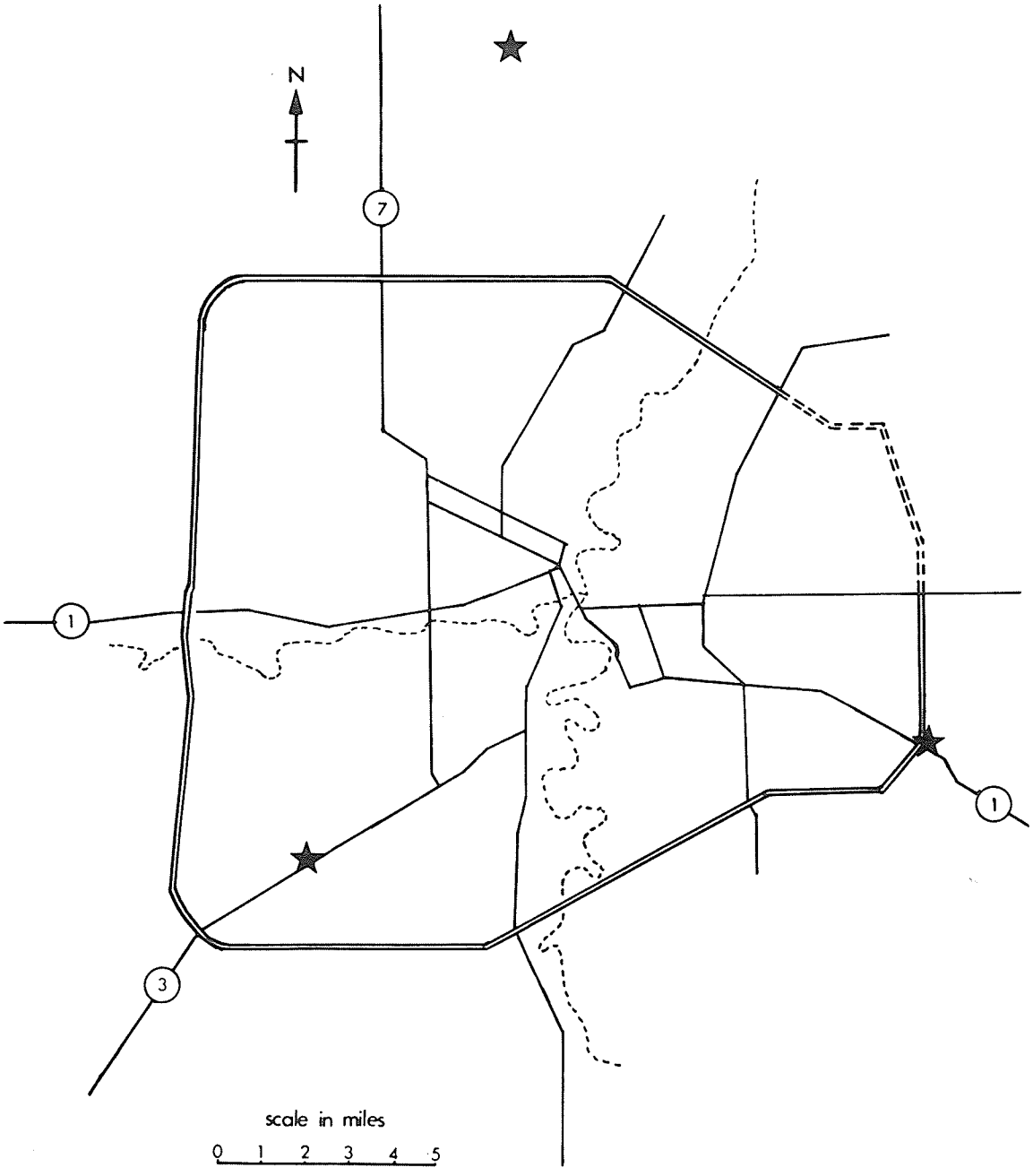


Figure 2

hatch occurred beyond this period. A technique proposed by Trpis (1970) gave excellent bleaching qualities for the egg chorion. Those eggs with mature embryos, having eye spots, a hatching spine and abdominal segmentation, were considered to be viable but in a state of diapause.

(c) Determination of mating success

Spermathecae were examined to determine percentage insemination or mating success. A random sample of females was aspirated from the swarm cage at regular intervals. The female imagos were anaesthetized with ethyl ether, wetted in 70% ethanol and rinsed in saline before dissection on a clean slide. The spermathecae were removed and examined under a cover slip by phase-contrast microscopy. A sample of females was usually taken on days 3, 7, and 14 following release of the adults into the swarm cage.

(d) Description of the 'Swarm Chamber'

All mating, of the laboratory colony of A. dorsalis, occurred within a controlled-environment-chamber (120x120x210 cm high). The chamber, which was constructed of 6-mil clear plastic on the inside, and 6-mil black plastic on the outside, was maintained at 70-80% R.H., $23\pm1^{o}C$ and light:dark conditions of either 12L:12D or 16L:8D.

The light source in the swarm chamber consisted of four 25W incandescent bulbs arranged in a square (50 cm to the side) above the ceiling. Simulated matudinal and crepuscular periods (dawn and dusk) were provided by a 60W incandescent bulb in the centre of the square. The simu-

lated dawn and dusk periods, each of 60 minutes duration, occurred prior to and directly after the lighted part of the daily photoperiod cycle.

The swarm chamber was entered through two 'doors'. Each door was fashioned from canvass and fitted with a curved zipper. The doors were 2' apart and the outer door could be closed before the inner one was opened to prevent the entrance of external light and the escape of adults. The small room, created by the two doors, also served to house any instruments needed to control the conditions within the swarm chamber.

Rehydrated apple was provided as a carbohydrate source for the adults.

2. COLONIZATION TECHNIQUES

The following sections describe handling and rearing procedures used for the various developmental stages of A. dorsalis.

(a) Eggs

Those eggs collected on sod samples (ca. 1x15x15 cm) which were removed from the margins of known oviposition sites in the field and returned to the laboratory, were stored, in situ, on the sod samples. By sealing the sod samples in plastic bags, eggs may be stored at a temperature of $5\pm1^{o}C$ for a period of one year or more. Eggs in sod samples were hatched simply by immersing the samples in dechlorinated tap water at room temperature.

Eggs from female imagos which had oviposited in the laboratory were stored on moist nylon cloth in covered petri dishes (Fig. 1e). The nylon cloth was placed on a pad of glass wool; both were wetted and the excess water was drawn off. This technique kept the eggs moist and drastically reduced contamination by molds and fungi (Kalpage, 1970). Eggs on pads were hatched in a nutrient broth solution (2:1000 w/w, powder in tap water) at room temperature.

All eggs that failed to hatch after 24 hours in the nutrient broth solution were bleached (Trpis, 1970) and examined to determine viability.

The oviposition substrate provided was paper towelling moistened with distilled water. In experiments using eggs from individual female imagos, a circular pad of paper towelling (30 mm dia.) was placed under a small screen cup which held the female imago. The screen cup was a 15 mm length of acrylic tubing (25 mm dia.) covered with fine mesh nylon screen. A moistened raisin or a piece of rehydrated apple was placed on the screen to serve as food for the imago.

For most experiments, eggs were collected from groups of females (20 females per cage) kept in somewhat larger acrylic oviposition cages (25x25x150 mm). These cages, having fine mesh nylon screen on two sides, allowed the imagos easy access to a blood source and to the oviposition substrate (Fig. 1d). An anaesthetized mouse was placed on each cage of female imagos on alternate days. A moistened raisin or rehydrated apple was provided following the blood meal, as this increases the survival and longevity of the insects.

(b) Larvae

Larvae were transferred from the nutrient broth solution to covered plastic pans (6x22x30 cm) containing ca. 1000 ml of distilled water (Fig. 1b). Larval density was kept low (50 larvae per pan) to minimize the inherent effects of crowding. The surface of the water, in the rearing pans, was towelled daily to remove any film of dust or scum. This technique prevented fouling of the larval media and greatly increased the percentage survival of the larvae.

Larval diet consisted of 70-150 mg of TetraMin Tube Food 66®, depending upon the instar stage of the larvae.

Experiments were conducted to determine the optimum temperature and the photoperiodic effect on the development and survival of instar IV larvae. Since the data recorded for the mature instar IV larvae is cumulative of the data for the previous larval instars, a statistical analysis is shown for this final larval stage only. Any analysis of the data given for the preceding larval instars would be redundant.

(c) Pupae

Pupae were removed from the larval pans daily, and were transferred to styrofoam food containers (50 pupae per 300 ml container) half-filled with distilled water. Higher concentrations of pupae caused an increase in pupal mortality.

The pupal containers were placed in acrylic cube cages (Fig. 1c) where the adults were allowed to emerge. Rehydrated apples and moist

paper-wicks provided the necessary food and water requirements of the adults. The moist paper-wicks helped to maintain the relative humidity, inside the cages, at 70-80%.

Experiments, similar to those described for instar IV larvae, were also conducted with pupae.

(d) Adults

Emerging adults were aspirated daily from the acrylic cube cages and released into the controlled environment swarm chamber.

Little successful copulation occurred during the early part of the adult life stage. The percentage insemination increased with time; maximum mating success occurred between days 7 and 14 of the confinement period (Table III).

The coupling of adults during flight was observed throughout the period that the adult population was held in the swarm chamber; the highest incidence of coupling occurred during the periods of changing light intensities (simulated dusk and dawn). It is assumed that these coupling responses, lasting not more than 5 seconds, resulted in female insemination. However, none of the females observed in the state of copulation, were captured to determine if insemination had taken place.

Following a two week confinement period in the swarm chamber, the adults were separated as to sex; the female imagos were placed in small acrylic holding cages (20 females per cage), as described in 'Methods and Materials', and were offered a blood meal. Oviposition began 5 days

after the initial blood meal, at which time a second blood meal was offered.

The eggs, deposited on the moist paper towels, were collected daily, washed and placed on nylon pads in sealed petri dishes (Fig. 1e). The petri dishes were stored in BOD incubators of the appropriate photoperiod regimes.

Attempts were made to colonize A. dorsalis by placing the adults in smaller swarm chambers, however no mating occurred despite efforts to maintain conditions as described for the larger swarm chamber.

Preliminary investigations were designed to evaluate the effect of photoperiod on mating (percentage insemination) and fecundity of A. dorsalis adults. Although it bears no relationship to the previous investigations, a study of the mating response of A. dorsalis, primarily due to the presence and absence of a population of A. vexans, is also noted in this dissertation.

3. INDUCTION, MAINTENANCE, AND TERMINATION OF DIAPAUSE

Investigations on the induction, maintenance, and termination of diapause were carried out using, primarily, photoperiod as the experimental variable. A few experiments were conducted to determine the effects of temperature on diapause.

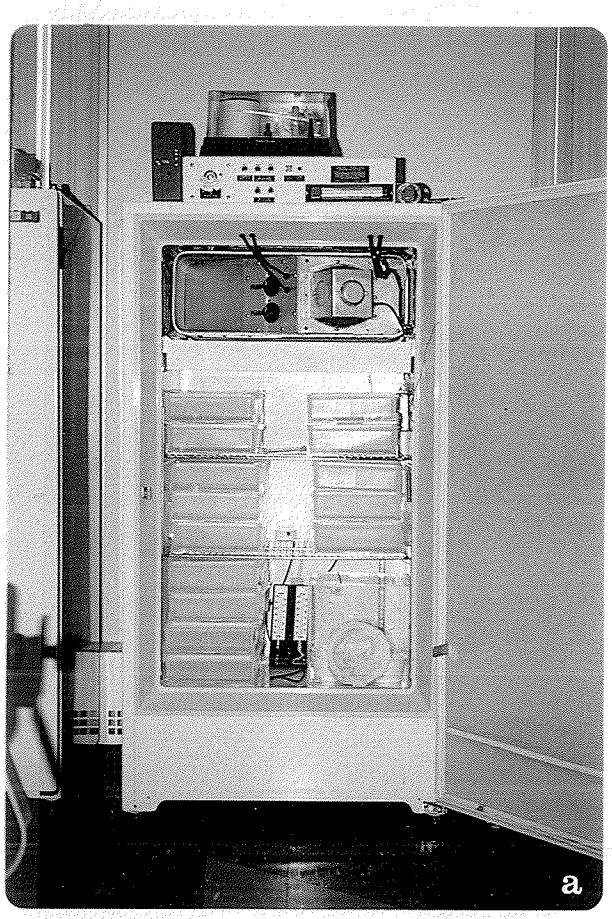
(a) Induction of Diapause

Preliminary experiments revealed that there was a high incidence of diapause in those eggs stored at short photoperiods (12L:12D) while

Figure 1. Apparatus used in laboratory colonization of A. dorsalis.

(a) B.O.D. incubator containing larval rearing pans on top shelves and adult emergence cage on bottom shelf.

(b) Larval rearing pan, tube of TetraMin 66 used for larval diet, paper towel for cleaning water in rearing pans, pupal container, wide-mouth eye dropper for transferring pupae from pans to styrofoam container, acrylic emergence cage containing moist paper wicks and rehydrated apple.


a


b

Figure 1. (continued)

(c) Acrylic adult emergence cage containing moist paper wicks, rehydrated apple, and pupal container. The aspirator is used to transfer adults from the emergence cage to the larger swarm cage.

(d) Plastic pan contains sufficient water to keep the paper towelling moist. The small acrylic cages (containing 20 female imagos each) are placed on the moist towels. Anaesthetized mice are offered as a blood meal source.


c

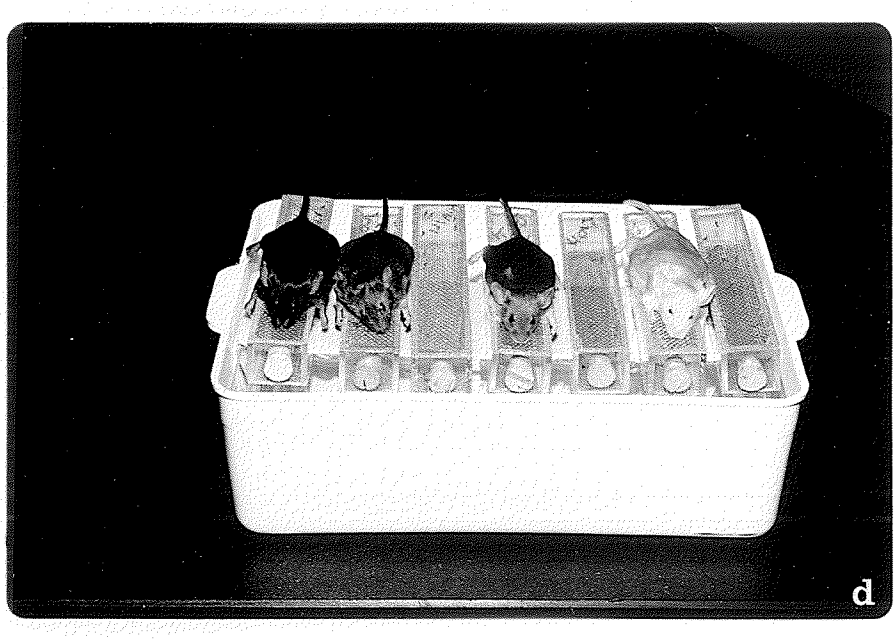

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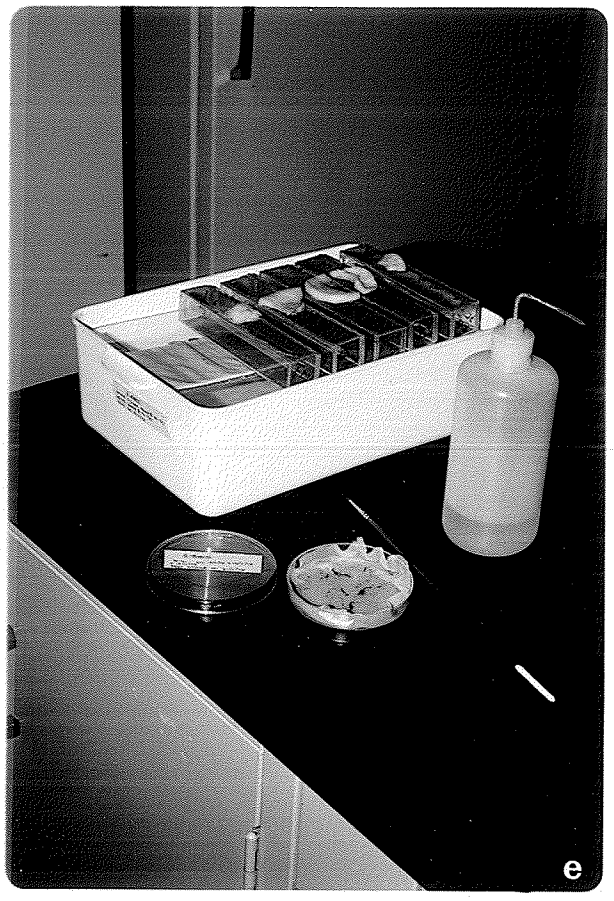
Figure 1. (continued)

(e) Plastic pan containing moist paper towelling, used as the oviposition substrate, and acrylic female-holding cages. The eggs oviposited on the moist towelling are washed onto the nylon pad of the petri dish for storage. The small paint brush is used to count eggs needed for experiments.


e

those eggs stored at long photoperiods (16L:8D) exhibited little or no diapause effect.

The following experiments were conducted, at 23±1°C, to determine the developmental stage(s) which respond to photoperiod and the daily photoperiod(s) capable of inducing diapause.

Eggs (< 24 hours old) were placed at various photoperiods for a period of 14 days (Induction exp. 1). At the end of the treatment period, the eggs were immersed in nutrient broth solution for 24 hours. The number of larvae and unhatched viable eggs were recorded for each treatment.

A second experiment (Induction exp. 2) was conducted to determine the effect of photoperiod on different stadia of A. dorsalis. A short photoperiod (12L:12D) and a long photoperiod (16L:8D) were used. Specific stages of the developmental cycle (larvae, pupae or adults) were transferred from short photoperiods to long photoperiods, and vice versa. After 14 days at the respective photoperiod, the eggs, laid by the adults used in these treatments, were immersed in nutrient broth solution.

(b) Maintenance of Diapause

Having determined the critical photoperiod and the developmental stage responsive to photoperiod, the following experiment was performed to determine the intensity of diapause in eggs stored for various time periods.

Eggs were stored for periods of 14, 30, 60, and 90 days at a

temperature and photoperiod of 23±1°C and 12L:12D, respectively. At the end of each trial period, the eggs were immersed in nutrient broth solution.

(c) Termination of Diapause

Eggs used in these experiments had been maintained at a diapause-inducing photoperiod (12L:12D) and a temperature of 23±1°C. To determine the effect of two different photoperiods on eggs in diapause, different batches of eggs were subjected to (a) a short photoperiod of 12L:12D and (b) a long photoperiod of 16L:8D (Termination exp. 1). At the end of the treatment period, the eggs were placed in the hatching medium. The following day, the number of larvae and unhatched viable eggs were recorded.

For the second experiment (Termination exp. 2), eggs which had been stored for periods of 30, 60, and 90 days under diapause-inducing conditions (12L:12D and 23°C) were transferred to 30±1°C incubators. The photoperiod remained the same for the treatment periods. At predetermined intervals, eggs were removed from the high-temperature incubators and placed at room temperature (23°C) for 1 hour before immersion in nutrient broth solution. The number of larvae and unhatched viable eggs were counted the next day.

Another experiment (Termination exp. 3) was designed to determine the effect of high temperature, at two different photoperiods, on diapausing embryos. Eggs which had been maintained at 12L:12D and 23°C

for 14 days, were transferred to incubators maintained at 30±1°C and were subjected to (a) a short photoperiod of 12L:12D, and (b) a long photoperiod of 16L:8D. At the end of a 14-day treatment period, the eggs were then subjected to the hatching stimulus.

CHAPTER IV

RESULTS

1. EFFECT OF PHOTOPERIOD AND TEMPERATURE ON REARING, MATING, AND FECUNDITY OF A. DORSALIS

(a) Effect of photoperiod and temperature on development in A. dorsalis

In order to establish a successful colony of A. dorsalis, and to provide research material for studies on diapause, the effects of photoperiod and temperature were investigated on the immature stages of A. dorsalis. Four temperatures (20°, 23°, 26°, and 29°C) and two photoperiods (12L:12D and 16L:8D) were used in all combinations as shown in Table I. All stages, from larva to adult were maintained at a constant photoperiod and temperature.

The results indicate that time of development, for all immature stages, was dependent upon temperature, i.e. at both photoperiods, larval and pupal development was more rapid at the highest temperature (29°C) than at the lowest temperature (20°C). Survival of the larvae and pupae was not greatly affected by the temperatures used, however, 23°C was most favorable for both photoperiods (Table I and Fig. 3). No records was made of the weight and size of the immature stadia, but larvae, pupae, and adults reared at 23°C appeared to be larger and healthier than those reared at the other experimental temperatures. With the exception of two conditions, the results in Table I indicate that

TABLE I

Effect of photoperiod and temperature on development and survival of A. dorsalis.

Development Stage	Temperature (°C)	12L:12D[1] Time (hours)[3] Mean ± s.d.[6]	12L:12D[1] % Mortality Mean ± s.d.	16L:8D[2] Time (hours)[3] Mean ± s.d.	16L:8D[2] % Mortality Mean ± s.d.
Larval Instar I	29	0+24	0	0+24	0
	26	0+24	0	0+24	0
	23	0+24	0	0+24	0
	20	0+24	0	0+24	0
new II	29	33±1.2	0	32± 2.4	7± 8.4
	26	38±2.0	0	45± 5.3	6± 8.2
	23	48±1.5	0	50± 3.4	3± 4.3
	20	59±1.2	0	51± 2.0	8±15.0
new III	29	53±1.2	0	62± 6.7	10± 9.2
	26	71±2.3	1±1.2	77± 8.0	12±11.5
	23	72±1.5	0	81± 5.7	4± 6.5
	20	115±3.1	0	118± 2.0	15±22.3
new IV	29	82±1.2	1±1.2	86± 6.1	11± 9.6
	26	102±2.1	1±1.2	111±11.7	15±15.3
	23	108±1.5	1±1.2	118±14.2	7± 9.0
	20	187±4.6	1±1.2	171± 8.8	20±22.4

TABLE I (continued)

Development Stage	Temperature (°C)	12L:12D[1] Time (hours)[3] Mean ± s.d.[6]	12L:12D[1] % Mortality Mean ± s.d.	16L:8D[2] Time (hours)[3] Mean ± s.d.	16L:8D[2] % Mortality Mean ± s.d.
mature IV (pharate pupa)	29	141±1.2a[4]	3±1.2a	138±10.0a	17±11.3ab
	26	164±2.0 b	5±6.1a	183±22.1 b	20±15.6ab
	23	167±2.3 b	4±5.3a	194±21.6 b	11± 8.9a
	20	s[5]341±3.1 c	16±2.0 b	s277±11.0 c	35±20.4 b
mature Pupa (pharate adult)	29	172±1.0a	s7±4.2a	168±12.1a	s24±10.6ab
	26	199±1.7 b	7±6.1a	220±24.7 b	23±14.6ab
	23	215±5.5 c	7±5.8a	228±15.7 b	15±10.6a
	20	s564±7.0 d	38±5.3 b	s393±23.2 c	40±17.4 b

1 Mean of 3 replications.

2 Mean of 6 replications.

3 Time for 50% of one instar or stage to molt to the next instar or stage.

4 Common letter following column indicates no significant difference at 5% level between pupal and larval instar IV developmental rates and mortality at different temperatures. Duncan's multiple range test (Duncan, 1955).

5 Letter s preceding column indicates a significant difference at 5% level between pupal and larval instar IV developmental rates and mortality at different photoperiods. F-test for analysis-of-variance.

6 Abbreviation: s.d., standard deviation.

Figure 3. The effect of photoperiod and temperature on development and survival of A. dorsalis. Mean median time between molts for larval instars (I-IV) and pupal stage (P) as shown for 20°C.

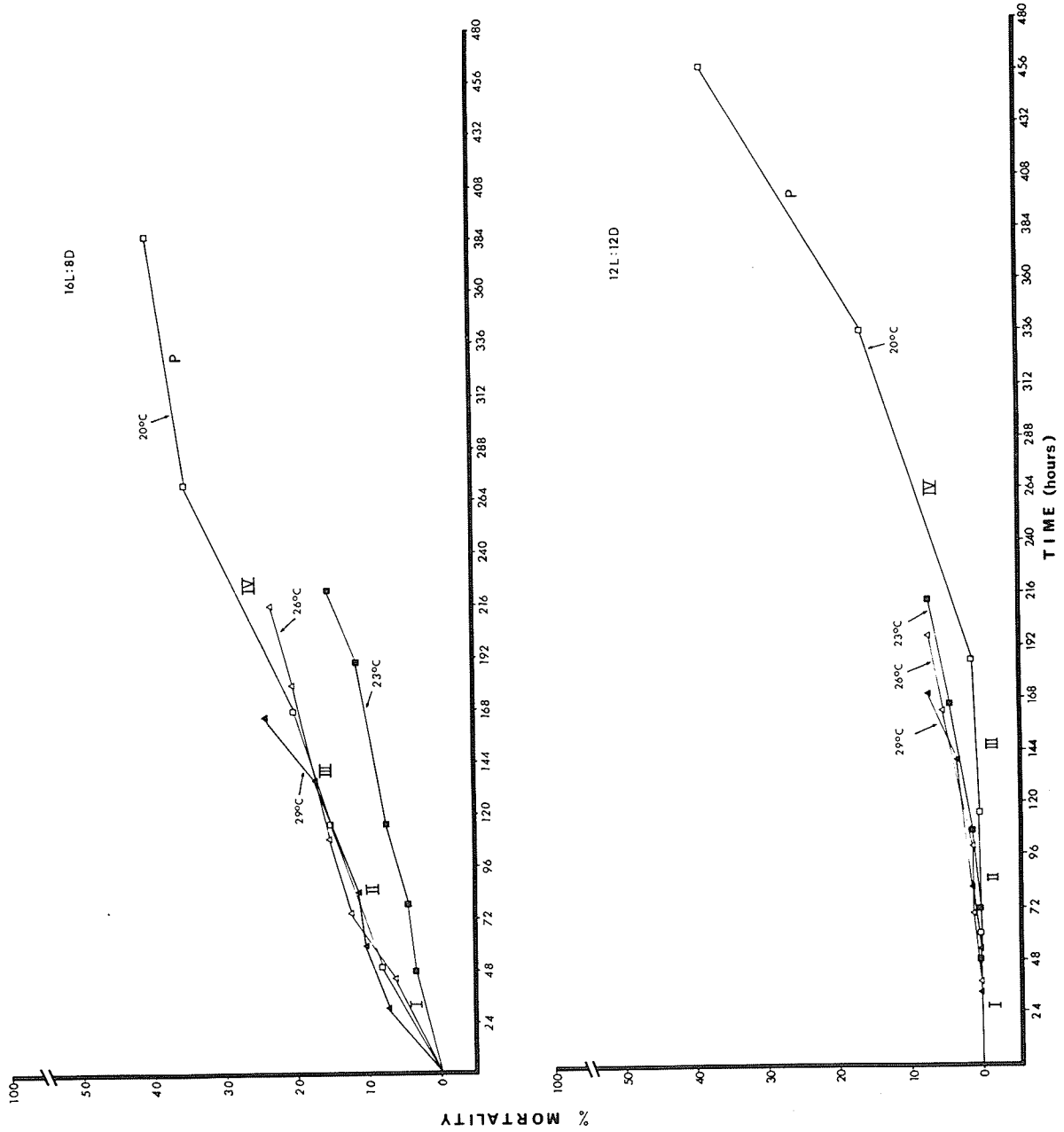

16L:8D
12L:12D
P
IV
III
II
I
20°C
26°C
23°C
29°C
% MORTALITY
TIME (hours)

Figure 3

there is no significant difference in developmental rates and mortality for the photoperiods and temperatures used. The two conditions indicating a significant difference were: (a) the pupal mortality rate at 29°C was greater at 16L:8D than at 12L:12D, and (b) the developmental rate for larvae and pupae at 20°C was greater at 12L:12D.

As previously noted (see 'Colonization Techniques' part b), a statistical analysis of the data recorded for the first three larval instars would be redundant of the analysis of the mature instar IV larvae.

(b) Effect of photoperiod on mating of A. dorsalis

In this experiment, the different photoperiods used were 12L:12D and 16L:8D. All experiments were conducted in the same cage, under similar conditions (70-80% R.H. and 23°C) with populations of the F_1 generation. The percentage insemination of the female imagos was used to determine mating success.

The results as shown in Table II would indicate that there is no effect on mating which could be attributed to differences in photoperiods.

(c) Mating of A. dorsalis in the presence and absence of A. vexans

For these experiments, all adult populations (parent generation) were maintained under similar conditions (70-80% R.H. and 23°C). Photoperiods of 12L:12D and 16L:8D were used, as noted in Table III. As for the previous experiment, insemination was used to determine mating success.

TABLE II

Effect of photoperiod on mating of A. dorsalis.

Photoperiod (L:D)	Generation	Total no. adults	Age (days)	% Insemination
16:8	F_1	210	3	-
			7	20
			14	20
16:8	F_1 [(a)]	1500	3	10
			7	25
			14	30
12:12	F_1	500	3	10
			7	10
			14	20
12:12	F_1	500	3	20
			7	20
			14	30

(a) 500 A. vexans adults were present in the cage with 1000 A. dorsalis adults.

TABLE III

Mating of A. dorsalis in the presence and absence of A. vexans.

Photoperiod (L:D)	Generation	Total no. adults	Age (days)		% Insemination	
			A. dorsalis	A. vexans	A. dorsalis	A. vexans
16:8	P^1	1500	absent	8	-	0
				12	-	1
				16	-	1.6
16:8	P^2	5000	absent	6	-	0
				11	-	0
				14	-	0
16:8	P^3	2000	14	absent	10	-
16:8	P^4	2200	14	16	30	10
16:8	P^4	600	7	9	20	5
12:12	P^4	750	3	5	0	0
			7	9	20	10
			14	16	20	10
12:12	P^4	1500	3	5	10	10
			7	9	20	10
			14	16	30	10

TABLE III (continued)

1 Field-collected as pupae on June 5, 1970; adult emergence began on June 6, 1970.

2 Field-collected as pupae on July 4, 1970; adult emergence began on July 5, 1970.

3 Field-collected as pupae on July 25, 1971; adult emergence began on July 26, 1971.

4 Field-collected as eggs during September, 1971; eggs maintained at 5^{o}C; experiments conducted February, 1972 through March, 1972.

Percentage insemination of A. dorsalis, with and without A. vexans present, is given in Table III. When populations of approximately equal number and age are considered, the results appear to indicate that the presence of A. dorsalis increased mating of A. vexans. However, there is no evidence to suggest that the percentage insemination of A. dorsalis is affected by the presence of A. vexans.

(d) Effect of photoperiod on fecundity of A. dorsalis

Virgin female imagos which had been given two blood meals within a 3 day period were placed in individual cages (as described in 'General Methods'). The adults were provided with moistened raisins on which they were able to feed ad lib and were placed at photoperiod regimes of 12L:12D and 16L:8D. The experiments were conducted at a constant temperature of 23°C. After a period of 14 days, all females were dissected and the number of eggs retained plus those laid per female were counted. The results of the experiment are summarized in Table IV and Appendix A.

The mean numbers of eggs per female at the short photoperiod (12L:12D) and the long photoperiod (16L:8D) were 93 and 103, respectively. There was no significant difference between the mean numbers of eggs per female at the two photoperiods.

2. PHOTOPERIOD INDUCTION, MAINTENANCE, AND TERMINATION OF DIAPAUSE

(a) Effect of different photoperiods, at a constant temperature, on diapause induction in A. dorsalis

The photoperiods used for this experiment ranged from 8L:16D through

TABLE IV

Effect of photoperiod on the fecundity of A. dorsalis.

Photoperiod (L:D)	No. females Dissected	No. eggs Laid	No. eggs Retained	Eggs per Female Mean ± S.E.*	Range Min.-Max.
12:12	18	1240	437	93.2 ± 6a	46-157
16:8	15	1034	510	102.9 ± 11a	31-163

* Treatments having common letter not significantly different at 5% level. Duncan's multiple range test (Duncan, 1955).

24L:0D per diem. The experiment was conducted at a constant temperature of 23°C. The purpose of this experiment was to determine what range of photoperiods caused the induction of embryonic diapause and to determine the critical photoperiod.

As the results in Table V and Fig. 4 indicate, a high percentage of embryonic diapause is induced at any photoperiod less than 14L:10D, for those photoperiods tested, while diapause is averted at any photoperiod exceeding 15L:9D. The critical photoperiod was indicated to be 14½L:9½D per diem.

(b) Effect on diapause, when different stadia of A. dorsalis were subjected to short photoperiods

The following experiments were conducted to determine which developmental stage(s) of A. dorsalis responded to photoperiod. A short photoperiod (12L:12D) and a long photoperiod (16L:8D) were used. All experiments were carried out at a constant temperature and humidity of 23°C and 70-80% R.H., respectively. The results of the experiments are summarized in Table VI.

From these experiments it was concluded that with regard to diapause, only the embryonic stage of A. dorsalis responded to photoperiod. A short photoperiod during the egg stage produced embryonic diapause in a high percentage (> 95%) of the eggs, whereas a short photoperiod during the larval, pupal, and adult stages, alone or together, produced virtually no diapause.

TABLE V

Effect of photoperiod on the induction of diapause in eggs of A. dorsalis at 23°C.

Photoperiod (L:D)	No. Replicated	Total no. Viable Eggs	No. eggs Hatched	No. eggs Unhatched	% Diapause*
8:16	2	48	0	48	100.0 c
10:14	2	53	0	53	100.0 c
12:12	2	102	9	93	91.4 c
13:11	2	54	1	53	98.5 c
13½:10½	2	42	1	41	97.9 c
14:10	2	33	4	29	90.9 c
14½:9½	2	55	32	23	42.1 b
14:9	2	75	69	6	9.5a
16:8	2	83	82	1	1.6a
18:6	2	45	43	2	4.5a
24.0	2	56	49	7	13.8a

* Treatments having common letter not significantly different at 5% level. Duncan's multiple range test (Duncan, 1955).

Figure 4. The effect of photoperiod on the incidence of embryonic diapause of A. dorsalis at 23°C.

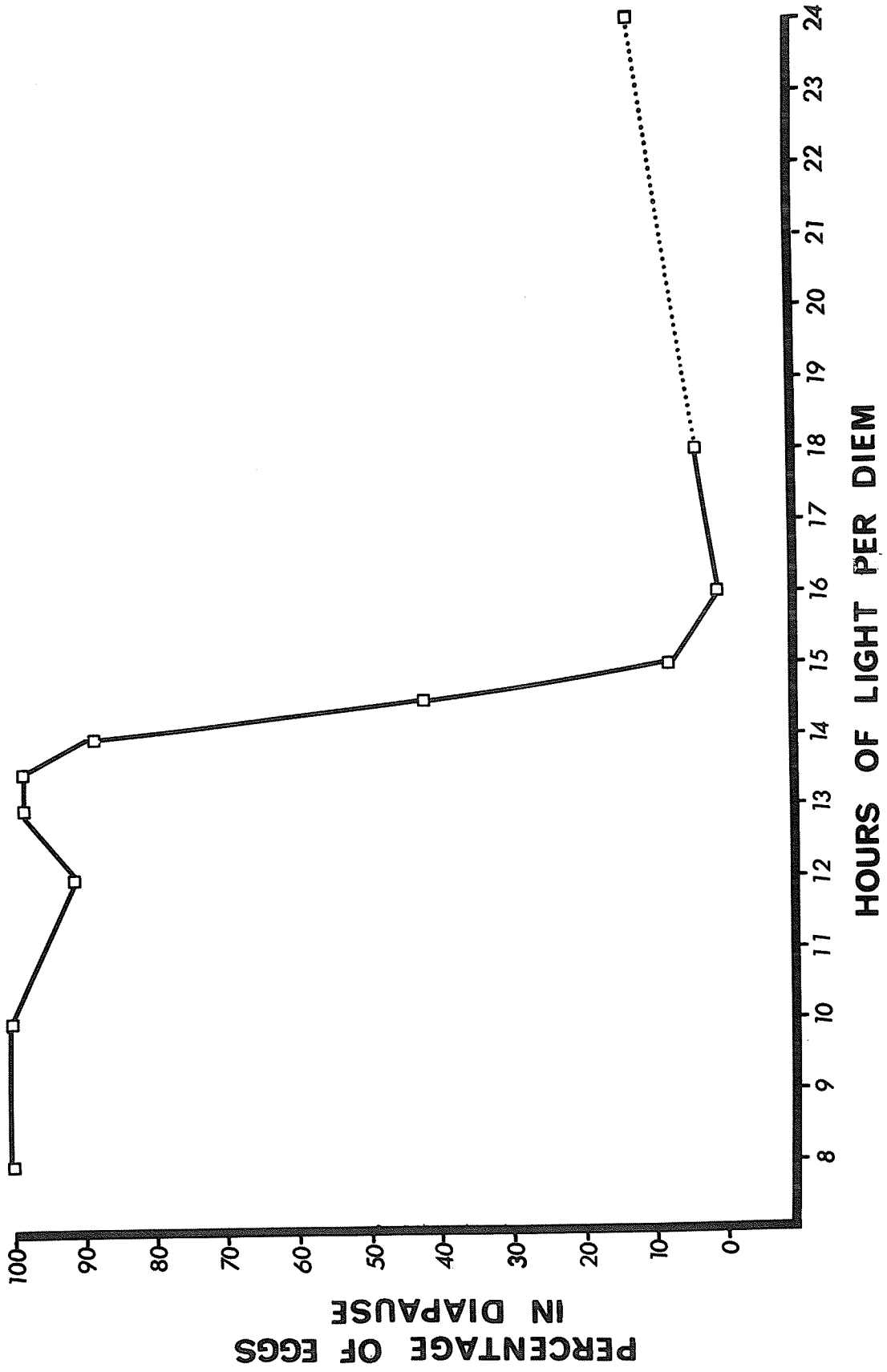

PERCENTAGE OF EGGS
IN DIAPAUSE
100
90
80
70
60
50
40
30
20
10
0
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
HOURS OF LIGHT PER DIEM

Figure 4

TABLE VI

Effect of long-day (16L:8D) and short-day (12L:12D) photoperiods applied to the developmental stages of A. dorsalis, on the production of embryonic diapause.

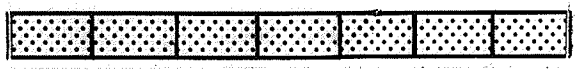
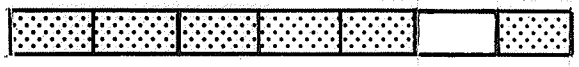
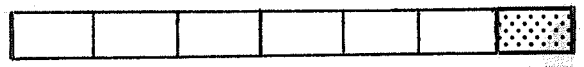
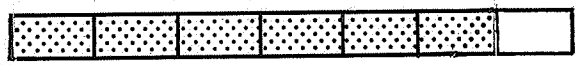
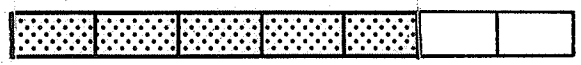
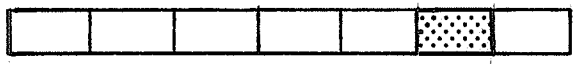
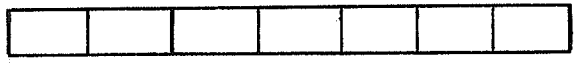
Larvel Instars I II III IV[2] P A E	No. Replicates	Total No. Viable Eggs	Total No. Eggs Hatched	No. Viable Eggs Unhatched	% Diapause[1]
	3	62	2	60	96.7 b
	3	101	3	98	97.0 b
	3	59	0	59	100.0 b
	3	126	4	122	96.6 b
	3	99	97	2	2.0 a
	3	141	138	3	2.1 a
	3	34	33	1	2.9 a
	3	98	95	3	3.3 a

TABLE VI (continued)

Note: Stippled areas denote short photoperiods (12L:12D) and non-stippled areas denote long photoperiods.

[1] Treatments having common letter not significantly different at 5% level. Duncan's multiple range test (Duncan, 1955).

[2] Roman numerals I - IV indicate larval instars; P=pupa; A=adult; E=F_1 embryo.

(c) Effect of short photoperiod on the maintenance of diapause in eggs of A. dorsalis at 23°C

For this experiment, all batches of eggs were maintained at a constant photoperiod and temperature of 12L:12D and 23°C, respectively. Upon completion of the treatment periods, the eggs were immersed in nutrient broth solution.

As the results shown in Table VII indicate, a very high percentage (> 90%) of the eggs remained in diapause even after 90 days, the duration of the experiment. Consequently, there was no significant difference in the percentage hatch between 14, 30, 60, and 90 day-old-eggs when maintained at 12L:12D and 23°C.

(d) Effect of long photoperiod on the termination of diapause in eggs of A. dorsalis at 23°C

All eggs used in this experiment were subjected to a diapause-inducing photoperiod of 12L:12D at 23°C for at least 14 days following oviposition. Egg batches were then transferred to a photoperiod of 16L:8D for periods of 0, 7, 12, and 14 days, as noted in Table VIII.

Exposure of diapause eggs to a long photoperiod (16L:8D) for a 14-day treatment period resulted in the termination of the diapause condition in a high percentage (> 75%) of the eggs. Those eggs exposed to the long photoperiod for periods of 0 and 7 days exhibited a significantly lower percentage hatch. The length of storage time at the short photoperiod had a significant effect on the percentage hatch. After 14 days at 16L:8D a significantly greater number of 28 (14+14) day-old-eggs

TABLE VII

Effect of the hatching stimulus on diapause eggs of A. dorsalis. Eggs maintained at a photoperiod of 12L:12D and 23°C.

No. days following Oviposition	No. Replicates	No. eggs Hatched	Total No. eggs Viable	% Hatch *
14	3	0	103	0 a
30	2	2	54	3.70 a
60	2	4	64	6.25 a
90	2	4	62	6.45 a

* Treatments having a common letter not significantly different at 5% level. Duncan's multiple range test (Duncan, 1955).

TABLE VIII

Effect of photoperiod on the termination of diapause in eggs of A. dorsalis.

No. days at 23°C & 12L:12D	No. days at 23°C & 16L:8D	No. Replicates	No. eggs Hatched	Total no. Viable eggs	% Hatch*
14	0	3	1	88	1.14a
14	7	2	23	55	41.82 b
14	12	3	33	38	86.84 cd
14	14	3	41	43	95.35 d
28	0	3	1	48	2.08a
28	7	3	50	87	57.47 b
28	14	3	64	84	76.19 c

* Treatments having common letter not significantly different at 5% level.
Duncan's multiple range test (Duncan, 1955) and F-test for analysis-of-variance.

hatched than 42 (28+14) day-old-eggs. The only difference was the length of storage at 12L:12D (see Table VIII).

(e) Effect of high temperature (30°C) on the termination of diapause in eggs of A. dorsalis

Following oviposition, eggs were maintained at a photoperiod of 12L:12D and 23°C. At intervals of 30, 60, and 90 days batches of these eggs were placed at 30°C. The photoperiod remained the same throughout the experiment. As noted in Table IX, eggs were sampled at various intervals to determine the percentage hatch.

From the results, summarized in Table IX, it can be seen that those eggs subjected to the high temperature for a period of 14 days showed a significantly higher percentage hatch than those eggs subjected to the same treatment for a shorter period. The results also indicate that there is no significant difference in the percentage hatch due to age, i.e. the percentage hatch of 44 (30+14) day-old-eggs was approximately equal to the percentage hatch of 74 (60+14), and 104 (90+14) day-old-eggs.

A photoperiod of 12L:12D and 23°C maintains embryonic diapause for 3 months or longer (Table VII), but at 30°C diapause is terminated at 12L:12D, indicating that the short photoperiod effect is subordinate to high temperature.

(f) Effect of different photoperiods and high temperature (30°C) on the termination of diapause in eggs of A. dorsalis

Diapause eggs which had been maintained at a photoperiod of 12L:12D and 23°C were used for this experiment. In order to determine whether

TABLE IX

Effect of high temperature (30°C) on the termination of diapause in eggs of A. dorsalis, maintained at 12L:12D.

No. days at 23°C	No. days at 30°C	No. Replicates	No. eggs Hatched	Total no. Viable Eggs	% Hatch*
30	4	2	3	55	5.45a
30	7	2	31	64	48.44 b
30	10	2	41	70	58.57 b
30	14	2	50	52	96.15 c
60	14	2	56	60	93.33 c
90	14	2	56	58	96.55 c

* Treatments having a common letter not significantly different at 5% level. Duncan's multiple range test (Duncan, 1955).

a simultaneous influence of high temperature and long photoperiod could increase the percentage hatch in diapausing eggs, batches of eggs were subjected to a high temperature of 30°C and a photoperiod of 12L:12D or 16L:8D for a treatment period of 14 days.

The results of the experiment are given in Table X and indicate that at a high temperature there is no significant difference in percentage hatch at the different photoperiods. The termination of embryonic diapause appeared to be dependent upon exposure to a high temperature rather than exposure to a long photoperiod, i.e. the influence of photoperiod is subordinate to the effect of high temperature.

TABLE X

Effect of high temperature (30°C) and photoperiod on the termination of diapause in eggs of A. dorsalis.

No. days at 23° & 12L:12D	No. days at Photoperiod		No. Replicates	No. eggs Hatched	Total no. Viable eggs	% Hatch*
	30°C	(L:D)				
14	14	16:8	2	42	44	95.45a
14	14	16:8	2	59	61	96.72a
14	14	12:12	3	45	49	91.84a

* Treatments having common letter not significantly different at 5% level.
F-test for analysis-of-variance.

CHAPTER V

DISCUSSION

1. EFFECT OF PHOTOPERIOD AND TEMPERATURE ON REARING, MATING, AND FECUNDITY OF A. DORSALIS

(a) Effect of photoperiod and temperature on development in A. dorsalis

The results, as summarized in Table I, and the observations made during the experiments indicate that the optimum temperature for development and survival of A. dorsalis in the laboratory is 23°C. Although the higher temperatures generally increased the rate of development, those individuals reared at 23°C appeared to be larger and healthier than those reared at higher temperatures. Since mating behaviour and fecundity of an insect are contingent upon its state of well-being, 23°C was selected as the optimum rearing temperature.

As no attempt was made to separate the different stadia as to sex, there is need for further discussion of the results summarized in Table I and Fig. 3. In field and laboratory populations observed during the course of this study, adult males emerge 2-3 days prior to adult females. Therefore, the development of male larvae and pupae proceeds more rapidly than the development of the female larvae and pupae. Since the results, as shown in Table I and Fig. 3, have been recorded as the first 50% of one stage of the population to reach the next stage, males comprise the majority of the individuals recorded. The results show, however, the influences of

temperature and photoperiod on the development and survival of A. dorsalis in the laboratory.

The influence of photoperiod on the developmental rates of the immature stadia was apparent only at 20°C. At this temperature the time of development at the short photoperiod (12L:12D) was greater than at the long photoperiod (16L:8D). The difference in developmental rates, however, was not reflected on the percentage mortality as neither photoperiod appeared to contribute significantly to the resulting mortalities. Percentage mortality at 29°C was significantly greater at the long photoperiod than at the short photoperiod during the pupal stage. However, these differences may be dependent upon possible deficiencies caused in the preceeding stages by the unusually rapid development at above-optimum temperatures. There was no significant differences in development rates or mortality due to photoperiodic treatments at the optimum temperature. The unusually large standard deviations obtained at 16L:8D may be, in part, attributed to a greater number of replications per trial or to a somewhat modified rearing technique. At the long photoperiod, the water in the larval pans was towelled on alternate days rather than daily as for the short photoperiod trials. It is suggested that this modification of technique plays no significant role in the experimental results.

Under optimum temperature conditions, completion of larval development took place in 7-8 days, while pupal metamorphosis required an additional 1½-2 days prior to adult emergence.

(b) Effect of photoperiod on the mating of A. dorsalis

From the results of the experiments summarized in Table II it is evident that the length of the photophase had no effect on mating of A. dorsalis. As previously described (see 'Colonization Techniques') the highest incidence of coupling occurred during the periods of changing light intensities (simulated dusk and dawn). This fact would suggest that the duration of the periods of changing light intensities, rather than the duration of photophase, would influence mating behaviour and success. The duration of dusk and dawn periods was kept constant for all populations, so the validity of this suggestion is still to be tested.

The percent insemination increased with time, and the maximum mating occurred between days 7 and 14 of adult life. It is difficult to determine from Table II whether population size is critical in influencing mating success.

(c) Mating of A. dorsalis in the presence and absence of A. vexans

During preliminary investigations with populations of A. vexans and A. dorsalis it was observed that the presence of A. dorsalis increased mating of A. vexans when the two populations were placed simultaneously in the same cage. Swarming of males was noted when A. dorsalis was present, but no swarming was observed when A. vexans was the only species in the cage. It appears that mating of A. vexans is induced by the presence of a species, like A. dorsalis, that swarms in a large cage.

Although A. dorsalis had mated successfully in a cage in the absence of A. vexans (see Table III), it was possible that the presence of A. vexans either inhibited or increased mating of A. dorsalis. The results shown in Table III may suggest that the presence of A. vexans increased the mating response in A. dorsalis, however, there is not sufficient evidence to warrant this conclusion. There is a need for further investigations to test the validity of the suggestion.

The relative ease of rearing A. dorsalis and A. vexans together, as well as the simplicity in identifying the mature and immature stages of each species, allows the maintenance of a continuous colony of the two species. The results of the mating experiments indicate that under laboratory conditions the two species exhibit a symbiotic relationship; the percentage insemination of neither species is hindered by the presence of the other species. Mating conditions in the field are undoubtedly quite different, and mating success in either species is independent of the other.

(d) Effect of photoperiod on the fecundity of virgin females of A. dorsalis

Although the actual fecundity of the female imago, i.e., the total number of eggs developed within the ovary, provides a reasonable estimate of the reproductive capacity of a species, this value is subject to several variables, including nutrition, temperature, humidity, mating and oviposition stimuli, and photoperiod.

Although the behavioural characteristics of the A. dorsalis adults appeared to be unaltered by either the short-day or the long-day photoperiod, it was deemed necessary to evaluate the photoperiodic influence on the physiological characteristics of the adults, which in all likelihood would be reflected in the development of the ovaries. By regulating all other variables under constant conditions in the laboratory, it was possible to isolate photoperiod and to assess the influence it exerted on ovarian development or fecundity.

The results as shown in Table IV and Appendix A denote that no significant difference in ovarian development exists due to photoperiodic effect. The wide range in the egg number per female shows primarily that the quantity of blood engorged by each female differed considerably, and this was responsible for the difference in the number of eggs produced (Volozina, 1961, 1963, 1967).

2. PHOTOPERIODIC INDUCTION, MAINTENANCE, AND TERMINATION OF DIAPAUSE

(a) Induction of Diapause

The role of photoperiod in the induction of embryonic diapause has been stressed as one of the essential factors governing seasonal cycles of many insects (Lees, 1955; de Wilde, 1962; Danilevskii, 1965; Beck, 1968). The action of photoperiod cannot be regarded as immediately favorable or unfavorable; the stimulus does not act directly upon the tissue cells but exerts its influence through the nervous system and endocrine organs (Danilevskii, 1965; Mansingh, 1971). It is neither

the absolute duration of photophase nor scotophase that appears to control diapause, but rather the duration of each relative to the other (Lees, 1955; Corbet, 1956; Danilevskii, 1965; Adkisson, 1966; Beck, 1968).

From Table V, it can be seen that, at a constant temperature of 23°C and at photoperiods of 8L:16D through 14L:10D a high percentage (> 90%) of A. dorsalis eggs are induced to diapause after 14 days. Photoperiods of 15L:9D through 24L:0D induced significantly fewer (< 14%) eggs to diapause under similar conditions. The effect of different photoperiods on the incidence of diapause is plotted as a diapause induction response curve shown in Fig. 4. A. dorsalis exhibits what Beck (1968) refers to as a "Type I" induction curve (long-day response) in which relatively long daylengths tend to favour continuous growth, while short daylengths favour diapause.

The critical photoperiod, defined as being the point on the response curve at which the transition from a long- to a short-day effect occurs (Beck, 1968), was indicated in the experiment reported herein as being 14½L:9½D per diem. The 50% response point on the population response curve was chosen to represent the critical photoperiod. The work of McHaffey and Harwood (1970) indicated that the "breakoff point for diapause induction is either at 11-hr. or somewhere between 11-hr. and 13½-hr. photoperiod" for A. dorsalis eggs in central Washington (46°N. lat.). This difference in the critical photoperiod among populations is due to the fact that insect species may consist of many geographical

strains (races), each of which exhibits a characteristic critical photoperiod (Danilevskii, 1965). These experiments on induction measure only the end result of a sequence of physiological responses which ultimately determine the response of the individual. This all-or-none response of eclosion for each egg may differ between the stimulus requirements needed to excite the hatching response in individual eggs (de Wilde, 1962).

Many investigators have demonstrated that the effect of photoperiod is cumulative, i.e. the photoperiodically sensitive period preceeds the stage associated with diapause. Anderson (1970) has shown that the late instars and subsequent stages of growth, including the developing F_1 embryo, are sensitive to length of day in A. sollicitans. The fourth larval instar, pupa, and adult of A. atropalpus are the stages sensitive to photoperiod (Anderson, 1968; Kalpage, 1970). Both embryonic and larval diapause has been noted to occur in A. triseriatus (Kappus and Venard, 1967; Clay and Venard, 1972), depending upon the geographical location of the strain and the conditions of diet and temperature; a slowed rate of larval development can aid in the induction of, as well as be a part of, the diapause condition (Clay and Venard, 1972). McHaffey and Harwood (1970) stated that there appeared to be a trend toward an increase in diapause of A. dorsalis eggs as the photoperiod to which the female imago was exposed decreased in length. Eggs from the 13½-hr. females were the most sensitive to a diapause-inducing photoperiod. McHaffey and Harwood (1970) have therefore deemed embryonic diapause in A. dorsalis to be, in part, subject to maternal influence.

From the results of the experiments summarized in Table VI it is evident that diapause induction in A. dorsalis eggs is not a cumulative effect of photoperiodic influence on the preceding developmental stages. Eggs subjected to a short photoperiod (12L:12D) exhibited a high percentage diapause (> 95%) while eggs subjected to a long photoperiod (16L:8D) exhibited a very low percentage diapause (< 5%). In contrast to what McHaffey and Harwood (1970) have found, the above results show that diapause is not subject to the influence of photoperiod on the preceding stages.

In order to relate the laboratory data to natural photoperiods as experienced in the field, a cycle depicting daylength (inclusive of civil twilight) was prepared as shown in Fig. 5. Allowing that the critical photoperiod is approximately 14½L:9½D per diem, the eggs of A. dorsalis could be expected to enter a diapause condition during the latter part of August or early September. The induction of diapause prevents unseasonably late egg hatch which could decimate the population. Winter would trap the developmental or adult stages before the new eggs could be laid. Diapause also permits the late summer eggs to undergo the appropriate physiological adjustments necessary to withstand severe winter conditions. A diapause condition is essential for the maintenance of prolonged cold-hardiness. It is well known that the lower the rate of metabolism, the greater is cold-hardiness, and intensity and duration of diapause (Mansingh, 1971). Those eggs failing to enter diapause due to photoperiodic influence may not be prepared to withstand the cold

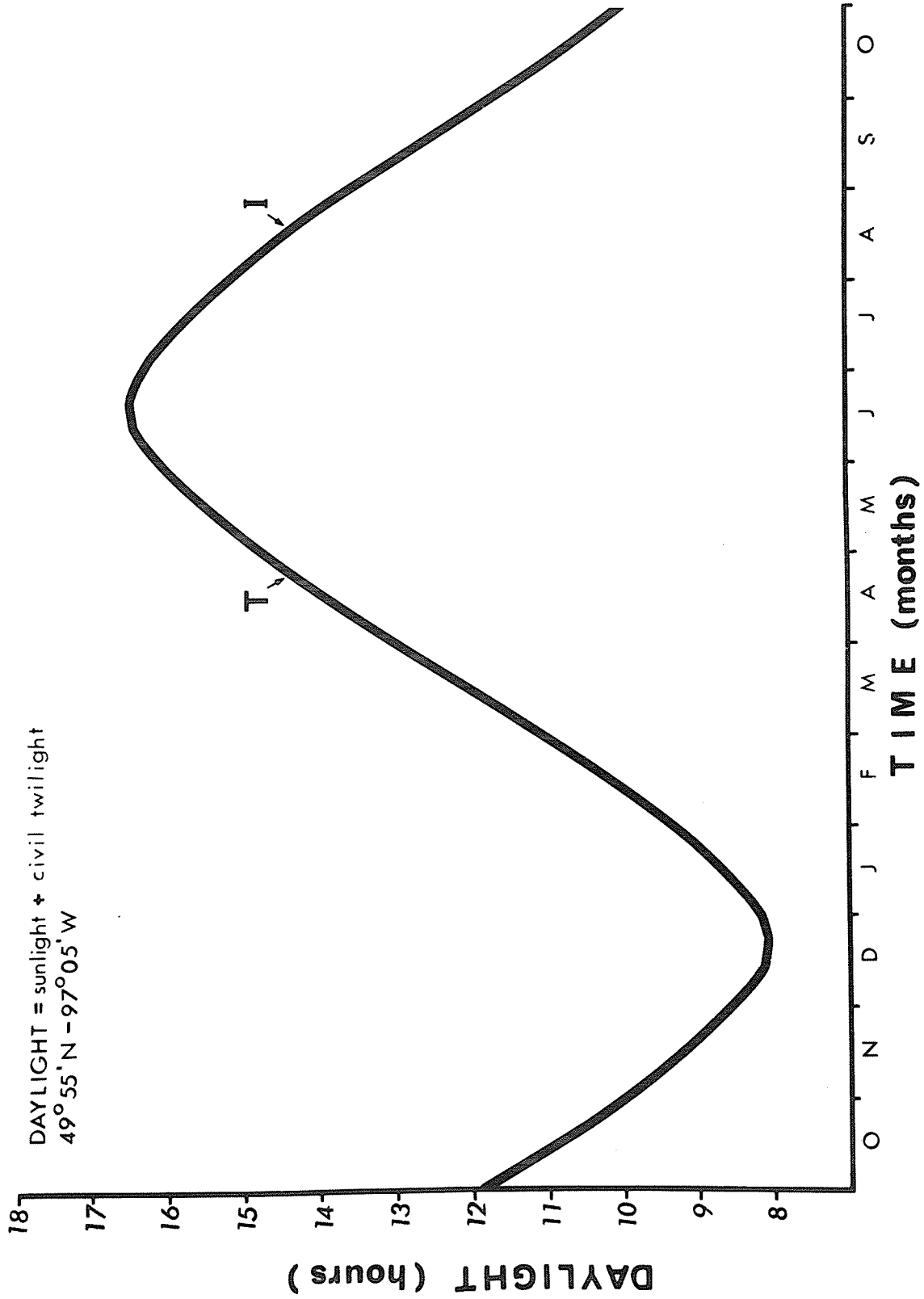

DAYLIGHT = sunlight + civil twilight
49° 55' N - 97° 05' W
DAYLIGHT (hours)
18
17
16
15
14
13
12
11
10
9
8
T
I
O N D J F M A M J J A S O
TIME (months)

Figure 5

temperatures in late September - early October, before there is sufficient snowfall to insulate the eggs from the nightly low air temperatures at this time of year. Experiments designed to test low temperature effects on non-diapausing and diapausing eggs are needed.

(b) Maintenance of Diapause

The duration of diapause provides some measure of the "intensity" of diapause in an insect species (Lees, 1955). The intensity of diapause is generally thought to be inversely proportional to metabolic and developmental rates, or directly proportional to the degree of suppression of these rates (Beck, 1968). The concept of "diapause development" has been defined by Andrewartha (1952) as the physiological development that occurs during diapause in preparation for the resumption of morphogenesis. Those definitions which have interpreted diapause as a state of "arrested" growth and development are inconsistent with the definition proposed by Andrewartha. Failure to define the processes as either anatomical or physiological may, in part, contribute to this contradiction. It is generally accepted that a state of "suppressed" metabolism and development does occur during the diapause condition. Beck (1968) has stated that the diapause state is rarely accompanied by morphological characteristics that permit immediate easy identification of either diapausing or nondiapausing insects.

The results of the experiment shown in Table VII indicate that eggs, maintained under a short photoperiod at 23^{o}C, remained in diapause for

a period of 3 months, as only a small percentage (< 10%) hatched when subjected to a hatching stimulus. The experiment was terminated at this point. The data suggests that diapause development (Andrewartha, 1952) in the eggs of A. dorsalis is very slow at 23°C.

(c) Termination of Diapause

Very little is known about the mechanisms by which either temperature or photoperiods hasten the completion of the diapause stage. The physiological relationships, for the most part, have not been investigated. Mansingh (1971) lends support to the view that diapause development or the "refractory phase" of diapause brings about the "activated phase" or the physiological ability to reactivate endocrine activity in diapausing individuals. The resumption of neuroendocrine activity appears to take place under favorable environmental conditions, gradually restoring all physiological and biochemical developmental processes to a 'normal' state.

Accordingly, diapause is not terminated immediately upon the return of favorable conditions; the activated condition of the individual and the nature of the stimuli determine the rate of diapause termination (Mansingh, 1971).

It is evident, in reviewing the results of the diapause-maintenance experiment, that eggs in the diapause condition could be activated only by subjecting them to an appropriate stimulus. Many investigators have demonstrated that prolonged exposure to low temperatures terminated the

diapause condition in the eggs of a number of insect species. In this laboratory, it has been observed that the embryonic diapause of a number of mosquito species, including A. dorsalis, could be terminated by subjecting the eggs to a temperature of 5°C for 3-4 months. The primary concern of this investigation was to determine an alternate method which would reduce the duration of the refractory phase of diapause eggs.

A preliminary experiment was performed to determine the effect of long-day photoperiod on the termination of diapause in eggs of A. dorsalis. Those diapause eggs which were not subjected to a long photoperiod treatment exhibited a very low percentage hatch (< 3%), as shown in Table VIII. Those diapause eggs which were subjected to 14 days of long-photoperiod treatment showed a high percentage hatch. It is interesting to note that the intensity of embryonic diapause appeared to be directly related to the duration of the short-photoperiod treatment, i.e. those eggs exposed to the diapause-inducing conditions (12L:12D and 23°C) for 14 days exhibited a significantly greater percentage hatch after the 14-day long-photoperiod treatment than did those eggs exposed to the diapause-inducing conditions for 28 days.

Previous investigations by Telford (1963) and Khelevin (1959) have neglected the role that photoperiod performs in the termination of embryonic diapause. Studies conducted by Baker (1935), Love and Whelchel (1955), Kappus and Venard (1967), Anderson (1970), and Kalpage (1970) have demonstrated that fully formed diapausing embryos of numerous multivoltine mosquito species are sensitive to photoperiod and that the

diapause condition can be terminated by exposure to long-day photoperiods. The results of the experiment, shown in Table VIII, clearly show an effect of long photoperiod at 23°C on the termination of diapause in eggs of A. dorsalis.

A second experiment was conducted to determine the effect of a high temperature on the termination of embryonic diapause. The results of the experiment, summarized in Table IX, indicate that an elevation in temperature to 30°C accelerates the processes of diapause development and neuroendocrine activity necessary for termination of diapause. High temperatures abbreviate the activated condition in diapausing individuals by increasing the relative rates of all the physiological and biochemical developmental processes (Mansingh, 1971). When comparing the effects of photoperiod and temperature on the termination of embryonic diapause (Table VIII and Table IX, respectively), it is difficult to determine whether or not high temperature terminates diapause more rapidly than long photoperiod. The final result is the same, when either long photoperiod at 23°C, or high temperature (30°C) is used separately on eggs which have been maintained at diapausing conditions for 14 days. However, when the two stimuli are separately applied to eggs which have been maintained at diapausing conditions for 28 days or longer, then high temperature (30°C) brings about diapause termination more quickly than long photoperiod (at 23°C).

A third experiment was carried out to evaluate the simultaneous effect of high temperature and long-day photoperiod on the termination

of embryonic diapause. In the previous experiment (Table IX) high temperature terminated the diapause condition in a high percentage of the eggs even at the diapause-inducing photoperiod. Thus at a long-day photoperiod, it would be expected that high temperature would either (a) terminate the diapause condition in a shorter period of time, or (b) cause a greater percentage hatch than was possible at a short-day photoperiod. However, the results as shown in Table X indicate that the incidence of photoperiod on the termination of diapause is masked by high temperature and eggs hatched as readily at 12L:12D as at 16L:8D.

As previously noted, embryonic diapause in A. dorsalis was also terminated by subjecting the eggs to a prolonged period of low temperature (5°C) even though the photoperiod was ca. 0L:24D per diem. This observation is in agreement with Horsfall (1956), Beckel (1958a), Khelevin (1959), Horsfall and Fowler (1961), Brust and Costello (1969), Anderson (1970), McHaffey and Harwood (1970), and Ellis (1973) who have demonstrated that diapause is terminated even under dark conditions. It is not known whether a long photoperiod at 5°C would speed up the termination of diapause. Preliminary experiments carried out on A. hexodontus Dyar in this laboratory, indicated that photoperiodic conditions did not affect the percentage hatch after 3-4 months (Brust, person. comm.). This supports the general thesis that photoperiod effects are masked at extreme high or low temperatures.

Although the influence of photoperiod on diapause termination appears to be subordinate to high temperature, this is of little ecological significance as high temperatures could only occur during seasons when daylength is favorable for growth conditions. Since cold temperatures probably terminate diapause before the appropriate photoperiod and temperatures occur in the spring, it is suggested that neither long photoperiod ($>$ 14½L:9½D per diem) nor high temperature play a vital role in terminating diapause in the eggs of A. dorsalis under field conditions. It is therefore proposed that studies be conducted to determine the specific time, at a short photoperiod and low temperature, required to terminate diapause in A. dorsalis eggs and to determine the stimulus required to induce a hatching response in the eggs.

CHAPTER VI

SUMMARY

1. The optimum temperature for development and survival of A. dorsalis in the laboratory is 23°C. Those individuals reared at this temperature appear to be larger and healthier than those reared at the other experimental temperatures. Under optimum temperature conditions, completion of larval development takes place in 7-8 days, while pupal metamorphosis requires an additional 1½-2 days prior to adult emergence.

2. At 23°C, there is no apparent influence of photoperiod on the developmental rates or mortality of A. dorsalis. The length of photophase had no effect on mating (percentage insemination) of A. dorsalis. The duration of the periods of changing light intensities (dawn and dusk) may influence mating behaviour and the success of copulation. There is no significant difference which can be attributed to photoperiodic influence in the number of eggs produced (fecundity) be female imagos.

3. The presence of A. dorsalis appears to increase the mating response in A. vexans when the two species are placed simultaneously in the same cage. There is no evidence to suggest that the mating of A. dorsalis is affected by the presence of A. vexans.

4. The local strain of A. dorsalis shows a high incidence of embryonic diapause at short photoperiods of 8L:16D through 14L:10D and a constant temperature of 23°C. A diapause condition is not evident in eggs at long photoperiods of 15L:9D through 24L:0D at 23°C.

5. The critical photoperiod, with regard to embryonic diapause, is indicated to be 14½L:9½D per diem at 23°C.

6. Diapause induction in A. dorsalis eggs is not a cumulative effect of photoperiodic influence on the preceeding developmental stages. Eggs subjected to a short photoperiod (12L:12D) for 14 days exhibited a high percentage diapause while eggs subjected to a long photoperiod (16L:8D) for a similar period of time exhibited a very low percentage diapause.

7. Diapause eggs, if maintained under diapause-sustaining conditions (12L:12D and 23°C), remain in a diapause state for a period of at least 3 months. High temperatures (30°C) culminate the diapause condition in eggs after 14 days. This evidence suggests that the "diapause development" processes (Andrewartha, 1952) are accelerated at a high temperature. The influence of photoperiod on the termination of diapause is masked by high temperature, i.e., the effect of photoperiod is subordinate to the effect of high temperature.

8. Long-day photoperiods (16L:8D) terminate embryonic diapause after a period of 14 days at 23°C.

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APPENDIX A

Effect of photoperiod on the fecundity of virgin females of A. dorsalis.

♀	Photoperiod (L:D) 12:12			♀	Photoperiod (L:D) 16:8		
	No. eggs		Total no.		No. eggs		Total no.
	Oviposited	Retained	Eggs		Oviposited	Retained	Eggs
1	85	0	85	1	163	0*	163
2	0	88	88	2	0	nme	0
3	0	50	50	3	37	0	37
4	84	0	84	4	53	0	53
5	157	0	157	5	109	0	109
6	111	0	111	6	14	47	61
7	46	0	46	7	0	134	134
8	105	0	105	8	29	2	31
9	79	0	79	9	63	73	136
10	54	26	80	10	85	0	85
11	80	0	80	11	106	0	106
12	99	29	128	12	0	nme*	0
13	34	51	85	13	107	0	107
14	77	0	77	14	15	120	135
15	103	0	103	15	128	0	128
16	123	0	123	16	125	0	125
17	3	89	92	17	0	134	134
18	0	104	104				

* No mature eggs.

APPENDIX B

Effect of photoperiod on the induction of diapause in the eggs of A. dorsalis at 23°C.

Photoperiod (L:D)	Sample	Total no. Viable eggs	No. eggs Unhatched	% Diapause
8:16	1	25	25	100.00
	2	23	23	100.00
10:14	1	32	32	100.00
	2	21	21	100.00
12:12	1	38	35	92.11
	2	64	58	90.63
13:11	1	21	21	100.00
	2	33	32	96.97
13½:10½	1	24	23	95.83
	2	18	18	100.00
14:10	1	22	18	81.82
	2	11	11	100.00
14½:9½	1	26	12	46.15
	2	29	11	37.93
15:9	1	30	5	16.67
	2	45	1	2.22
16:8	1	52	0	0.00
	2	31	1	3.23
18:6	1	24	1	4.17
	2	21	1	4.76
24:0	1	35	3	8.57
	2	21	4	19.05

APPENDIX C

Effect of long-day (16L:8D) and short-day (12L:12D) photoperiods applied to the developmental stages of A. dorsalis, on the production of embryonic diapause.

Developmental Stages exposed to photoperiod					
L:D - 12:12	L:D - 16:8	Sample	Viable Eggs	Unhatched Eggs	% Diapause
1,2,3,4,P,A,E**		1	22	22	100.00
		2	30	28	93.33
		3	10	10	100.00
1,2,3,4,P,E	A	1	30	29	96.67
		2	35	33	94.29
		3	36	36	100.00
A,E	1,2,3,4,P	1	18	18	100.00
		2	24	24	100.00
		3	17	17	100.00
E	1,2,3,4,P,A	1	34	32	94.12
		2	47	46	97.87
		3	45	44	97.78
A	1,2,3,4,P,E	1	13	1	7.69
		2	4	0	0.00
		3	17	0	0.00
1,2,3,4,P	A,E	1	45	1	2.22
		2	53	0	0.00
		3	43	2	4.65
1,2,3,4,P,A	E	1	25	0	0.00
		2	30	1	3.33
		3	44	1	2.27
	1,2,3,4,P,A,E	1	30	2	6.67
		2	37	0	0.00
		3	31	1	3.23

** Numbers indicate larval instars; P=pupa; A=adult; E=F_1 embryo.

APPENDIX D

Effect of the hatching stimulus on diapause eggs of *A. dorsalis*, maintained at 12L:12D and 23°C.

Age of Eggs	Sample	No. eggs Hatched	Total no. Viable eggs	% Hatch
14	1	0	26	0.00
	2	0	33	0.00
	3	0	44	0.00
30	1	0	24	0.00
	2	2	30	6.67
60	1	1	28	3.57
	2	3	36	8.33
90	1	2	33	6.06
	2	2	29	6.90

APPENDIX E

Effect of photoperiod on the termination of diapause in eggs of *A. dorsalis* at 23°C.

No. days at 12L:12D	No. days at 16L:8D	Sample	No. eggs Hatched	Total no. Viable eggs	% Hatch
14	0	1	0	42	0.00
		2	0	22	0.00
		3	1	24	4.17
14	7	1	14	28	50.00
		2	9	27	33.33
14	12	1	10	11	90.91
		2	12	15	80.00
		3	11	12	91.67
14	14	1	16	17	94.12
		2	14	14	100.00
		3	11	12	91.67
28	0	1	0	11	0.00
		2	1	17	5.88
		3	0	20	0.00
28	7	1	11	26	46.92
		2	15	25	60.00
		3	24	36	66.67
28	14	1	22	28	78.57
		2	27	34	79.41
		3	15	22	68.18

APPENDIX F

Effects of high temperature on the termination of diapause in eggs of A. dorsalis, maintained at 12L:12D.

No. days at 23°C	No. days at 30°C	Sample	No. eggs Hatched	Total no. Viable eggs	% Hatch
30	4	1	2	27	7.41
		2	1	28	3.57
30	7	1	19	34	58.82
		2	11	30	36.67
30	10	1	16	31	48.39
		2	25	39	61.54
30	14	1	22	24	91.67
		2	28	28	100.00
60	14	1	25	27	92.59
		2	31	33	93.94
90	14	1	30	31	96.77
		2	26	27	96.30

APPENDIX G

Effect of high temperature and photoperiod on the termination of diapause in the eggs of *A. dorsalis*.

No. of days at		Photoperiod		No. eggs	Total no.	%
23°C & 12L:12D	30°C	(L:D)	Sample	Hatched	Viable eggs	Hatch
14	14	16:8	1	22	22	100.00
			2	20	22	90.91
14	14	16:8	1	33	34	97.06
			2			96.30
14	14	12:12	1	13	15	86.67
			2	8	8	100.00
			3	24	26	92.31