THE ORIGIN OF SPRING FLIGHTS OF *HELIOTHIS PUNCTIGERA* WALLENGREN IN SOUTH AUSTRALIA.

by

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SUMMARY

The origin of moths comprising spring flights of *Heliothis punctigera* in South Australia had been unexplained and this study was concerned with examining likely sources.

To determine if local weather conditions were consistent with the timing of such flights, an attempt was made to develop a model based on the rate of pupal development during winter. This involved studies on diapause and post-diapause development. The diapause development portion of the model was not formulated because of its complex nature. A preliminary model was developed for post-diapause development and provides a basis for field verification.

A cursory assessment of synoptic weather patterns indicated that migration from outside the area of study was unlikely.

Field studies on mortality factors of each stage of the life cycle, with special emphasis on the larval stage, indicated that *H. punctigera* survives within the area of study; but there were insufficient data to indicate that the spatially contracted over-wintering population accounts for the widespread occurrence of moths in spring.

No firm conclusion as to the origin of these moths was made and the shortcomings of the project are discussed.

DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text.

I consent to the thesis being made available for photocopying and loan if applicable, if accepted for the award of the degree.

S.E. LEARMONTH

JUNE 1986

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Finally, for providing unflinching moral support for "the duration", a special thanks to my parents, the Hittmanns and especially Jill.

SPECIAL NOTE OF THE AUTHOR

The field and laboratory work of this thesis were carried out over the period March 1975 - March 1977, with the laboratory studies on diapause beginning in mid-1975. Subsequent published work relevant to these studies and in some notable cases negating them, presented a problem in chronology when referring to them. Such papers are therefore only cited in discussions of results and not in the introductory sections where literature relevant to experiment design or likely treatment effects is normally included.

CHAPTER 1 INTRODUCTION AND OBJECTIVE OF THE STUDY

1.1 INTRODUCTION

This study is concerned with an investigation into some aspects of the ecology of *Heliothis punctigera* Wallengren (Lepidoptera: Noctuidae) in South Australia, relating especially to the origin of the flights of moths appearing in spring each season. Although the economic importance of this species throughout Australia is now well known, its identification in the past has been confused.

1.1.1. Historical review of the identification of *H. punctigera*

The name *Heliothis* was first used validly by Ochsenheimer in 1816 and *H. punctigera* was named and described by Wallengren in 1860. Despite this detailed description of the species, Hampson in 1903 (in Common 1953) considered it synonymous with *H. armigera*, which was the name given to the majority of what are now considered to be distinct species of *Heliothis*. Hampson (and previously Aurivillius 1897) further pointed out that *obsoleta* had priority over *armigera* and questioned the validity of the genus, preferring to place it in *Chloridea*. Tams (in Common 1953) rejected the changes Hampson made to *H. armigera*, and thereafter use of this name, though somewhat inconsistently, again became widespread. Much of the literature on the genus therefore refers to the same species by various names (e.g. *H. armigera*, *H. obsoleta*, *C. armigera*, *C. obsoleta*), while at times the same name is used to refer to what are now considered to be distinct species. The Australian *Heliothis* fauna was examined by Common (1953) who described and illustrated four distinct species, *H. armigera* (Hübn.), *H. assulta* Guen., *H. punctigera* Wallengr., and *H. rubrescens* (Walk.). It consequently became obvious that many reports concerning *H. armigera* in Australia up to that time were in fact referring to either or both *H. armigera* and *H. punctigera*, locally the two most important species of *Heliothis* economically. However, because of differences in both their distribution in Australia and, to a certain extent, the species of food plants on which they

have been found (Common 1953, Kirkpatrick 1961b), it is obvious which species is being considered in some of the reports written before 1953. Morphological comparisons of the two species were also reported by Kirkpatrick (1961a).

Following Common's work, new species previously thought to be synonymous with H. armigera have been described e.g. an important species of Heliothis occurring in North America was found to be distinct from H. armigera and Todd (1955) showed that it should be referred to as H. zea. A comprehensive study of the world Heliothis fauna has been made by Hardwick (1965) in which he described eleven new species and two new sub-species and also advocated placing the group in a new genus, Helicoverpa, because of differences between members of this group and the type species of Heliothis. This suggestion has not been widely accepted.

Most recently, in relation to a rule in scientific nomenclature that adjectival specific names must agree in gender with generic names, Todd (1978) suggested that the true name for the species should be H. punctiger. The matter was subsequently discussed by the International Commission on Zoological Nomenclature (Anon 1985) where it was agreed that the gender of the generic name was feminine, effectively reversing Todd's finding. The correct name therefore remains unchanged as H. punctigera.

1.1.2 Distribution and economic importance of H. punctigera

H. punctigera is an endemic Australian species and occurs throughout the continent except in the dry regions (of the interior and on the coast). The only records of occurrence outside Australia are the Cocos Islands (Common 1953), Norfolk Is. (Holloway 1977) and New Zealand (see section 2.3). In South Australia it is the most abundant member of the genus and the only economically important species; H. rubrescens also occurs here, but in low numbers. Recordings of H. armigera have been made in South Australia, but so infrequently that, for practical purposes, this species is regarded as not occurring here.

As is the case for other members of the genus, the economic importance of H. punctigera in South Australia arises from the feeding of larvae on the flowers and fruiting bodies of certain agricultural crops and other plants, such as ornamentals, that are valued by man. In South Australia, it is of major importance in the lucerne-seed and field-pea industries and of occasional importance in clover-seed crops, ornamental plants, tomatoes, and, during major outbreaks, in fruit and almond orchards. In other states, it is found on cotton, linseed, lupins, peanuts, safflower, soybeans, sunflower and tobacco, as well as lucerne, field peas etc. Apart from these crops and ornamental plants, H. punctigera has also been found on a number of weeds (e.g. see Richards 1968).

Lea (1928) mentions that docks, thistles, capeweed and stinging nettles are attacked by this species in South Australia. Following field and laboratory observations made during the present study, other species of weeds can now be added to the list for South Australia (see Section 3.4.2).

1.1.3 Description of the study area

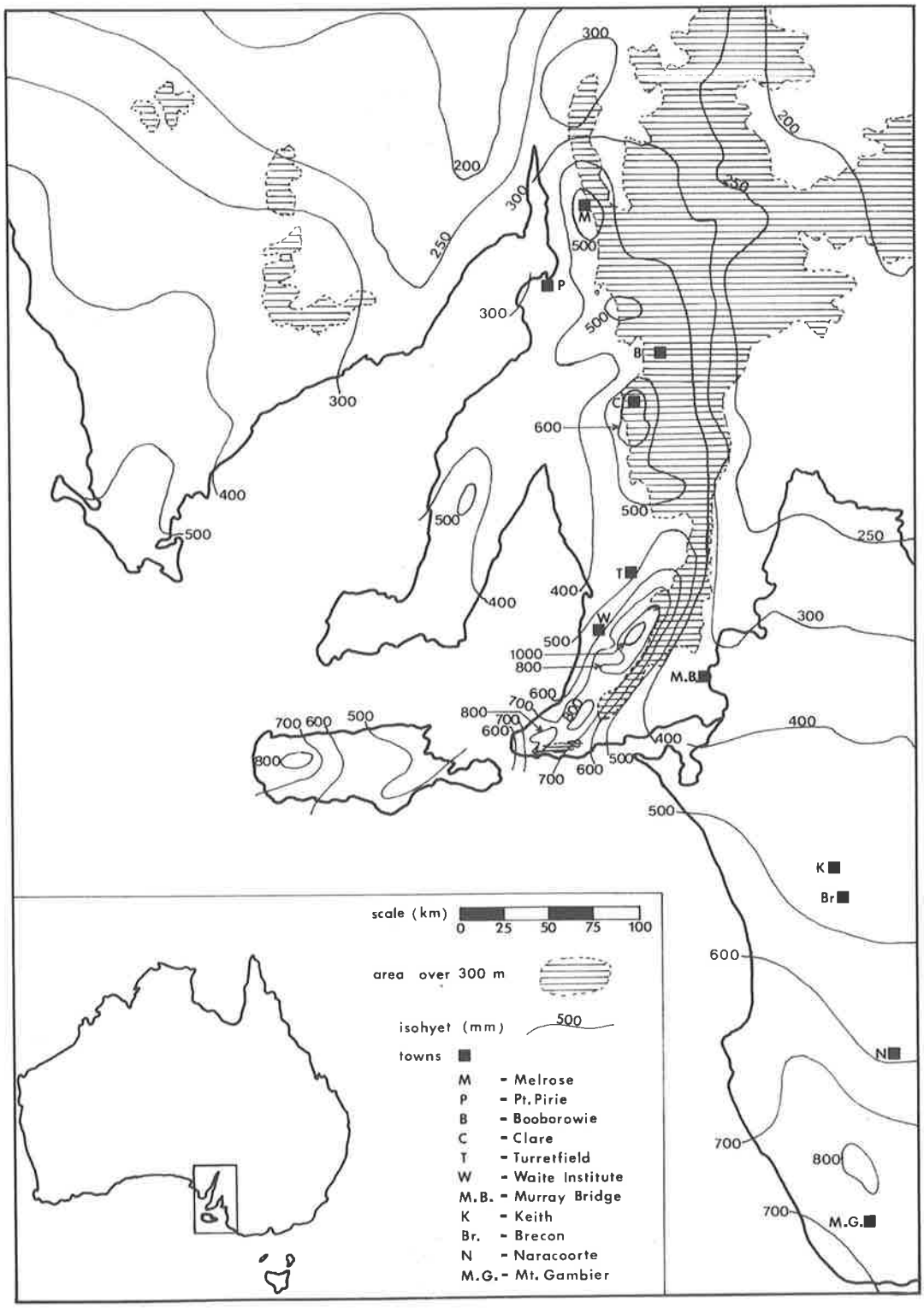
1.1.3.1 Climate

The location of the study area together with elevation contours and average rainfall isohyets are shown in Figure 1.1. The area is generally of low relief, and is dominated by the Mt. Lofty - Flinders Ranges system, which nowhere exceeds 1200 m. The influence of these ranges on the distribution of rainfall is obvious in Figure 1.1. Rainfall, bourne on westerlies blowing over the Southern Ocean, tends to be higher on the western side of the ranges, and within them, than on the plains to the east. A feature having an ameliorating effect on the overall climate in the study area is the Southern Ocean, which separates the Australian continent from the Antarctic polar land-mass, producing a more temperate climate compared with the areas at the same latitude in the northern hemisphere.

FIGURE 1.1

Land over 300 m in height and isohyets

in the area of study in South Australia.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M.G.
scale (km)
0 25 50 75 100
area over 300 m
isohyet (mm)
500
towns
M - Melrose
P - Pt. Pirie
B - Booborowie
C - Clare
T - Turretfield
W - Waite Institute
M.B. - Murray Bridge
K - Keith
Br. - Brecon
N - Naracoorte
M.G. - Mt. Gambier

The climate consists of hot, dry summers and cool but not severe winters, with most of the rainfall occurring in late autumn and winter (May to August). The average monthly rainfall for some selected stations in the study area (see Table 1.1) is indicative of its seasonal distribution. The winter months (June to August) are the wettest, with rainfall decreasing through September and October. Falls in November to March are slight, but rarely absent. Such falls, however, are not significant from an agricultural point of view because of the high evaporation rate during this period. The first useful falls, which end this dry period, usually occur in April to May.

Temperature records show that warm to hot weather is usually experienced between December and February with temperatures often higher inland than near the coast. Temperatures decline during March and continue to do so through April and May when the first frosts occur. The three coldest months are June, July and August and, in the colder regions, the mean temperature for these months is about 10°C, which is low enough to slow the growth of vegetation, but not to stop it altogether. Temperatures begin to rise in September and continue to do so until November. Plants begin to grow rapidly on stored moisture as temperature increases. Frosts can occur in this period resulting in some damage to plants. The monthly average maximum and minimum air temperatures for 5 stations in the study area are shown in Table 1.1.

1.1.3.2. Vegetation

Certain aspects of this climate, especially rainfall, have a profound effect on the occurrence and growth-habit of plants in the study area. The native and exotic annuals, as well as some native perennial species in unusually dry seasons, are dependent on rainfall and, to some extent, temperature, for continued growth. As rainfall decreases and temperatures rise, they begin to dry off so that usually by November, these plants have produced seeds or drought tolerant bulbs (e.g. soursob), which enable them to survive the

Table 1.1. Mean monthly rainfall, and mean daily minimum and maximum air temperatures for selected stations in South Australia (Anon. 1975).

(i) Mean Monthly Rainfall

Month and Rainfall (mm)

Station	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Year
Pt. Pirie	19	19	18	29	39	41	33	36	34	31	23	21	343
Clare	26	26	24	48	75	80	81	80	71	55	36	30	632
Waite Inst.	24	27	21	56	79	73	85	73	63	55	39	30	625
Keith	19	24	21	35	57	52	54	57	51	43	32	26	471
Mt. Gambier	25	34	33	62	76	78	101	92	67	62	45	37	712

(ii) Mean Daily Minimum Air Temperature

Month and Temperature (°C)

Station	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Year
Pt. Pirie	17.3	17.6	15.8	13.2	10.3	8.4	7.5	7.7	9.3	11.6	13.7	15.7	12.3
Clare	13.9	13.6	11.5	8.6	5.8	4.0	3.4	3.7	5.0	7.5	9.5	11.8	8.2
Waite Inst.	16.3	16.4	15.4	12.9	10.6	8.6	7.8	8.1	9.3	10.9	12.7	14.7	12.0
Keith	13.1	13.5	11.6	9.7	7.5	5.7	5.5	5.6	6.7	8.0	9.6	11.4	9.0
Mt. Gambier	10.8	11.4	10.2	8.6	6.9	5.2	4.7	4.9	5.7	6.7	7.8	9.5	7.7

(iii) Mean Daily Maximum Air Temperature

Month and Temperature (°C)

Station	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Year
Pt. Pirie	31.4	31.1	28.6	24.6	19.6	17.2	15.9	17.2	20.0	24.4	26.7	28.8	23.8
Clare	30.1	29.1	26.5	22.2	16.9	14.6	13.2	14.2	17.0	21.4	24.7	27.0	21.4
Waite Inst.	27.8	27.5	25.6	21.4	17.8	15.1	14.2	15.1	17.6	20.3	23.3	25.8	21.0
Keith	29.9	29.9	26.7	22.9	17.8	15.6	14.8	15.8	18.1	21.8	24.4	26.8	22.0
Mt. Gambier	25.6	25.3	23.1	19.9	15.8	14.0	13.1	14.0	15.6	18.1	20.4	22.5	19.0

rigors of the South Australian summer. Other plants, however, continue growing throughout the season. The latter category includes irrigated exotic species and both exotic and native perennials growing under conditions of natural rainfall. One such exotic species is of particular interest (see below-section 1.1.5). This plant is the perennial legume lucerne (Medicago sativa). The next section describes the way in which it is managed in this environment.

1.1.4. Management of lucerne

In South Australia, lucerne is used as a source of fodder for animals or to produce seed (see Figure 3.1 for areas of production in South Australia). It is grown as a perennial (e.g. some seed crops are more than fifteen years old) and if managed correctly, can be cut and allowed to regrow many times during the life of the crop.

There is little growth of lucerne through the winter months and it is not until spring, with the higher temperatures, that appreciable growth occurs. Crops begin to flower in October and the first cut (or grazing) occurs in November when crops are in full bloom. Up to this time, both seed and fodder crops have been managed in the same way, but after this first cut, the seed crop is fenced off from stock and left to flower and set seed. Crops are reaped in the North around early March and progressively later towards the South. Fodder crops, however, are cut or grazed in cycles throughout the season when they reach full bloom, once every four to five weeks during summer.

Seed and fodder crops are grown under both irrigated and dryland conditions. Irrigation water is obtained using bores to tap underground supplies and also from the River Murray and associated fresh water lakes near its mouth. The water is applied through overhead sprinklers or by flooding.

Dryland lucerne has been established in situations where the extensive root system which the plants are capable of producing enables them to utilise

water occurring in soils of alluvial flats adjacent to streams (e.g. in the mid-North) or where the water table is reasonably high (e.g. in the South-east). Naturally these crops depend on rainfall for good yields, but rarely does crop growth cease entirely, even in unusually dry seasons.

Because lucerne provides an important source of food for both moths and larvae, the effect of the two crop management systems on the abundance of flowers and vegetative growth through the season will have a considerable influence on the activity of H. punctigera. Usually fodder lucerne crops are subdivided into a number of paddocks which are cut or grazed in rotation so that some lucerne is almost always in flower. This can be compared with the situation in seed lucerne where peak flowering occurs around mid-January, and thereafter the number of flowers declines as seed pods develop and the crop matures. By March, flowers in seed lucerne crops are rare. Superimposed on this situation with respect to availability of food, is the occurrence of the different stages of H. punctigera in the field and this, or course, is related to the time required to complete the life cycle, as well as other ecological influences on H. punctigera within the study area.

1.1.5. Seasonal occurrence of H. punctigera

With the exception of a thesis by Cullen (1969) on the reproduction and survival of H. punctigera in South Australia, no detailed studies on the ecology of this insect were found in the literature. However, where light-trap data have been collected and field observations made on larvae and adults by various workers (Greenup, Kay, Wardhaugh, pers. comms.), the seasonal occurrence of H. punctigera can be described at least in general terms.

In the Eastern states of Queensland and New South Wales, moths are scarce and larvae absent during winter. Following winter, a flight of moths (usually small when compared to later flights during early summer) occurs in August or September. The larval progeny of such flights feed mainly on

abundant weed species and produce the second generation of moths, which is usually much larger than the first. These moths oviposit on weeds and, more importantly, on agricultural crops which have become suitable for oviposition and larval development. During autumn, the number of moths and larvae decline and eventually few can be found in the field. At this time, H. punctigera pupae enter a state of diapause and do not emerge as moths until the following spring.

The above outline is consistent with the situation in South Australia as proposed by Cullen. However, since his discussion and conclusions define the problem which is the subject of the present study, it is considered desirable to elaborate on the ecology of H. punctigera as he described it.

According to Cullen, the appearance of H. punctigera in South Australia begins each season with a flight of moths in spring. Subsequently, larvae are abundant on a wide variety of plant species. These larvae pupate over a considerable period of time, but a large proportion enter pupal diapause. The remainder undergo pupal development to produce a flight of moths usually in November. As stated above, many annual food plants of H. punctigera have begun to dry off about this time. Lucerne, remaining green, therefore becomes the most important food plant until the end of the season.

The number of third generation moths emerging in December is increased with the emergence of moths from those first generation pupae which entered diapause. Cullen suggests the high soil temperatures experienced at this time cause the termination of the diapause. Two or three more generations occur until early autumn, when the abundance of the insect declines in the two situations investigated by Cullen - seed and fodder lucerne crops. The reasons for this decline vary according to the situation.

In seed lucerne, with the scarcity of flowers (see section 1.4 above), the fertility and hence the effective fecundity of moths, decline. Subsequently, larval abundance declines also. In fodder crops, with a

continual supply of food (i.e. flowers), moth fertility and fecundity achieve their full potential. Consequently, larvae are abundant here; however, an increasing proportion of them succumb to a naturally occurring nuclear polyhedrosis virus (NPV) as the season progresses, and during autumn almost all larvae are affected. Therefore, in both seed and fodder crops the abundance of H. punctigera was thought to be low during autumn.

This field work was complemented with laboratory studies on the effect of temperature and photoperiod on the induction of diapause. From these studies it was suggested that diapause induction in the field occurs during mid-autumn i.e. when few larvae could be found in the field. Cullen concluded that the abundance of overwintering pupae in the study area was not sufficient to account for the number of moths present in the following spring flights. Possible explanations of this anomaly were proposed by Cullen - (a) long range migrations of moths from outside the study area during spring, (b) modifications in the diapause induction mechanisms under field conditions such that diapause is induced earlier in the season when larvae are more abundant and (c) the existence of areas near or within the study area where H. punctigera larvae are abundant in autumn and the dispersal of moths from the resultant over-wintering pupae produces the spring flights. None of these possibilities was investigated by Cullen.

1.2. OBJECTIVE OF THE STUDY

The objective of the present study was to identify the origin(s) of moths comprising the spring flights in South Australia. The explanations examined were that moths migrate into the area of study each spring, supplementing a very small resident population, or that, unbeknown to Cullen, survival of H. punctigera was high enough in some areas within South Australia to produce a large overwintering population.

CHAPTER 2 TIMING OF SPRING FLIGHTS AND MIGRATION

2.1. INTRODUCTION

The procedures that have been employed to study the migration of a scattered population of a wide-ranging insect in a direct manner (e.g. capture, mark, release, recapture, and the use of radar), require a large expenditure of time and labour. These resources were limited in the present study and the hypothesis of migration to explain the annual spring flights of *H. punctigera* in South Australia had to be tested using phenomena which are less directly related to migration.

One such phenomenon is the occurrence of discrepancies in the timing of the flights. For example, Helm (1975) concluded that the noctuid *Persectania erwingii* migrates from South Australia to Tasmania because moths were found in Tasmania earlier than could be explained by field temperatures there. In addition to this approach, there are many reports in the literature (including the above study by Helm) where an analysis of synoptic weather conditions enabled workers to confirm both the existence of a mechanism to account for a migration, and also the most likely source of the immigrants.

These two aspects - the timing of spring flights and studies of synoptic weather patterns - are considered below.

2.2. THE TIMING OF SPRING FLIGHTS

2.2.1. Introduction

The study by Helm on *P. erwingii* was made less complex because of the absence, in this species, of any over-wintering adaptive mechanisms such as diapause, and it was possible to estimate the timing of moth flights by considering normal development-temperature relationships only; such is not the case for *H. punctigera*.

In a study on the ecology of *H. punctigera* in South Australia, Cullen (1969) found that this species undergoes a facultative diapause in autumn. It was

also shown that field temperatures were not so low during winter to stop normal pupal development completely. Such pupae not entering diapause would subsequently emerge as moths during winter. However, few moths are caught in light traps during this time (see Appendix 7), and if H. punctigera were to survive in South Australia, the catches of larger numbers of moths in spring would have necessarily emerged from pupae that had entered diapause in the preceding autumn. In this report, field observations on the presence of larvae confirmed a decline in their abundance in autumn (see Section 3.4.3.4) and collections of pupae during winter confirmed the presence of a pupal diapause (see Figure 2.1).

Information on the time of year at which pupae enter diapause and the rate at which pupal development is completed in the field, provides the basis on which the timing of emergence of moths locally can be determined. Such a theoretical assessment could then be compared with more empirical evaluations e.g. examination of light trap records.

The timing of pre-winter diapause induction of H. punctigera in the field was discussed by Cullen (1969), but his conclusions were principally based on results of laboratory experiments. Attempts during the present study to corroborate his conclusions using field cages were unsuccessful because of a high mortality rate of larvae.

With respect to the duration of the pupal stage for diapausing pupae, it is necessary to consider it as two separate components of development - diapause and post-diapause (Phillips and Newsom 1966, Cullen 1969, see also Appendix 4).

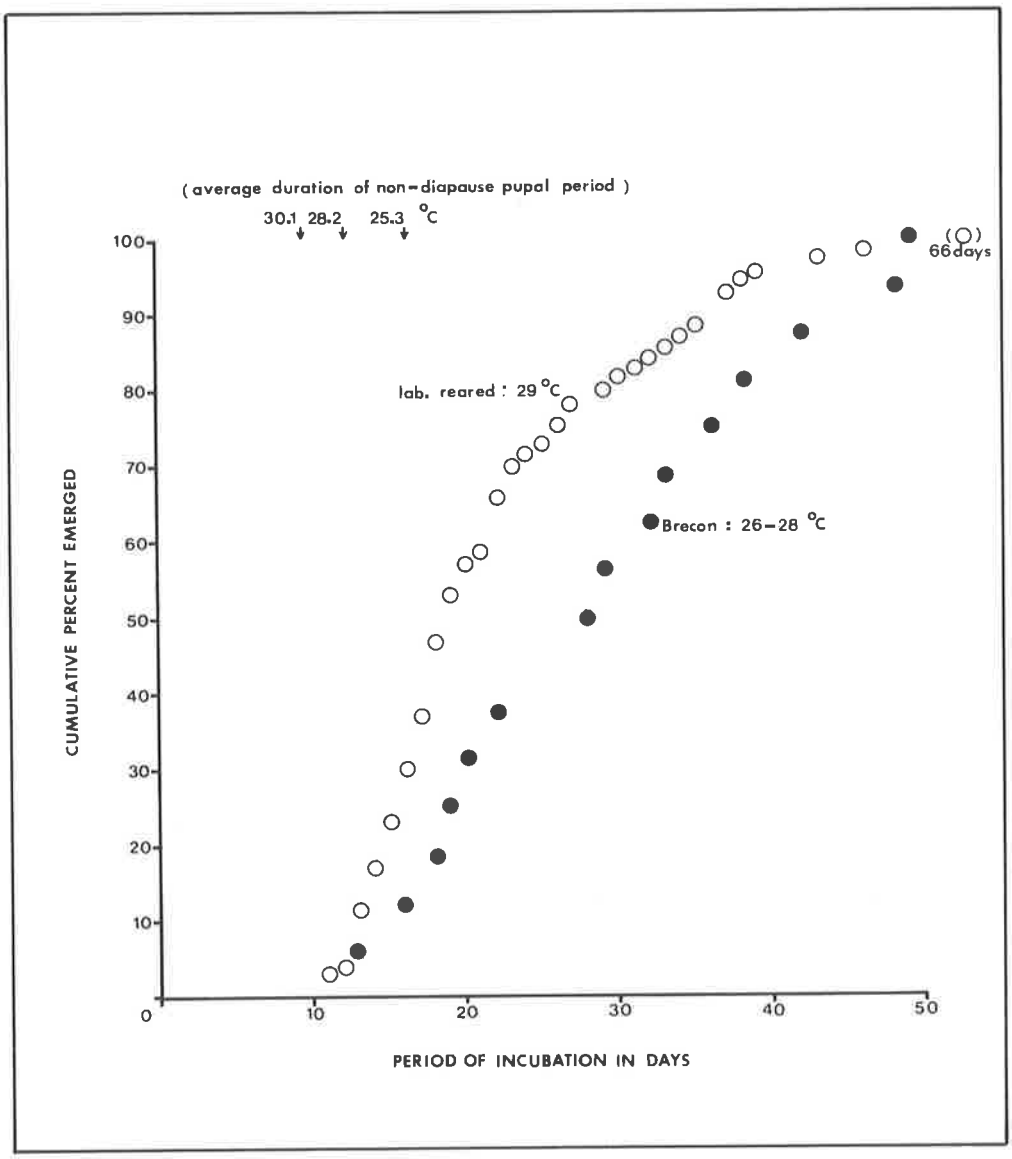
2.2.2. Completion of diapause development

Introduction

The completion of physiological processes associated with the termination of diapause has been termed diapause development by Andrewartha (1952). Reviews of the literature on the rate of diapause development in insects have been conducted by many authors (e.g. Andrewartha 1952, Lees 1955, De

FIGURE 2.1

The cumulative proportion of pupae reaching the adult stage plotted against the duration of exposure to laboratory rearing temperatures - (i) pupae collected under seed lucerne at Brecon 20.iv.1975 and reared at 26-28°C; (ii) pupae induced to enter diapause in the laboratory (see text) and reared at 29°C. Also shown are the mean durations of the pupal stage for non-diapause pupae reared at constant temperatures of 25.3, 28.2, 30.1°C (Cullen 1969).


(average duration of non-diapause pupal period)
30.1 28.2 25.3 °C
(○) 66days
lab. reared : 29 °C
Brecon : 26–28 °C
CUMULATIVE PERCENT EMERGED
PERIOD OF INCUBATION IN DAYS
0
10
20
30
40
50
60
70
80
90
100

Wilde 1962, Danilevskii 1965, *Beck 1968). In general, for insects that undergo diapause during the pupal stage, the most important environmental parameters which affect the rate of diapause development are temperature and, with fewer examples, photoperiod and humidity.

Though relatively little work has been done on the rate of diapause development in members of the genus Heliothis, the same conclusions apply. Diapausing pupae of Chloridea obsoleta (syn. H. armigera) resumed normal development and emerged most rapidly after being exposed to 11°C for two months (Goryshin 1958). Working with the same species, Kuznetsova (1972) found that the rate of diapause development was greatest at a temperature of between 10 and 15°C. Low temperature (less than 15°C) was the most stimulating factor in causing the termination of diapause in H. zea (Roach and Adkisson 1971).

Cullen (1969) found that diapausing pupae of H. punctigera completed development in the shortest time when exposed to high temperature, while exposure to low temperature caused a small but inconsistent stimulatory effect. The relationships between the other environmental parameters - photoperiod (e.g. Roach and Adkisson 1971, Kuznetsova 1972) and humidity (e.g. Jones 1937, Bourke and Gellatley 1962) - and the rate of diapause development of Heliothis spp., have been found to be more tenuous.

Experiments were designed to investigate in more detail the relationship between the rate of diapause development in H. punctigera and certain environmental parameters. After the success of Andrewartha et al. (1974) in obtaining a more intense diapause in Phalaenoides glycinae by artificial means, their procedure was duplicated on H. punctigera in order to broaden this study.

Provided suitable quantitative data were obtained, relationships between the

* Footnote:-

Beck subsequently published a second edition in 1980.

various parameters tested and the rate of diapause development would be investigated so that the timing of diapause termination in the field could be calculated.

Procedure

Considerable variation was expected in the response of diapausing pupae to the proposed treatments (see references above), necessitating the use of large numbers of pupae. For this to be practicable, larvae were mass-reared in the laboratory (see Appendix 1) and induced to enter diapause artificially. This was achieved by exposing the adult and egg stages to a regime of 28°C and a photoperiod of 14 hours light/10 hours dark (14L:10D), and the larval stage to 19°C and 12L:12D (Cullen 1969).

Parent moths were collected as pupae from two sources - diapausing pupae obtained from the field and caused to emerge in the laboratory at 26-28°C (see Figure 2.1); the other source was a laboratory strain reared under non-diapause inducing conditions. The history of moths used in the experiment was recorded and specific matings (single pairs) were conducted. This precaution was taken to guard against possible heritability of various intensities of diapause (Phillips and Newsom 1966, Morris and Fulton 1970). For each larva, the histories of its parents and the day of oviposition within the egg laying period of its parents were recorded.

When larvae began to burrow into the vermiculite they were transferred to constant darkness still at 19°C. (Unless otherwise stated, after burrowing, all insects were held in constant darkness, except for brief periods during observations). Larvae were examined daily and the date of pupation noted. Pupae were held at 19°C for six* days (except in Experiment 3 - see below), after which time they were examined to determine whether they were in

* Footnote:-

Pupae were held at 19°C for six days after pupation to overcome the possibility of the treatments affecting diapause induction. This reasoning has been vindicated to some extent as a result of later work by Browning (1979 - see Table 3).

diapause (see Appendix 4), sexed and allocated at random to the various treatments (for explanation of the randomisation procedure, see Appendix 2).

Over the time that pupae were formed, they were allocated to nineteen treatments in all, but during three different periods. The resultant groups of treatments are considered as separate experiments.

The first experiment was designed to examine the affect of various times of exposure to low temperature on the rate of diapause development. Pupae were exposed to either constant low temperature or varying low temperature. The latter treatments were based on the average mean 2.5 cm soil temperature for the months of April, June and September (15, 10 and 15°C respectively) and the average minimum 2.5 cm soil temperature for the same months (10, 7 and 10°C) (see below for discussion of why this depth was used). After the initial six day period at 19°C, pupae were exposed to 25°C (the control treatment), left at 19°C, transferred to 15°C or exposed to temperatures of 7, 10, 15°C or variations of these temperatures before being placed at 25°C until moth emergence i.e. pupae were exposed to the following temperatures before being placed at 25°C - 7°C for 30 days and 60 days; 10°C for 30, 60 and 90 days; 30 days at 10°C then transferred to 7°C for 30 days and back to 10°C for a further 30 days; 30 days at 15°C then transferred to 10°C for 30 days and back to 15°C for a further 30 days.

In the second experiment pupae were exposed to high temperature (29°C) or placed at 25°C to examine the effect of high humidity and long days on diapause development. High humidity was achieved by soaking the vermiculite in the rearing vials with a 3 per cent sodium chloride solution giving a humidity of > 90%. Pupae were exposed to high humidity either on the day of transfer to 25°C or thirty days after transfer. The treatment involving exposure to long days was achieved by rearing pupae outside the lightproof cabinet (see Appendix 1) within a constant temperature room set at 14 hours light:10 hours dark - equivalent to the daylength occurring in mid-spring. A fifth group of pupae transferred to 25°C acted as a control treatment.

All pupae in the third experiment were wounded to study the effect of low temperature on a more intense diapause (Andrewartha et al. 1974). On the day of pupation, pupae were wounded (see Appendix 3 for a description of the method used) and allocated to four treatments - three involved exposure to 15°C for ten days then (i) transfer to 25°C, (ii) left at 15°C for a further ninety days before being transferred to 25°C and (iii) transferred to 10°C for ninety days before being placed at 25°C; the fourth (control) treatment involved an initial six days' exposure to 19°C then transfer to 25°C.

These treatment details and the number of pupae allocated to each of them are presented in Table 2.1.

After the initial six day period at 19°C, pupae allocated to 15, 19, 25 and 29°C were examined every 60, 30 and 14 days and daily respectively, until normal development had resumed. Those pupae held at low temperatures initially (7, 10, 15°C), were not examined until after being placed at 25°C - the final rearing temperature. When a pupa had completed diapause, it was aged as described in Appendix 4 and the date of diapause termination calculated. The incidence of mortality was also recorded.

Results

The level of mortality was generally low, but extended exposure to low temperature and wounding resulted in slight increases (Table 2.1). A complete record of the duration of the pupal diapause in all treatments is given in Appendix 5.

After about 550 days' exposure to 15°C (treatment 3, experiment 1) only 3% of pupae had resumed normal development. To determine the developmental stage of the remainder, they were divided into four groups and randomly allocated to rearing regimes of 15, 19, 25 and 29°C. The duration of exposure required to terminate diapause at these temperatures was obtained as described above.

Table 2.1 The treatments in the experiment on diapause termination, the number of pupae allocated to each treatment, their rate of mortality and the number in diapause at the conclusion of the experiment.

Experiment 1. Effect of low temperature

Treatment no.	Intervals in the pupal stage (days) and temperatures (°C) to which pupae were exposed					No. pupae allocated (% mortality)		No. pupae left in diapause[a]
	0-6	6-36	36-66	66-96	>96			
1 (control)	19	25	25	25	25	141	(0)	0
2	19	19	19	19	19	140	(10)	4
3	19	15	15	15	15	142	(20)	110[b]
3-1[b]	-	-	-	-	-	19	(0)	19
3-2	-	-	-	-	-	31	(3)	6
3-3	-	-	-	-	-	30	(3)	0
3-4	-	-	-	-	-	30	(3)	0
4	19	10	25	25	25	144	(3)	0
5	19	10	10	25	25	134	(8)	0
6	19	10	10	10	25	140	(11)	0
7	19	7	25	25	25	147	(5)	0
8	19	7	7	25	25	136	(7)	0
9	19	15	10	15	25	136	(7)	0
10	19	10	7	10	25	134	(10)	0

[a] as at 22.iii.1977 (termination of the experiment)

[b] as at 12.i.1977 (after failing to complete diapause at 15°C, pupae were reallocated to treatments 3-1, 3-2, 3-3, 3-4 :- 15, 19, 25, 29°C respectively.)

Table 2.1 (cont'd.).

Experiment 2 Effect of high temperature (T), daylength (L:D) and humidity (H).

Treatment no.	Intervals in the pupal stage (days) and the conditions to which pupae were exposed (main conditions underlined)									No. pupae allocated (% mortality)	No. pupae left in diapause[1]
	0-6			6-36			36-emergence				
	T(°C)	L:D(hrs)	H	T	L:D	H	T	L:D	H		
11 (control)	19	0:24	low	25	0:24	low	25	0:24	low	48 (6)	0
12	19	0:24	low	25	16: 8	low	25	16: 8	low	77 (3)	0
13	19	0:24	low	25	0:24	high	25	0:24	high	63 (3)	0
14	19	0:24	low	25	0:24	low	25	0:24	high	44 (7)	0
15	19	0:24	low	29	0:24	low	29	0:24	low	72 (0)	0

Experiment 3 Effect of low temperature on wounded pupae.

Treatment no.	Intervals in the pupal stage (days) and temperatures (°C) to which pupae were exposed				No. pupae allocated (% mortality)	No. pupae left in diaspause[1]
	0-6	6-10	10-100	100-emergence		
16 (control)	19	25	25	25	63 (21)	0
17	15	15	25	25	62 (10)	0
18	15	15	15	25	82 (20)	0
19	15	15	10	25	75 (23)	0

[1] as at 22.iii.1977 (termination of the experiment).

The means and coefficients of variation of the diapause periods at the final temperatures of incubation for all treatments are given in Table 2.2. The latter parameter is compared with figures obtained by Holtzer et al. (1976) working with H. zea. It is apparent that the emergence of H. zea within treatments in those experiments was more synchronous than for H. punctigera here.

The cumulative proportion of pupae completing diapause was plotted against duration of exposure at the final rearing temperatures (see Figures 2.2 A-E). With the exception of continuous exposure to 15 or 19°C (treatments 3 and 2), all treatments resulted in a reduction of the diapause period at the final rearing temperature when compared to the control treatment of continuous exposure to 25°C.

An inexplicable bimodal pattern of diapause termination for pupae exposed to 19°C experiment 1 (Figure 2.2.A) was observed. This also occurred in other treatments, but to a much lesser extent.

Of the low temperature treatments examined in experiment 1, 7°C had the greatest effect on terminating diapause (see Table 2.2; Figure 2.2.B). Exposure to 29°C in experiment 2 produced a similar result (Figure 2.2.C). Other treatments, in experiment 2, suggested a slight stimulatory effect of humidity especially when it was increased soon after pupation. Exposure to the long day photoperiod did not reduce the duration of diapause. This latter result is in general agreement with other workers where photoperiod had an inconsistent effect (e.g. Roach and Adkisson 1971, Kuznetsova 1972).

An unexpected effect of wounding pupae was to reduce the diapause period (see Figure 2.2.D; treatment 1 compared with treatment 16). However exposure of pupae to 15°C at pupation increased the diapause period as expected (treatment 17; see Figure 2.2.D). Results of these treatments also suggested that diapause termination occurred slightly more rapidly at 10°C than 15°C (treatments 18 and 19 respectively; see Figure 2.2.D).

Table 2.2 The average duration of the diapause period and coefficient of variation for treatments in the experiment on diapause termination.

Treatment no.	*Mean diapause period	Coefficient of variation (%) Present data	Holtzer et al.(1976)
Expt. 1			
1	88.2 b (g,k)	50	# 32.6
2	220.8 a	49	-
3-1	¶ -	-	-
3-2	+ 35.7	56	-
3-3	15.5	53	-
3-4	6.2	50	-
4	44.5 cd	83	-
5	32.2 de	101	-
6	38.0 d	80	-
7	47.5 cd	93	@ 29.4
8	18.3 f	134	$ 16.3
9	53.7 c	65	-
10	30.8 e	92	-
Expt. 2			
11	82.4 g	63	-
12	82.0 g	64	-
13	63.0 h	68	-
14	73.6 gh	69	-
15	13.6 i	115	-
Expt. 3			
16	44.4 m	69	-
17	62.3 l	63	-
18	30.4 n	79	-
19	16.5 o	102	-

* means followed by the same letter were not significantly different at the 5% level using an l.s.d. test; means in experiment 2 and 3 also compared with treatment 1 (assigned letters g and k respectively).

\# 23°C used instead of 25°C

@ 38 days at 8°C then to 23°C

$ 75 days at 8°C then to 23°C

¶ as at 22.iii.77 all pupae still in diapause

\+ as at 22.iii.77 6 pupae still in diapause

FIGURE 2.2

Diapause termination experiments.

The cumulative proportion of pupae completing diapause plotted against the duration of exposure at the final rearing temperature.

Numbers on graphs refer to treatment number.

FIGURE 2.2 A

Effect of low temperature.

Expt. 1.	Symbols	Treatment no.
	.....	1. 25°C control
	.....	2. 19°C
	O O O O	4. 30 days at 10°C, then 25°C
	□ □ □ □	5. 60 days at 10°C, then 25°C
	▲ ▲ ▲ ▲	6. 90 days at 10°C, then 25°C.

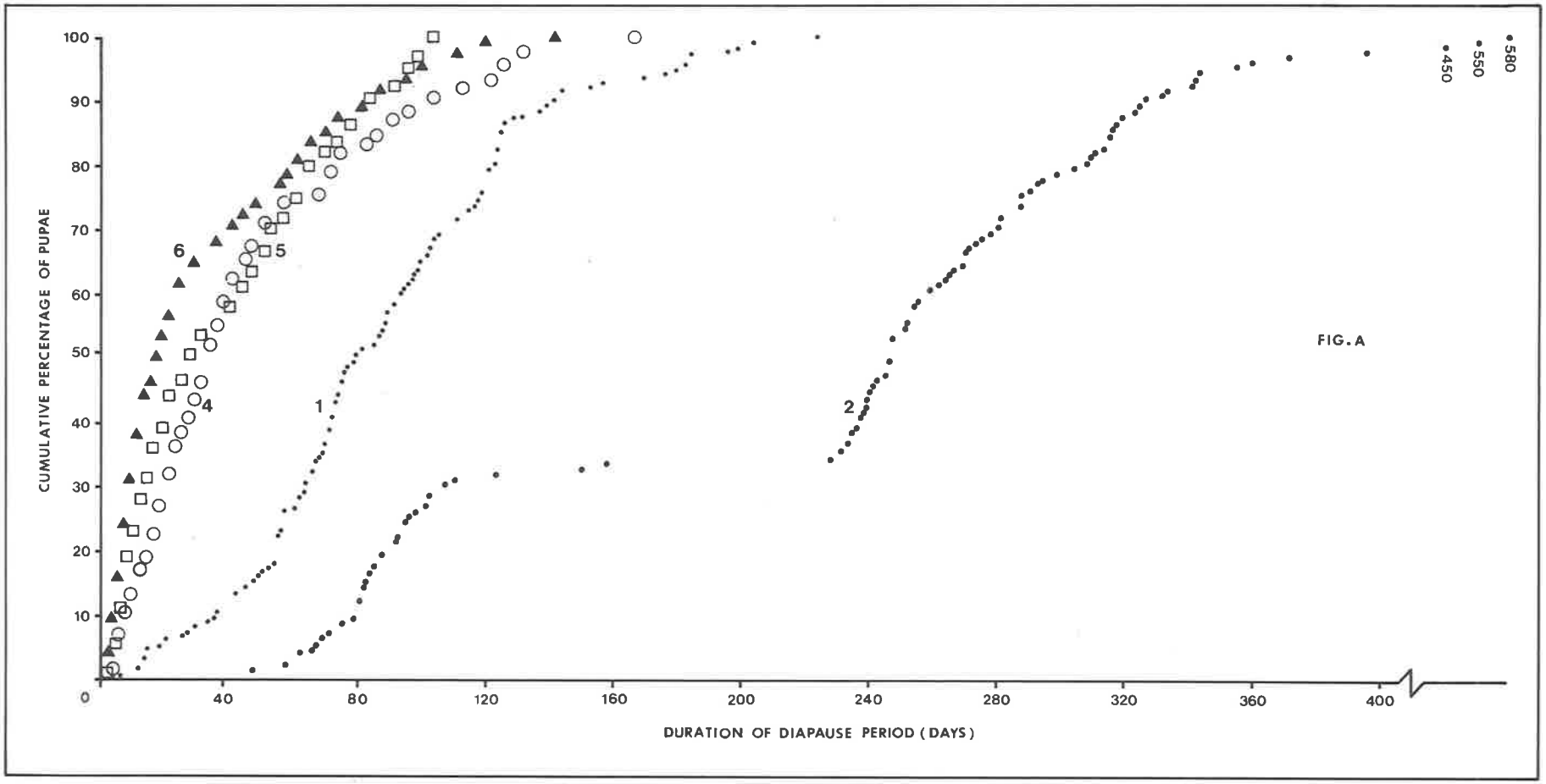

CUMULATIVE PERCENTAGE OF PUPAE
DURATION OF DIAPAUSE PERIOD (DAYS)
0
10
20
30
40
50
60
70
80
90
100
40
80
120
160
200
240
280
320
360
400
1
2
4
5
6
450
550
580

FIG. A

FIGURE 2.2 B.

Expt. 1 (cont'd.) Effect of low temperature.

Symbols	Treatment no.
.....	1. 25°C control
▲ ▲ ▲	7. 30 days at 7°C, then 25°C
.....	8. 60 days at 7°C, then 25°C
□ □ □	9. 30 days at 15°C, 30 days at 10°C, 30 days at 15°C, then 25°C
○ ○ ○	10. 30 days at 10°C, 30 days at 7°C, 30 days at 10°C, then 25°C.

FIGURE 2.2 C.

Expt. 2 Effect of long days, humidity, and high temperature.

Symbols	Treatment no.
.....	1. 25°C control (Expt. 1)
■ ■ ■	11. 25°C control (Expt. 2)
□ □ □	12. photoperiod of 16L:8D at 25°C
▲ ▲ ▲	13. high humidity at 25°C
△ △ △	14. high humidity after 30 days at 25°C
.....	15. 29°C.

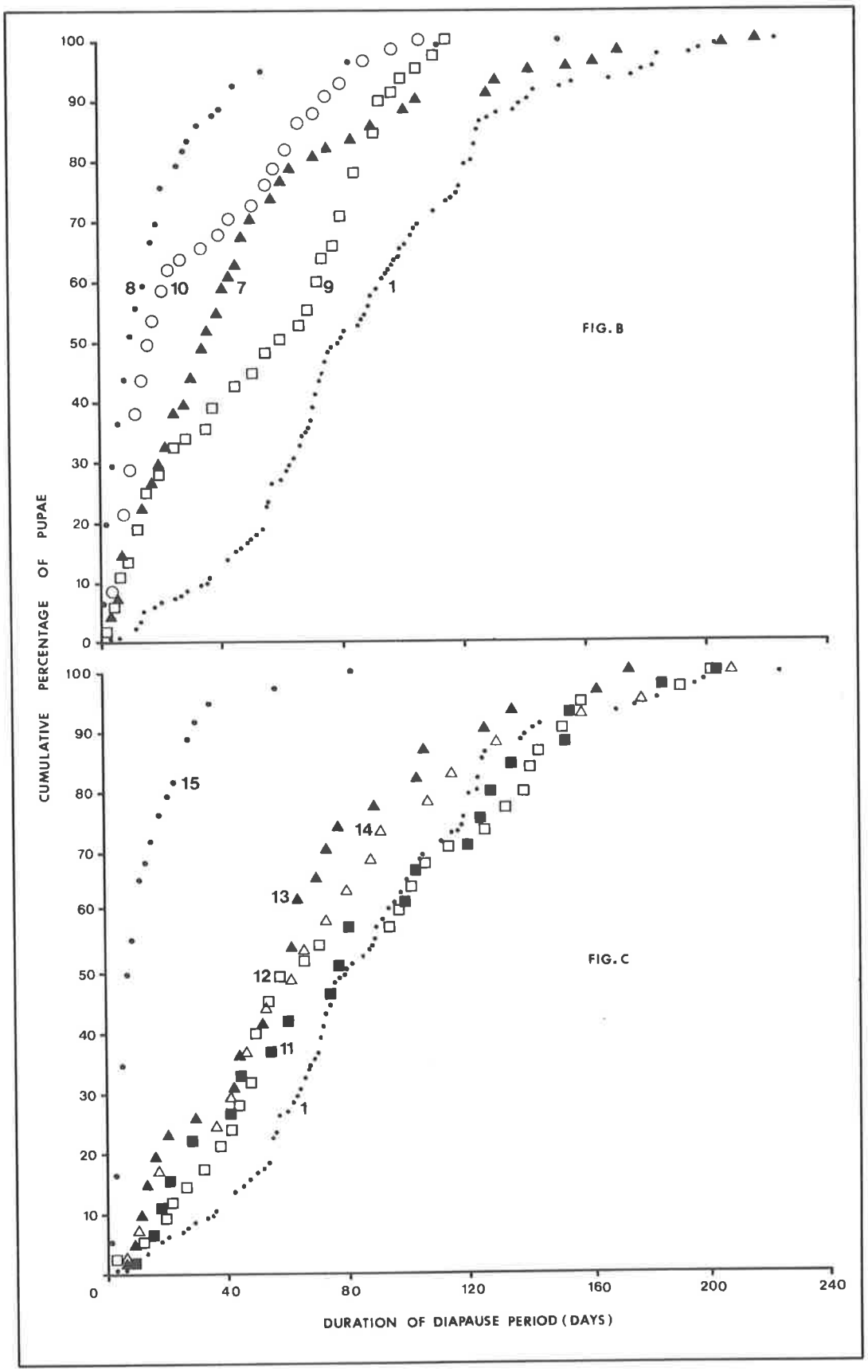

CUMULATIVE PERCENTAGE OF PUPAE
DURATION OF DIAPAUSE PERIOD (DAYS)
8
10
7
9
1
FIG. B
15
14
13
12
11
1
FIG. C

FIGURE 2.2 D

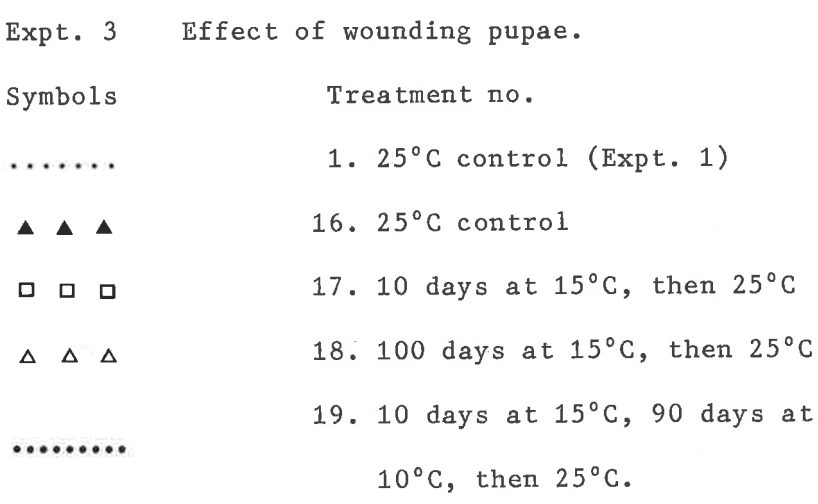
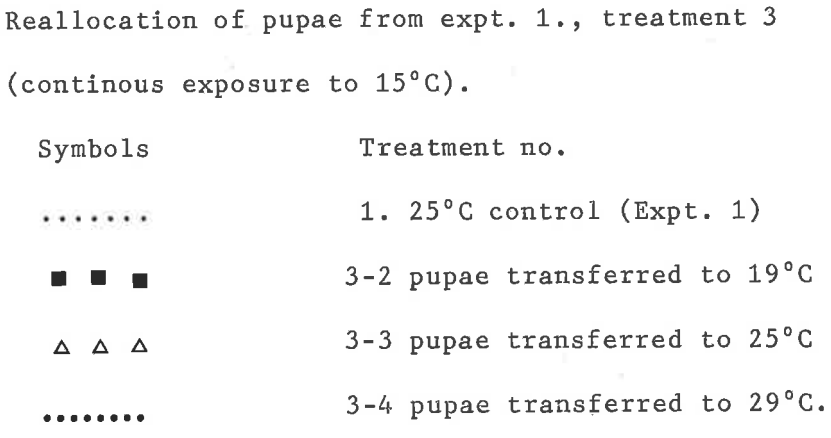
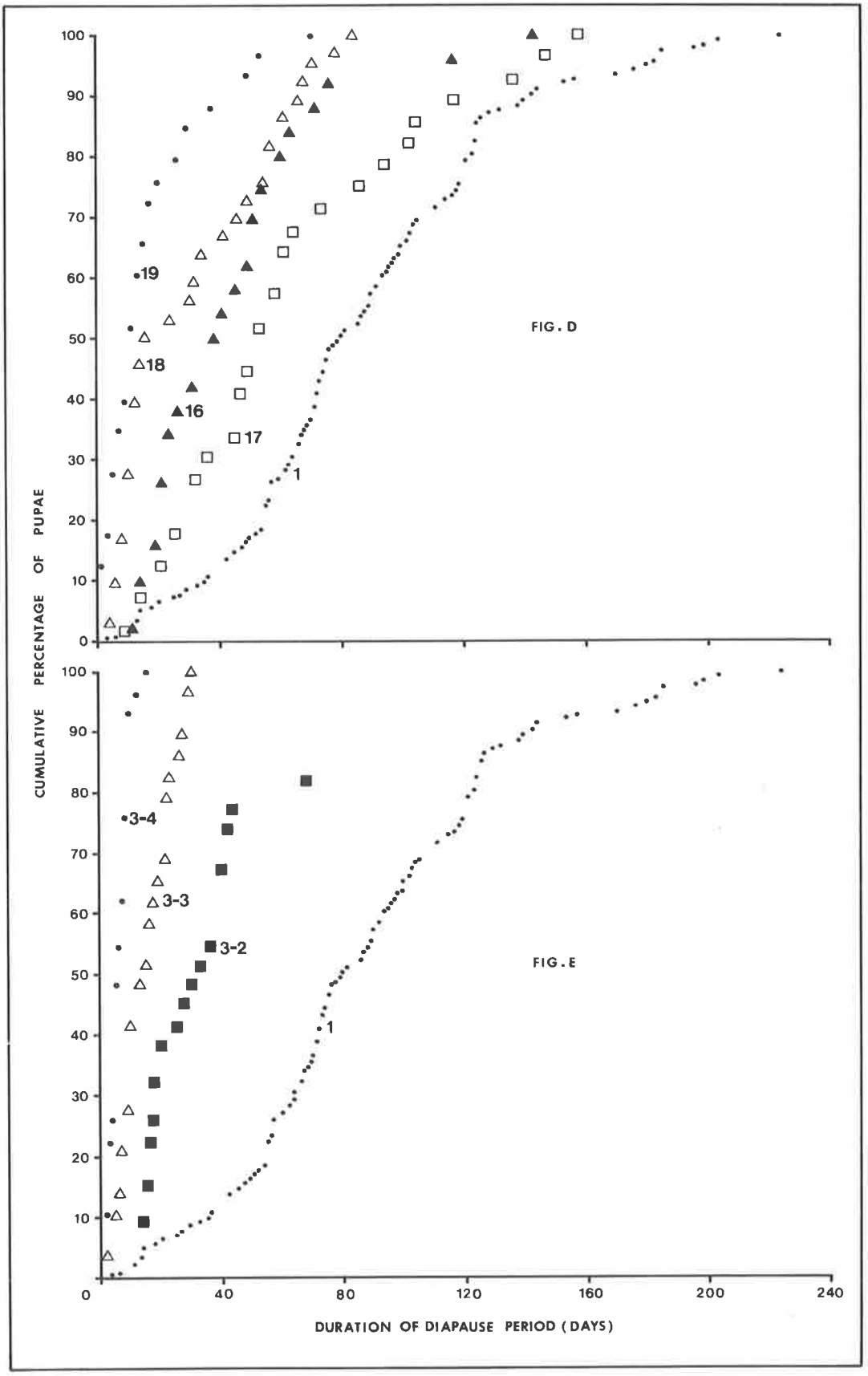


FIGURE 2.2 E




CUMULATIVE PERCENTAGE OF PUPAE
DURATION OF DIAPAUSE PERIOD (DAYS)
19
18
16
17
1
FIG. D
3-4
3-3
3-2
1
FIG. E

Reallocation of the pupae in treatment 3 to other treatments revealed that the long exposure of the diapausing pupae to 15°C had all but caused the completion of diapause (Figure 2.2.E). None of the pupae left at 15°C had completed diapause by the conclusion of the experiment.

Diapause intensity* was found to have been unaffected by either the history of the parents (but see Herzog and Phillips 1976) or the day in the oviposition cycle in which a pupa had been in its egg stage (see Appendix 6). For some treatments, a significant difference in the diapause period between the sexes was obtained. In these treatments, male pupae consistently completed diapause sooner (see Appendix 6). This was the reverse of the situation found for H. zea (Holtzer et al. 1976).

Discussion

It is apparent that there is no simple relationship between temperature and the rate of diapause development in H. punctigera. Therefore the aim of the above series of experiments was not achieved.

An examination of the data in more detail is made in an attempt to gain a clearer understanding of the nature of diapause termination as suggested by the above data.

For this, certain assumptions are necessary - (a) diapause intensity of a pupa was not increased after the initial six day exposure to 19°C i.e. maximum diapause intensity was achieved by this time and diapause development procedes thereafter; (b) the variation in diapause intensity within treatments was approximately the same for all treatments (random allocation of pupae to treatments should have ensured this): therefore the variation in diapause intensity of pupae in the 45-55 per cent decile of

* Footnote:-

Diapause intensity is defined for this study as the duration of the pupal stage prior to the completion of diapause i.e the diapause period, and for treatments where pupae had been exposed to various temperatures, this duration was taken as commencing at the time pupae were transferred to the final rearing temperature.

intensity for each treatment is approximately the same (see below); (c) the rate of diapause development at the final rearing temperature of 25°C is constant and is the same for all such treatments i.e. it is independent of the diapause intensity of pupae at the time of exposure.

The mean diapause periods at the final rearing temperatures of pupae in the 45-55 percent decile (see Table 2.3) are used to eliminate the extremes of the range in diapause periods and hence, reduce the coefficient of variation (Table 2.3 cf. Table 2.2). Using these values, an interpretation of the way in which diapause may have been completed in the above experiments, is given.

Table 2.3 The mean and coefficient of variation of diapause periods at the final rearing temperature for the 45 - 55% decile of pupae.

Treatment	No. pupae	Mean diapause period (days)	Coeff. of variation (%)
1 (control)	14	80.2	6.1
2	12	246.3	1.2
4	14	33.6	4.8
5	12	17.2	10.2
6	13	28.0	10.6
7	14	35.6	7.5
8	13	10.6	8.0
9	13	60.4	9.7
10	12	17.0	7.5
11 (control)	5	76.8	3.0
12	7	63.0	9.3
13	6	59.5	6.6
14	4	61.3	8.4
15	7	7.9	11.4
16 (control)	5	38.2	7.0
17	6	53.3	2.8
18	7	19.3	29.9
19	6	10.8	9.1

The intensity of the diapause of a pupa at the time of exposure to its final rearing temperature is, from the assumptions above, either at its maximum i.e. 100%, or something less than that (e.g. for pupae exposed to low temperature). In the latter case, the intensity of the diapause at that

time can be quantified as the ratio of the durations of (a) exposure at the final rearing temperature to (b) that of pupae continually exposed to that temperature before diapause termination occurred i.e. the latter durations are those recorded in the control treatments - numbers 1, 11, 16 for experiments 1, 2, 3 respectively. This ratio is calculated using the mean diapause periods in Table 2.3.

Graphs depicting diapause termination were then constructed in the following way. For each treatment the line for the appropriate control treatment was drawn first and it connects the points (a) 100% diapause intensity (i.e. start of exposure) and (b) the mean duration of exposure prior to diapause termination (using data in Table 2.3). Parallel "diapause - decay" lines (assumption (c) above) were drawn for other treatments in backwards fashion i.e. beginning at the mean exposure period for diapause termination in that treatment.

The rate of diapause development during exposure to low temperature i.e. the slope of the line, was obtained by joining points of appropriate diapause intensities - either back to 100% diapause intensity (i.e. start of exposure) or to that intensity indicated by another treatment using the same low temperature but at a shorter duration of exposure.

In experiment 1 the rate of diapause development at each of the temperatures 7 and 10°C is shown to vary, depending on the duration of exposure (although the decline is less obvious at 7°C) (see Fig. 2.3.A, B). While this may be an artifact related to the method of interpretation, a change in the rate of diapause development as diapause intensity declines has been suggested by Tauber and Tauber (1976). This was interpreted as diapuase termination being a dynamic process, with the optimal temperature for diapause development changing as diapause is completed. The ecological relevance of this was that temperature could thus act to maintain diapause - a low optimal temperature in autumn to prevent early diapause termination and a suggested higher optimal temperature in late winter to serve as a mechanism

FIGURE 2.3

The change in diapause intensity with duration of exposure to various temperatures in the experiment on diapause termination (numbers next to lines are treatment numbers).

For figures A-D, duration of exposure was measured from six days after pupation; for figure E, it was measured from the day of pupation.

A. Experiment 1; control - treatment 1; exposure to 10°C - treatments 4, 5, 6.

B. Experiment 1; control - treatment 1; exposure to 7°C - treatments 7, 8.

C. Experiment 1; control - treatment 1; exposure to varying low temperatures: 15°C, 10°C, 15°C - treatment 9; 10°C, 7°C, 10°C - treatment 10.

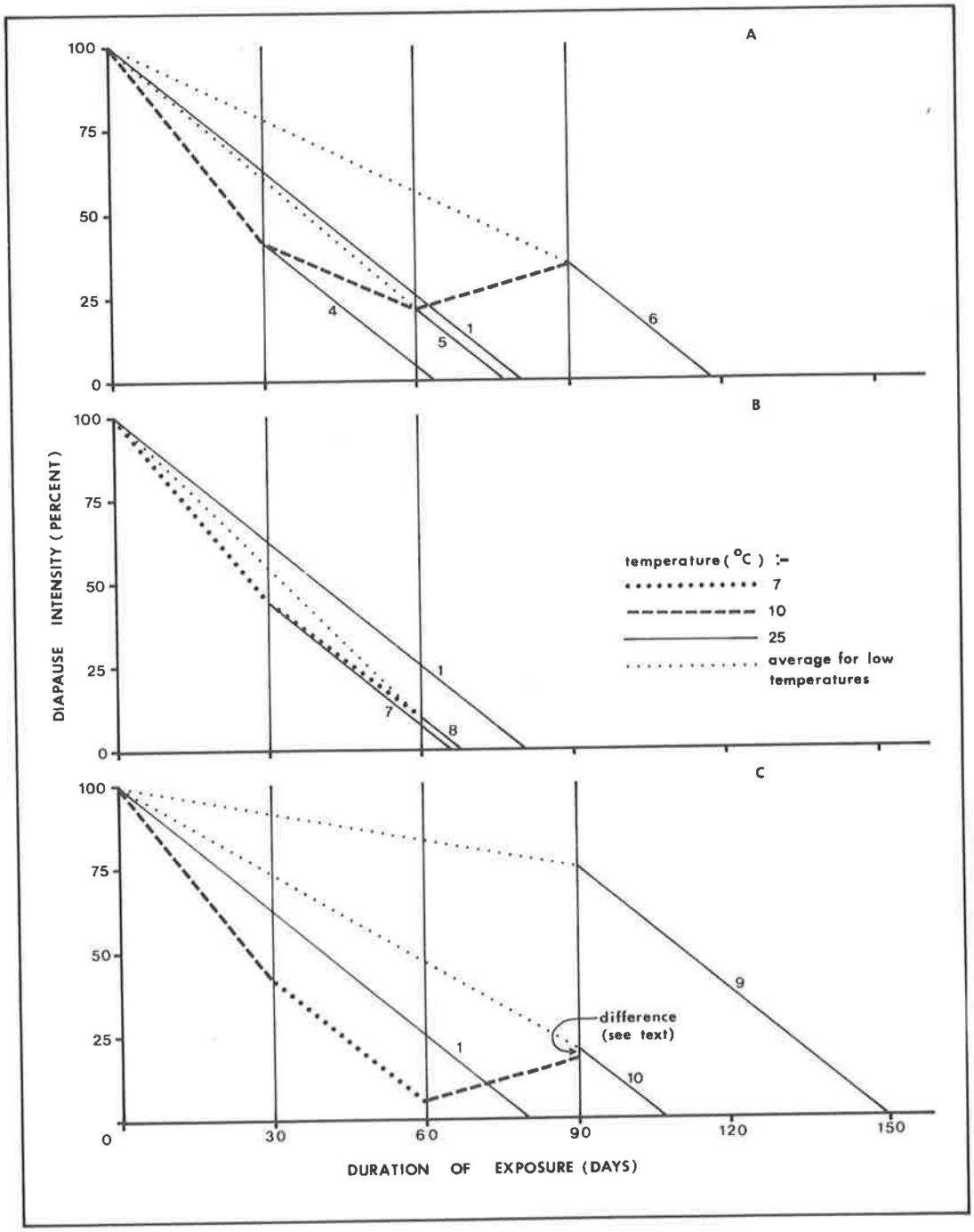
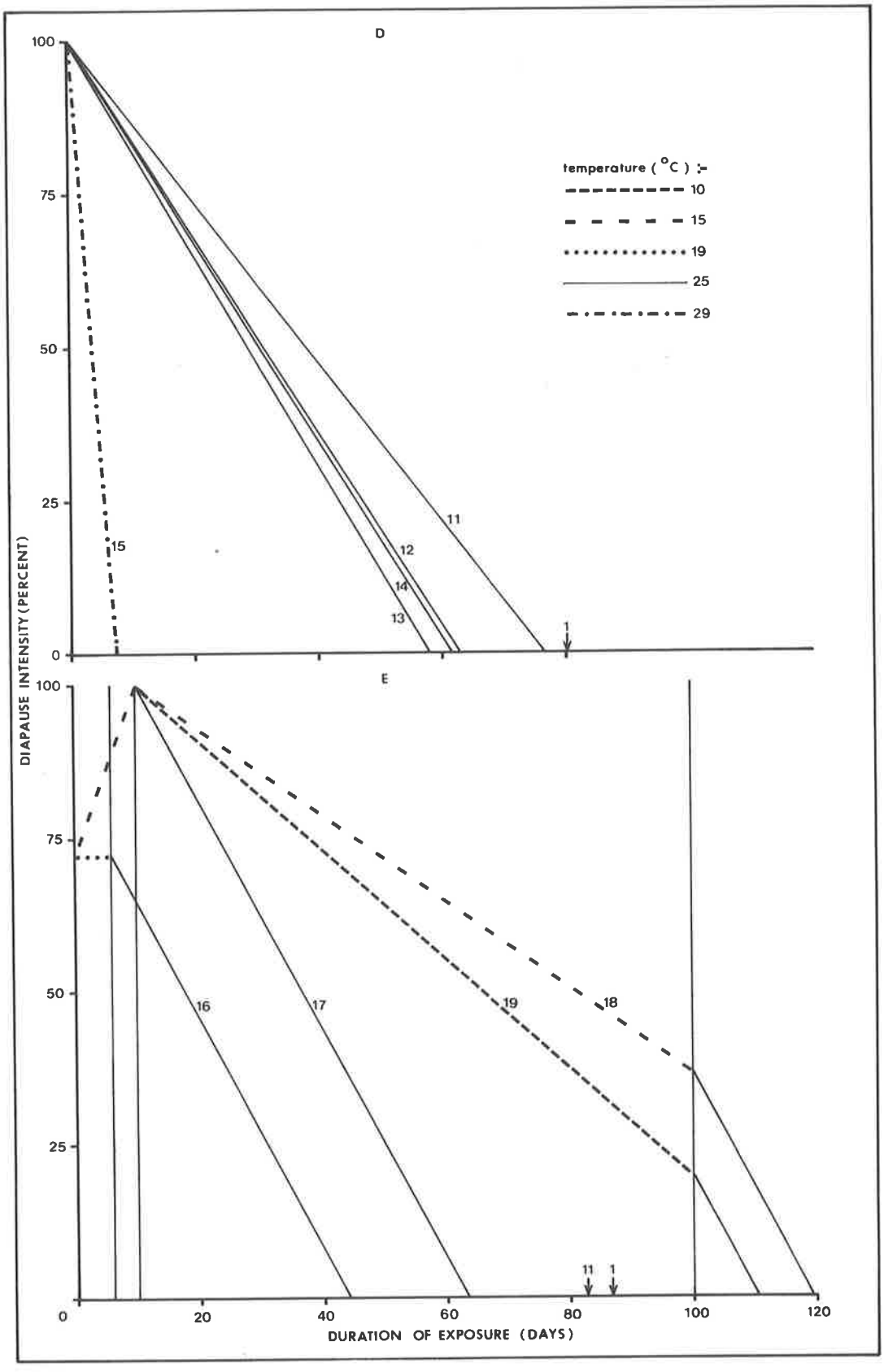

A
B
C
100
75
50
25
0
DIAPAUSE INTENSITY (PERCENT)
4
5
1
6
7
8
1
9
1
10
difference
(see text)
temperature (°C) :-
7
10
25
average for low
temperatures
30
60
90
120
150
DURATION OF EXPOSURE (DAYS)

FIGURE 2.3 (cont'd).

D. Experiment 2; control - treatment 11 (mean time to diapause termination for control treatment in experiment 1 indicated); exposure to long days - treatment 12; exposure to high humidity - treatments 13, 14; exposure to 29°C - treatment 15.

E. Experiment 3 (wounded pupae); control - treatment 16 (mean time to diapause termination for control treatments in experiments 1(1) and 2(11) indicated); exposure to 15°C for first ten days of the pupal stage - treatment 17 (then placed at 25°C); treatment 18 (held at 15°C for a further 90 days, then to 25°C); treatment 19 (transferred to 10°C for 90 days, then to 25°C).


D
100
75
50
25
0
temperature (°C) :-
10
15
19
25
29
11
12
14
13
15
1
E
DIAPAUSE INTENSITY (PERCENT)
16
17
19
18
11
1
0
20
40
60
80
100
120
DURATION OF EXPOSURE (DAYS)

for a gradual transition into the postdiapause growth period. Under such an adaptation, the rate of diapause development would decrease with time (and perhaps actually be reversed, as appeared to be the case for 90 day exposure to 10°C - see Figure 2.3.A). Examples of such a decrease under constant temperature conditions are found in the gypsy moth, Porthetria dispar (Masaki in Tauber and Tauber 1976) and the fall webworm, Hyphantria cunea (Morris and Fulton 1970). This was also demonstrated for diapausing pupae of H. zea exposed to 3°C for varying periods of time (Holtzer et al. 1976), but this report and a more recent one by Wilson et al. (1979) concerning H. armigera, propose a different hypothesis on diapause termination. They suggest that diapause in Heliothis consists of two phases - the first with an optimum development rate at low temperature (less than 18°C) and upon its completion, a second phase - completed most rapidly at higher temperatures and with the developmental threshold at about 18°C. This has the same effect, ecologically, as the diapause maintenance phenomenon proposed by Tauber and Tauber (1976).

Interpretation of the results of exposure to varying low temperatures in experiment 1 is shown in Figure 2.3.C. Because there was no indication of the rate of diapause development that occurs at 15°C (treatment 3), the average rate only at the low temperatures is indicated for treatment 9. Here, the decline in diapause intensity of approximately 25% after exposure to low temperature was more likely due to the effect of exposure to 10°C rather than 15°C (Figure 2.3A and Table 2.3 - treatments 18 and 19).

The component rates associated with the reduction in diapause intensity indicated for treatment 10 were obtained from the equivalent periods and temperatures in Figures 2.3 A, B. The resulting small difference between the measured (i.e. using the mean exposure period and parallel diapause decay line at 25°C control) and expected (using corresponding components) diapause intensities on transfer to 25°C, provides some degree of support for this method of interpretation.

The minor effect of humidity and the dramatic effect of high temperature on the rate of diapause development in experiment 2 are demonstrated in Figure 2.3 D. The fact that the lines representing diapause decay in treatments 11 to 14 (all at 25°C) are not parallel, is a contradiction of assumption (c) above; but an assessment of the importance of this deviation must consider the minor ecological significance of these parameters reported in the literature (see above). Exposure of pupae to 29°C resulted in a rapid resumption of normal development (roughly equivalent to the effect of exposure to 7°C for 60 days). This phenomenon was also demonstrated for H. punctigera by Browning (1979). It could therefore be inferred that exposure to high temperature results in a high rate of diapause development, but it is possible that it is merely a diapause-averting temperature.

An increase in diapause intensity as a result of exposing newly-formed, wounded pupae to 15°C compared to 19°C is shown in Figure 2.3 E, treatments 17 and 16 respectively. Wounding pupae appeared to result in an increase in the rate of diapause development - treatments 1 and 11 (controls in experiment 1 and 2 respectively) compared to treatment 16. Also, a higher rate of diapause development at 10°C compared to 15°C is suggested (treatments 19 and 18).

The limitations of the above representation have already been alluded to (e.g. experiment 2) - a stimulatory (although minor) effect of humidity in treatment 13 implies a higher rate of diapause development at 25°C and hence, modification of assumption (c) above is required.

A further shortcoming of the above interpretation is the absence of information on the effect of low temperature at the time of pupation on diapause induction and intensity. Exposure of pupae to 15°C on the day of pupation was shown to induce a more intense diapause than exposure to 19°C. The effect of exposure to low temperature after the initial six days at 19°C on diapause intensity is not known, although results here (see treatments 4-8) suggest diapause intensity has been determined by the end of this period

(also, see footnote on page 13).

By far the most important shortcoming of the above interpretation is its failure to incorporate the phenomena of maintaining diapause (Tauber and Tauber 1976) or phases of diapause (Holtzer et al. 1976, Wilson et al. 1979) which provided an interesting extension to the study of diapause.

At continuous exposure of 15°C, diapausing pupae failed to resume normal development; similar observations were reported for H. zea (Holtzer et al. 1976) and both H. zea and H. virescens (Phillips and Newsom 1966) where pupae could be held in diapause at 18°C for extended periods without resumption of normal development. However this does not necessarily mean that the rate of diapause development at these temperatures is close to zero. When the above diapausing pupae (of both H. punctigera and H. zea) were transferred to higher temperatures, it was found that the diapause had been all but completed.

Should the phenomenon of phases of diapause be demonstrated as applicable to H. punctigera, an explanation for diapause maintenance at such temperatures will have been found.

Also, while the rate of diapause termination in phase 1 is yet to be studied, it has already been demonstrated that models on phase 2 development alone have a role in the prediction of the timing of moth emergence (Logan et al. 1979, Cunningham et al. 1981). The most interesting conclusion from the goodness of fit achieved between the model and field data is that the range of diapause intensities that are obviously inherent in Heliothis spp. may no longer be a barrier to understanding the diapause. Rather than looking for mechanisms that result in strict synchronisation of emerging moths (which is probably an essential mechanism for the survival of insects that occur in colder climates), the natural variation in emergence times can be measured, modelled and predicted as a field phenomenon. With respect to the economic entomologist, it then remains a matter to ascertain which part

of the emergence curve and its temporal occurrence are most relevant in pest outbreak situations.

More work is required before the true nature of the effect of temperature on the termination of diapause in H. punctigera is elucidated and it should be performed in conjunction with observations in the field, as suggested by Tauber and Tauber (1976). The studies by Rabb et al. (1975) and Wilson et al. (1979) on termination of diapause of H. zea and H. virescens and H. armigera respectively, provide examples of the type of back-up field experimentation required to complement any laboratory work.

Conclusions

While low temperature, high temperature, and to a lesser extent high humidity (and wounding) were found to stimulate diapause termination in H. punctigera, the results from treatments were inconsistent and did not allow the development of a model that could be used to predict the timing of diapause termination under field conditions.

2.2.3 Completion of post-diapause development

Introduction

This section examines the duration of the pupal stage after the completion of diapause, which in H. punctigera is confirmed by the migration of pupal stemmata (Cullen 1969), as is the case for other species of Heliothis (e.g. see Phillips and Newsom 1966). The duration of the pupal stage from diapause termination to adult emergence is temperature dependent, but the relationship between temperature and the rate of this development had not been quantitatively examined.

It was therefore necessary to undertake such an examination. The results could then be incorporated into a model to estimate the timing of emergence of moths, based on local field temperatures.

Development of a model

Laboratory studies under two constant temperature regimes showed that the duration of pupal development from the migration of the pupal stemmata to adult emergence in diapausing and non-diapausing pupae, expressed as a proportion of the total normal pupal period (i.e. for non-diapausing pupae) is approximately constant at 93% (see Appendix 4). Using this proportion in conjunction with the relationship between rate of development of non-diapausing pupae and temperature established by Cullen, the duration of the pupal stage following diapause could now be defined for any temperature to which pupae were exposed. Naturally, these temperatures are related to the soil depth at which pupation occurs.

Pupae collected in the field during the present study were found at depths of from 2.5 cm to about 7.5 cm and most commonly at about 5 cm (see Figure 3.15 and for a discussion on pupal depth of Heliothis spp. see Eger et al. 1983, Wilson 1983). Soil thermograph data collected at the Waite Institute are for depths of 2.5 cm and 15 cm, neither of which appeared to be appropriate. Russell (1973) stated that the amplitude of diurnal temperature fluctuations decreases with increasing depth of soil and discussed the effect of vegetation and soil type on relative temperatures, but makes no mention of the effect of these factors on the actual temperatures at different depths. Foster and Fye (1973) have developed a formula enabling the calculation of soil temperature at various depths, but the complexities involved in applying it severely restrict its practical application. After an unsuccessful search of the literature, the effect of temperature on the rate of pupal development was determined for the two depths for which data were available to quantify the magnitude of any differences in development rates between them.

The possible methods for calculating the amount of development per day for poikilotherms were considered (Andrewartha and Birch 1954). The procedure where per cent development per day is calculated using the two-hourly mean

soil temperature was adopted. Because there is only a slight variation in the timing of the daily minimum and maximum temperatures at 2.5 and 15 cm, the starting time used in the calculation was the same time of day for each depth. The period of interest was from autumn (March) to early spring (September).

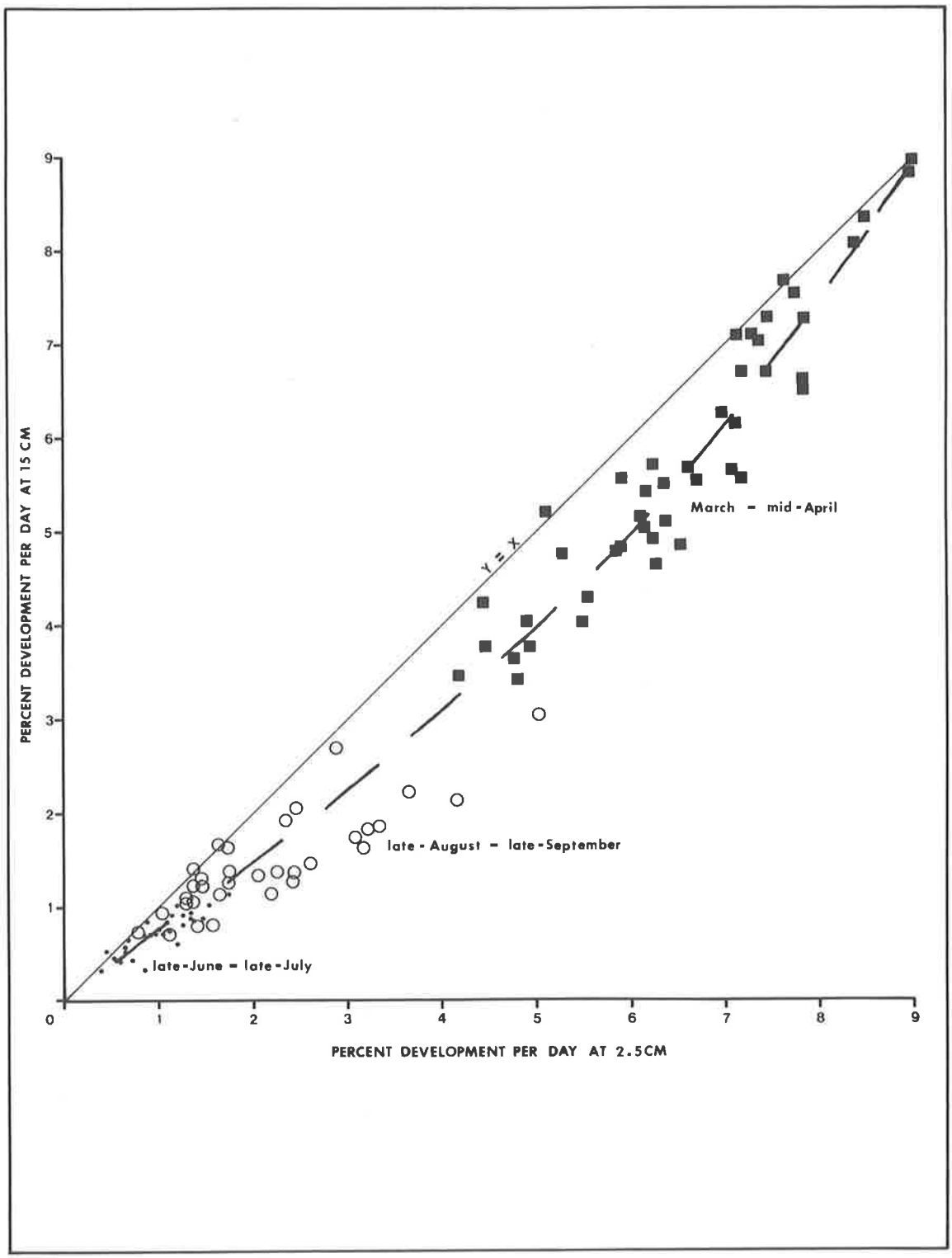
Per cent development per day for corresponding days at the two depths is plotted in Figure 2.4. Over the period of interest, a curvilinear relationship is apparent, but for selected periods within it, relationships exist that are almost linear. Development at 2.5 cm was usually greater - an indication of how much greater can be seen by the deviation of the fitted curve (drawn by eye) from the y = x line superimposed on Figure 2.4. Also, the proportion of development that took place at 15 cm compared to that at 2.5 cm changed over the period (see Table 2.4). Assuming that diapause is not completed prior to May (when some larvae of H. punctigera had been observed in the field, see section 3.4.3.4) but is completed by late June, pupae at 15 cm would have undergone approximately 74% of the development

Table 2.4 The ratio of post-diapause normal pupal development completed by a pupa at a depth of 15 cm to that completed by a pupa at 2.5 cm for the period March to September, 1976 (see Fig. 2.4 also).

PERIOD	(percent development/day at 15 cm ÷ percent development/day at 2.5 cm) x 100%
March 1 - 31	90.2
April 1 - 15	81.8
June 23 - 30	78.2
July 1 - 20	76.6
August 23 - 31	67.7
September 1 - 27	73.5
March to September	79.8
June to September	74.2

FIGURE 2.4

Percent development per day of post-diapause pupae at a soil depth of 15 cm plotted against that for pupae at 2.5 cm on the same day. (The relationship at different periods from autumn to spring is indicated).


PERCENT DEVELOPMENT PER DAY AT 15 CM
PERCENT DEVELOPMENT PER DAY AT 2.5CM
Y = X
March - mid-April
late-August - late-September
late-June - late-July
0
1
2
3
4
5
6
7
8
9

completed by pupae at 2.5 cm by late September. Although this represents a considerable "error" (which in fact merely reflects the effect of depth on temperature and hence rate of development), it must be remembered that no pupae had been found as deep as 15 cm and that pupae occurring closer to the surface would be exposed to temperatures that would reduce this "error" and more closely approximate the 2.5 cm data. Temperature data for 2.5 cm is used for all calculations below, but the variation in depth of pupation as well as the effect of soil type and vegetation cover and hence temperature to which pupae are exposed, requires further investigation if a more precise model is to be developed.

In order to reduce the amount of time required to calculate percent. development per day, a relationship between some easily derived parameters of the thermograph plot and daily percent development was investigated.

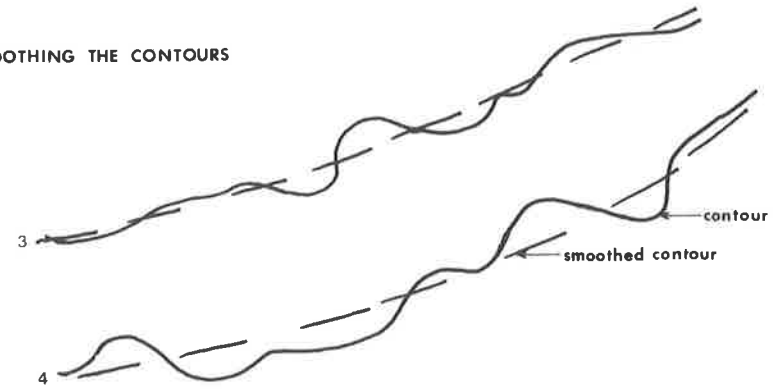
Using the 2.5 cm soil thermograph data for autumn - spring 1976, percent. pupal development per day was calculated based on two-hourly mean temperatures (see above, in this section). These data were displayed in a two dimensional table, the horizontal and vertical axes being the daily minimum and maximum temperatures respectively.

A diagrammatic representation of the procedure used to delineate areas of equal percent. development in the model, is given in Figure 2.5. Contour lines of percent. development per day were drawn and then smoothed. Adjacent contours were usually at intervals of 1% development, since this was the most convenient gradient to use when drawing the original contours; however for practical purposes, greater refinement was desirable and gradients of 0.5% were chosen (at low temperatures, smaller gradients were appropriate). This closer gradient was achieved by drawing a line half-way between adjacent contours (of 1% gradient). Areas of equal percent. development (as opposed to a contour) were delineated by two lines placed equidistant from adjacent contours, the area so contained being equivalent to the percent. development of the contour line at its mid-point.

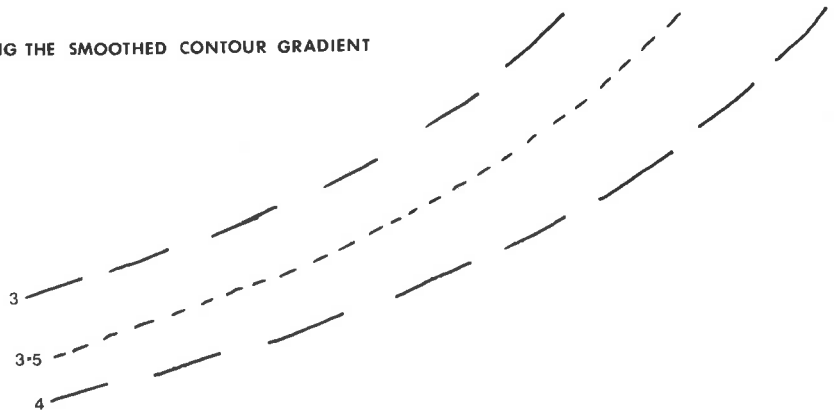
FIGURE 2.5

A diagrammatic representation of the method used to delineate areas of equal percent. development per day in constructing a model on post-diapause pupal development and soil maximum and minimum temperatures.

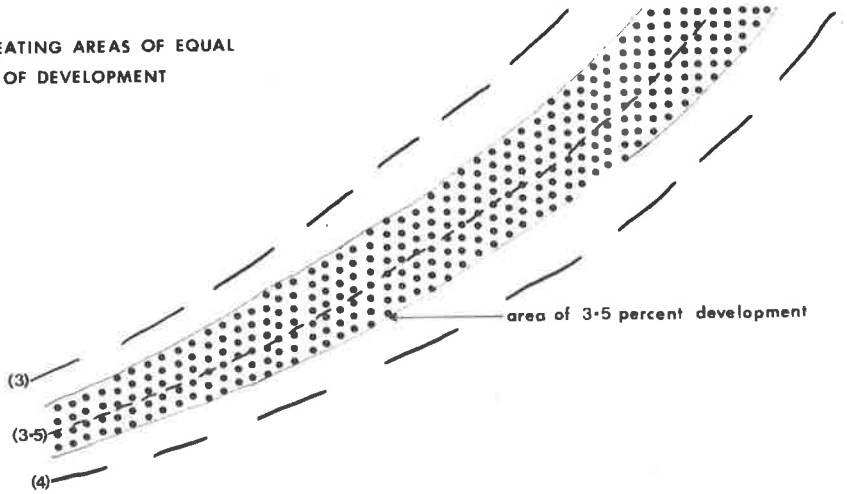
1. SMOOTHING THE CONTOURS



2. HALVING THE SMOOTHED CONTOUR GRADIENT



3. DELINEATING AREAS OF EQUAL RATES OF DEVELOPMENT



The model that was produced is shown in Figure 2.6. To use the model, the daily minimum and maximum 2.5 cm soil temperatures are obtained and the area in which these two lines intersect represents the percentage development of pupae for that day e.g. on a day with a minimum of 10°C and a maximum of 25°C, pupae will complete 2% of their development.

For both the two-hourly mean temperatures and the minimum-maximum temperature model, the time to completion of the pupal stage was taken on that day on which the cumulative percent. development was equal to or greater than 93% (see above and Appendix 4).

The accuracy of the model was determined by comparing the duration of the post-diapause pupal stage obtained either by using the model (i.e. based on daily maximum and minimum temperature) or based on two-hourly mean temperatures from the thermograph plot. The results are presented in Figure 2.7, where assumed diapause termination dates are at seven-day intervals. The difference resulting from the two methods was considered small enough to warrant the adoption of the model.

Given the timing of diapause termination in the field, it was therefore possible to estimate the time at which pupal development was completed using daily maximum and minimum 2.5 cm soil temperatures.

2.2.4 The timing of spring flights - discussion and conclusions

The relationship between environmental conditions and the rate of diapause development for *H. punctigera* was not elucidated in the studies undertaken here. Therefore it was not possible to develop the necessary model to determine whether local conditions explain the timing of flights of moths recorded in the area of study by a light trap. Nevertheless, an examination of the timing of diapause termination in the field could be made using the model developed for normal, post-diapause development.

Records of the nightly catch of *H. punctigera* moths in a light trap at

FIGURE 2.6

The model on percent. development per day of post-diapause pupae: the daily minimum and maximum soil temperatures at 2.5 cm and areas of equal percent. development per day (see text for explanation).

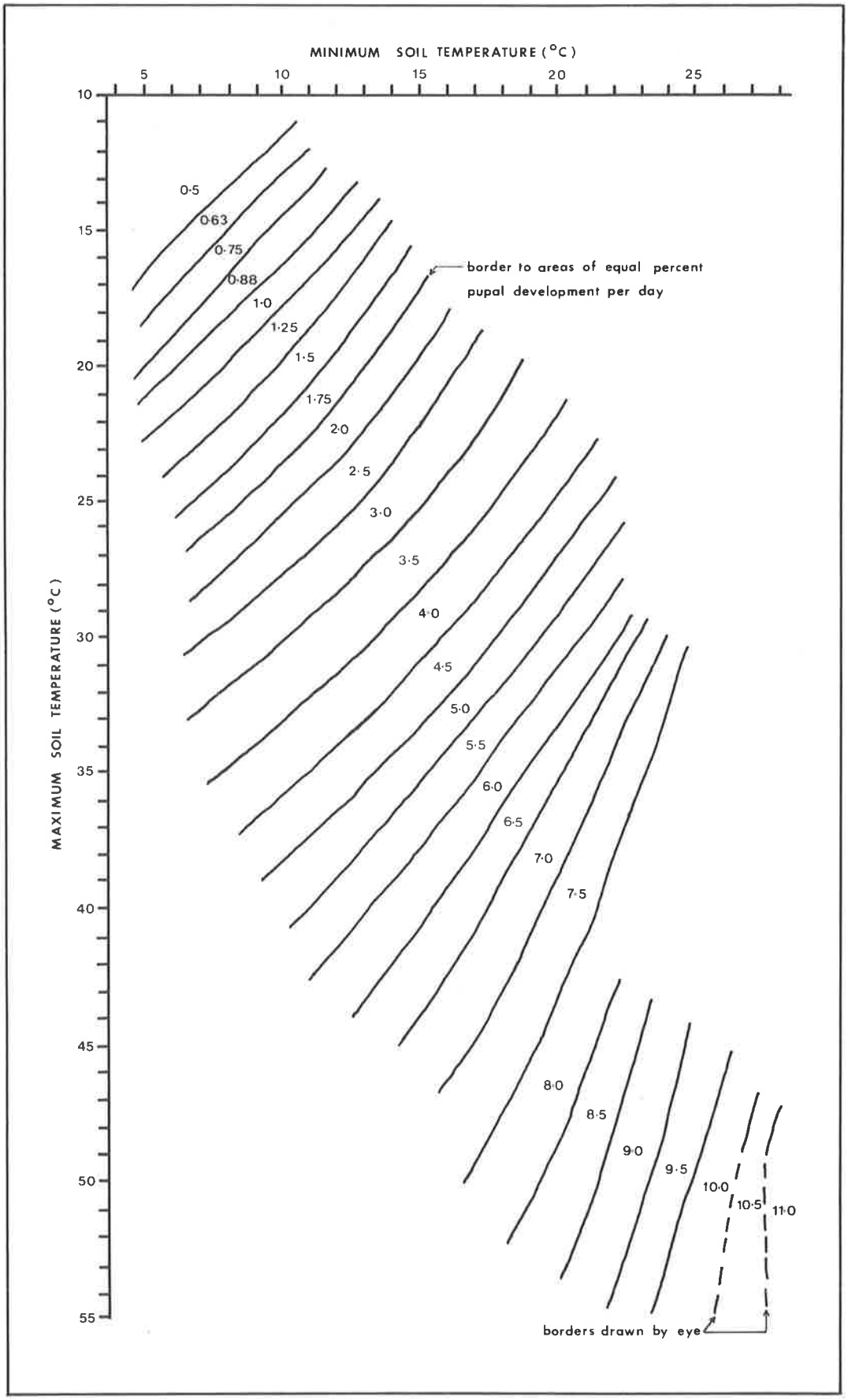
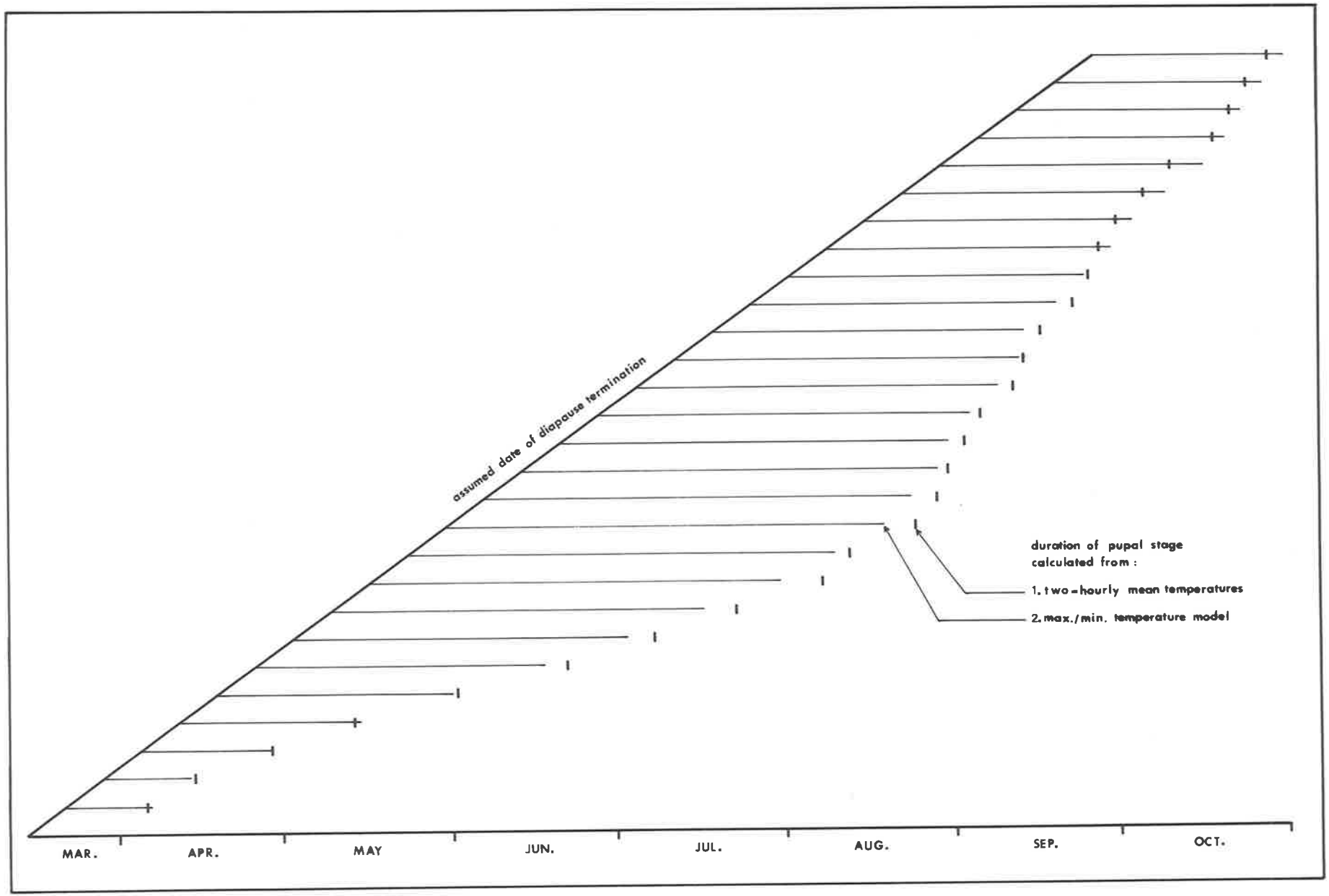

MINIMUM SOIL TEMPERATURE (°C)
5
10
15
20
25
MAXIMUM SOIL TEMPERATURE (°C)
10
15
20
25
30
35
40
45
50
55
0·5
0·63
0·75
0·88
1·0
1·25
1·5
1·75
2·0
2·5
3·0
3·5
4·0
4·5
5·0
5·5
6·0
6·5
7·0
7·5
8·0
8·5
9·0
9·5
10·0
10·5
11·0
border to areas of equal percent pupal development per day
borders drawn by eye

FIGURE 2.7

A comparison of the duration of the post-diapause pupal stage of *H. punctigera* in the field at various assumed dates of diapause termination, where duration of the pupal stage is determined from two-hourly mean temperatures of the soil thermograph and daily minimum and maximum soil temperatures using the model.


assumed date of diapause termination
duration of pupal stage
calculated from :
1. two-hourly mean temperatures
2. max./min. temperature model
MAR.
APR.
MAY
JUN.
JUL.
AUG.
SEP.
OCT.

Turretfield had been collected by the South Australian Department of Agriculture since 1960 (P. Birks pers. comm.). Peak flights of moths defined as catches of larger numbers of moths in one night or over a number of consecutive nights compared with nights before and after, are indicated on the trap catch data in Appendix 7. Eighty-seven such flights occurred over the periods 1960 to 1973. With the model on post-diapause pupal development and the dates of peak moth emergence (taken as the day preceding the peak moth catch in the light trap), timing of diapause termination in the field was calculated. This is represented in Figure 2.8 with each line representing a cohort of moths; the length of the lines and their starting point on the left, correspond to the duration of the post-diapause period and the time of diapause termination respectively.

The frequency in each of the months from April to October at which development resumed in diapausing pupae was:

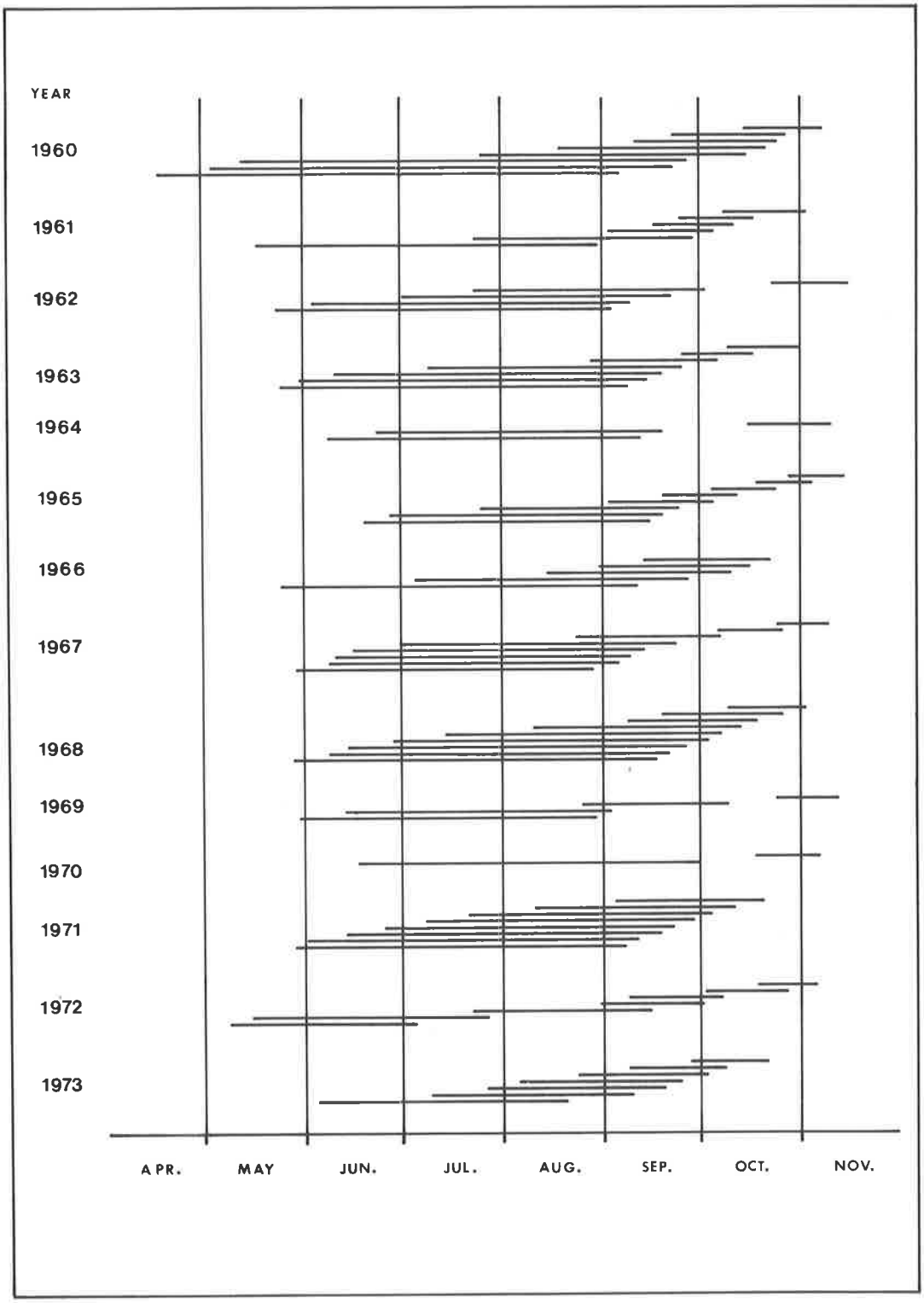
April	1
May	10
June	21
July	15
August	10
September	15
October	15

The variation in the timing of diapause termination in Figure 2.8 could be a reflection of a variation in the timing of diapause induction and in some cases, may represent non-diapausing pupae. Also the later flights of moths (after late October) may, in some years, be the progeny of moths emerging in early spring.

Also evident in Figure 2.8 is a degree of synchronisation of moth flights within some years when compared to the duration over which diapause termination occurred.

FIGURE 2.8

Times of diapause termination in the field based on peak catches of H. punctigera at a light trap, and the duration of the post-diapause pupal stage obtained from the model on rate of development. The duration of this stage is indicated by the length of the lines; the left- and right-hand points of the lines indicate the time of diapause termination and time of moth emergence respectively.


YEAR
1960
1961
1962
1963
1964
1965
1966
1967
1968
1969
1970
1971
1972
1973
APR.
MAY
JUN.
JUL.
AUG.
SEP.
OCT.
NOV.

While the model on post-diapause pupal development of H. punctigera requires verification in the field, it will hopefully provide a basis for the much larger task of developing a model that incorporates diapause development. Only then will an examination of the timing of spring flights (from field cage or light trap data) with respect to local weather conditions in the context of defining the origin of spring flights be possible.

The final section of this chapter considers the aspect of long range migration.

2.3 MIGRATION

Introduction

If spring flights of migratory moths into the area of study were to occur, their origin would be either in regions of higher temperature (and hence earlier emergence than moths of local origin) or where survival of the species was higher e.g. in areas with a uniform or summer rainfall pattern. Maps produced by the Australian Bureau of Meteorology indicate the location of such regions, and their distance from the area of study in South Australia precludes their arrival here (at least en masse) by any means other than with the aid of strong winds associated with synoptic weather patterns.

The review of insect migration by Williams (1958) and a subsequent account by Johnson (1969), provide many examples of long distance movement of insects. Some notable cases involved moths migrating in association with synoptic weather patterns. Studies in Australia and New Zealand (e.g. Tomlinson 1973, Hughes and Nicholas 1974, Farrow 1975) confirm the existence of wind-assisted migration in this region. With the capacity of H. punctigera to undertake strong climbing flights in flight chamber experiments (Laughlin pers. comm.) and the ability of Heliothis spp. to undertake sustained active flight (Haile et al. 1975), it is not surprising

that migrations of H. punctigera have been reported (Holloway 1977, Fox 1978, Drake et al. 1981). Examples of long distance migration of other members of the genus can be found in a recent review (Reed 1982).

The aim of the current investigation was to determine whether migratory flights of H. punctigera into the area of study occur during spring.

Procedure

Use of radar has enabled direct "observation" of migratory flights of insects (Roffey 1972, Schaefer 1976, Drake et al. 1981, Riley et al. 1983) and, on at least one occasion, assessment of their source (Drake and Farrow 1983). A more common method of studying insect migration and the one used here, has been to construct trajectories based on associated synoptic weather patterns (French and White 1960, French 1969, Brown 1973).

The methods used to construct such trajectories have been described by Petterson (1956) and Saucier (1965). The Australian Bureau of Meteorology has incorporated such procedures into a computer program (Air Mass Trajectory or AMTRAJ System) which calculates trajectories of air parcels over Australia. Given the location and time of arrival of an air parcel, together with a base of meteorological data, a backtrack trajectory of the parcel can be computed.

The effect of vertical motion on the parcel, after its height at the arrival point had been specified, is taken into account in the trajectory analysis so as to maintain the parcel at this height; however there is no provision for incorporating an insect's flight speed. While it is widely believed that large insects such as locusts and moths must continue to beat their wings to remain aloft during migrations (French 1969, but see discussion by Johnson 1969 and Taylor 1979 on gliding by locusts), there is a difference of opinion as to whether flight speed of an insect should be incorporated into the analysis (e.g. Mikkola and Salmensuu 1965, French 1969, Tomlinson 1973, Domino et al. 1983, Riley et al. 1983). In this study, the program

was used without alteration i.e. H. punctigera was assumed to migrate passively.

Because of the extensive light trap data collected at Turretfield in South Australia, this location was selected as the destination of the migrants (and to represent the area of study). Migrating moths were assumed to arrive about half an hour before sunrise i.e. when light intensity was increasing and likely to induce moths to alight (Dreisig 1980). The dates of the migratory flights were defined as capture of large numbers of moths, with very few or no moths being caught during the night or nights prior to the very marked increase (Persson 1976, Tucker et al. 1982). These dates are indicated on the trap catch data in Appendix 7.

The day on which a migratory flight occurred was taken as the day before the one on which the flight was recorded in the light trap (moths would be unavailable for capture until the night following their arrival). This is consistent with the assumption of migrating moths arriving near sunrise.

Little work has been reported on flight times for Heliothis spp. to indicate the interval over which the trajectories should be run. An upper limit would certainly be less than the 4.1 days Cullen (1969) recorded as the longevity of unfed adults in field cages. The longest periods of continuous flight of two other noctuids in flight mill experiments give some indication e.g. 36 hours for Mythimna (Leucania) separata (Hwang and How 1966, in Johnson 1969) and 20 hours for Spodoptera exempta (Tucker et al. 1982). However the possibility of migration occurring over a number of consecutive nights interspersed by resting periods during the daylight hours with or without feeding cannot be ruled out. Unlike transoceanic migration, moths migrating over land can alight, and for the present study this was assumed to occur. The trajectory program was therefore run over four nights (the longest interval for each program run) prior to the arrival of each migratory flight.

The large number of reports where take-offs of migratory moths were either observed or indicated (e.g. Schaefer 1976, Drake et al. 1981, Morton et al. 1981, Riley et al. 1983) suggests that dusk (taken as half an hour after sunset) was the most appropriate time to terminate the backtrack trajectory of analysis for each night of travel i.e. the likely time of commencement of migration.

The final aspect of migration to be considered was the height at which it occurred, since variations in both wind speed and direction at different levels within the atmosphere have obvious importance in any trajectory analysis. The lower limit is defined by the upper level of the friction layer i.e. where wind speed and direction are quite variable due to both the presence of topographic features and the frictional forces between the wind and the earth's surface. In this layer, while trajectory analysis is possible, the effect of these frictional forces on wind characteristics is such that accurate trajectories may not be obtained (using the rules of synoptic weather analysis) and in any case would be less likely to result in long distance displacement of a mass of insects. Only above the friction layer can the laws of balanced frictionless flow (gradient flow) be applied and trajectories accurately determined from synoptic weather maps. The upper limit of this friction layer is about 1,000 m (McIntosh 1972, Anon 1977), but in practice it has been found that many insects migrate at lower levels (Farrow 1975, Drake et al. 1982, Tucker et al. 1982, Riley et al. 1983). In the absence of thermals, the upper limit of the friction layer can be less than 1,000 m; however, the characteristics of the wind at 1,000 m should be representative of wind above the friction layer. To further complicate this aspect, some reports have indicated that the level at which migration occurs can vary within one night (Schaefer 1976, Drake and Farrow 1983).

The upper level at which migration could occur, apart from any consideration of vertical winds to carry the insects higher, is air temperature, which

usually decreases with height. Carpenter et al. (1981) determined the threshold temperature for flight of Heliothis sp. as 6.7°C. The average air temperatures at 8.30 am for various altitudes at Adelaide in the period of interest are given in Table 2.5. At levels greater than 3,000 m low air temperatures would preclude flight; migration would most likely take place at levels between 100 m (i.e. above the more important of the destabilizing effects of the friction layer) and 1,500 m and on some occasions, higher still.

Table 2.5 Mean air temperature for various levels at Adelaide Airport from July to November. (Period of record 1957-1984, Bureau of Meteorology).

	Month and *Air Temperature (°C)				
+ Level (m)	July	August	September	October	November
150	9.6(2.4)	10.9(2.6)	13.3(3.0)	16.1(3.9)	18.3(4.7)
1020	4.6(2.7)	5.1(3.4)	6.3(4.4)	8.8(5.4)	11.1(6.2)
1500	2.0(2.9)	2.4(3.6)	4.0(4.6)	6.7(5.2)	9.0(5.9)
3050	-5.3(3.0)	-5.2(3.1)	-3.3(3.8)	-1.0(3.6)	1.4(3.7)

* Standard deviation of temperature is given in parenthesis.

\+ Temperatures are recorded at constant air pressures rather than actual height; the levels in the table are mean values but the variation in height is usually small (from 10-40 m).

For the present study, migrations were assumed to occur at a level of 1,000 m. On one occasion, a trajectory for an air parcel at 3,000 m was determined for comparison.

Details of the assumed migration for which trajectory analyses were carried out are given in Table 2.6.

Table 2.6 Details of suspected migratory flights for which backtrack trajectory analyses were undertaken.

Year	Date	Start time (sunrise)	Stop time (sunset)	Level (m)
1972	15 Sept	0530 CST (2000 +GMT)	1800 CST (0830 GMT)	1000
	1 Oct	0530 CST (2000 GMT)	1900 CST (0930 GMT)	1000
1973	19 Sept	0530 CST (2000 GMT)	1800 CST (0830 GMT)	1000
	19 Sept	0530 CST (2000 GMT)	1800 CST (0830 GMT)	3000
1974	8 Oct	0530 CST (2000 GMT)	1900 CST (0930 GMT)	1000
	22 Oct	0530 CST (2000 GMT)	1900 CST (0930 GMT)	1000

+ GMT (Greenwich Mean Time) is 9.5 hours behind CST (Central Standard Time).

Results and Discussion

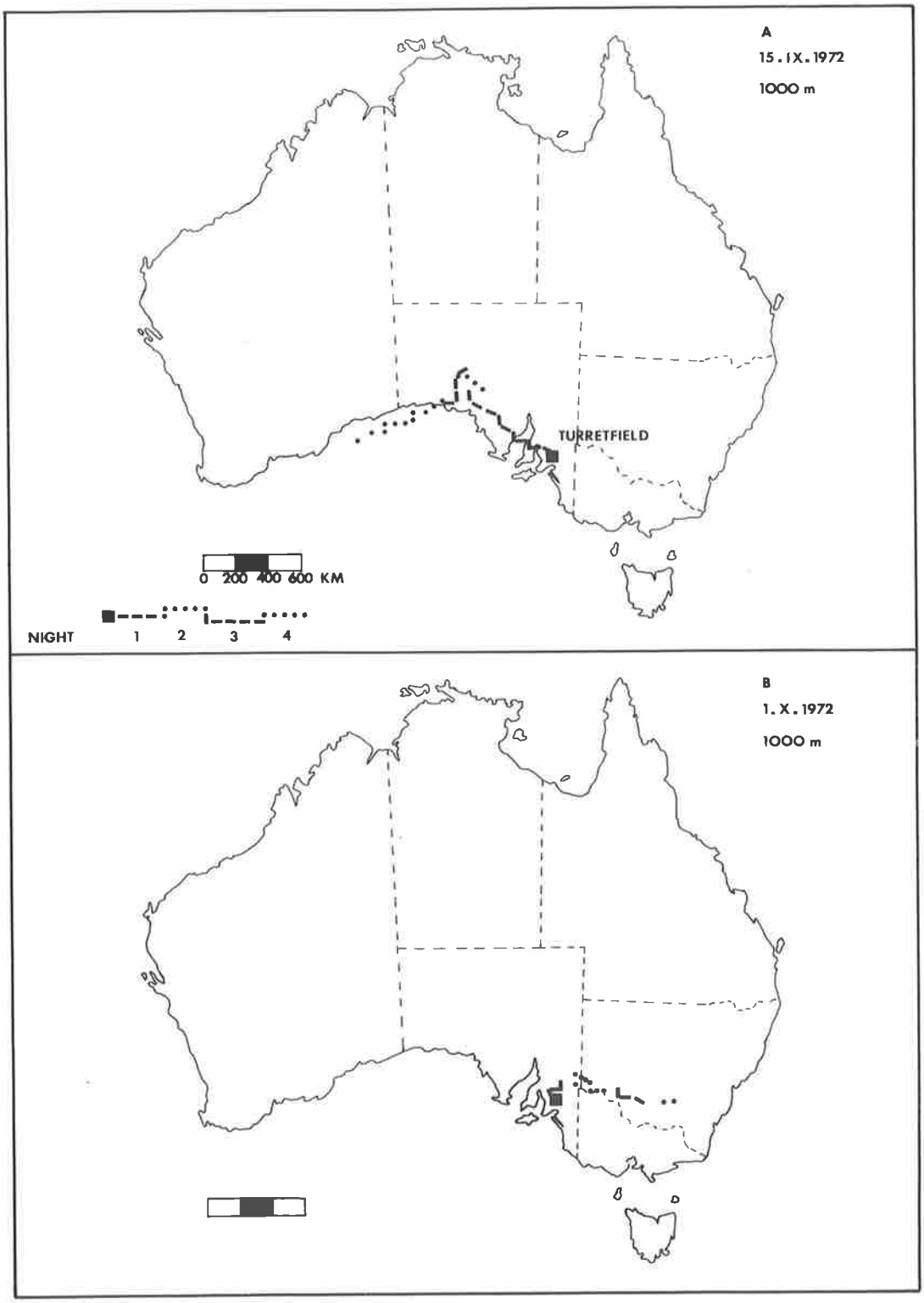
The primary objective of the trajectory analyses was to indicate direction and therefore, those portions that track over water are included despite their biological impossibility. The trajectories determined for the assumed migratory flights are given in Fig. 2.9. All but one (see Fig. 2.9B) originated to the west of the area of study, and when the height of moth travel of 3,000 m was used instead of 1,000 m the trajectory was considerably longer; but low temperatures at this time of year may preclude migrations at such heights.

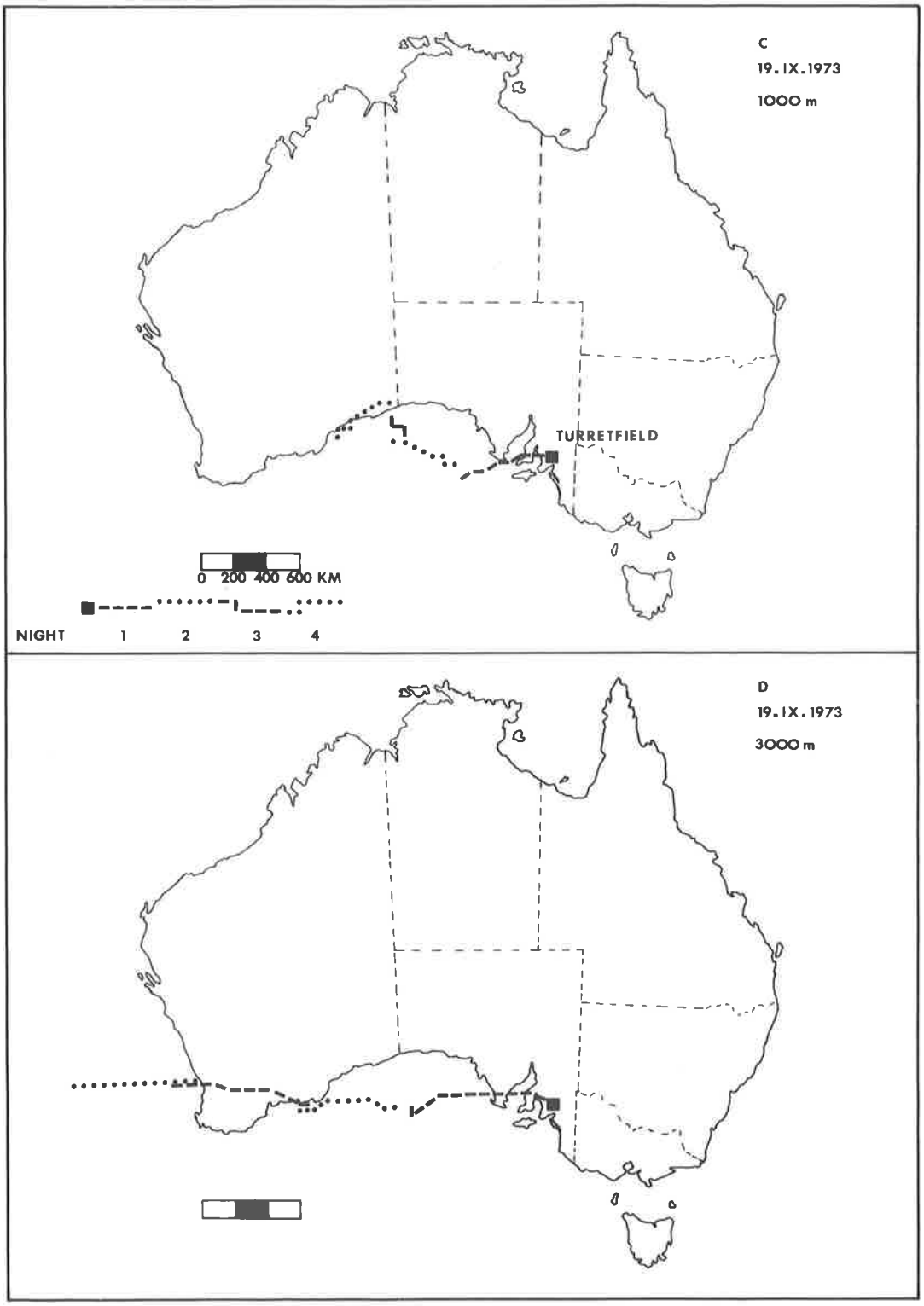
While the trajectories indicate that long distance passive travel is possible, those which began west of the area of study would be rejected. Despite the fact that H. punctigera occurs in this region of Australia (often in sufficient numbers to be classified as a pest), it is climatically similar to the area of study and the ecology of the species there is very similar to the situation in South Australia (P. Michael pers. comm.).

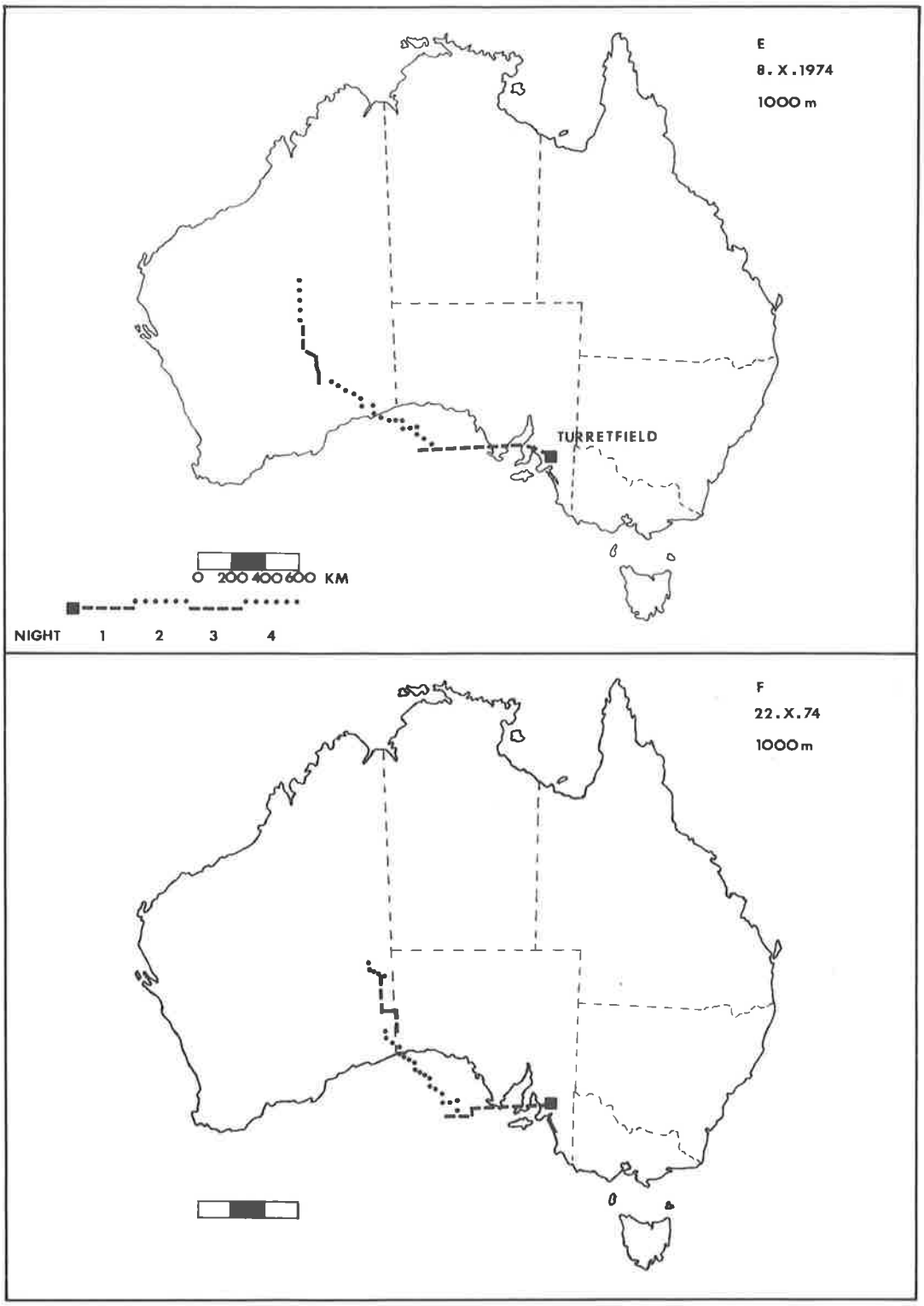
On the other hand, the origin indicated for the arrival on 1.10.72 is more likely because it represents a source where the climate is different. In this region, rainfall is more evenly distributed throughout the year and therefore, likely to promote the survival of H. punctigera in the summer-

FIGURE 2.9 A-F

Trajectories of air parcels arriving at Turretfield, coinciding with the times of assumed moth migration (see Table 2.6 for details of each migration).


A
15.IX.1972
1000 m
TURRETFIELD
0 200 400 600 KM
NIGHT
1
2
3
4
B
1.X.1972
1000 m


C
19.IX.1973
1000 m
TURRETFIELD
0 200 400 600 KM
NIGHT 1 2 3 4
D
19.IX.1973
3000 m


E
8. X .1974
1000 m
TURRETFIELD
0 200 400 600 KM
NIGHT 1 2 3 4
F
22. X. 74
1000 m

autumn period. Nevertheless, consideration of other factors discussed below suggests that there is little likelihood that such areas constitute a source of migrants.

This example where an easterly source of migrants was indicated occurred as a result of a high pressure system moving South-east across the Great Australian Bight to pass eventually over Tasmania. Such an occurrence often results in easterly winds over Southern mainland Australia. In an examination of weather during winter in Australia by Gentilli (1971), the normal path for high pressure cells is over the Southern portion of mainland Australia. Such a pattern typically results in westerlies occurring in the area of study and was present for all or a portion of the duration of the other trajectories examined here.

Low pressure systems (and associated fronts) have been implicated in many studies of migration (see references above). This has also been demonstrated in Australia and investigations by Helm (1975) and Drake et al. (1981) are relevant. Their work showed that under synoptic weather conditions during spring, the most likely source of moths, including H. punctigera, present in North-west Tasmania was from a region on mainland Australia that included the area of study and North-western Victoria. Considering the distance involved in these studies (about 800 km), it would seem unlikely that the moths had arrived in this region as a result of a prior long distance flight from a different climatic zone i.e. the moths were indigenous to the area of study.

Conclusions

This aspect of migration was only cursorily examined here and much remains to be done before a full understanding of this phenomenon as it relates to the ecology of H. punctigera in the area of study is achieved. Nevertheless, tentative conclusions are made.

Preliminary results of trajectory analyses and an examination of both the weather systems of Australia and reports in the literature indicated that conditions favouring the migration of H. punctigera into the area of study during spring from the most likely source areas do not occur frequently or consistently. Therefore it is unlikely that this mechanism explains the relatively regular occurrence of spring flights of moths into the area of study.

The most likely source of moths comprising the spring flights would therefore appear to be from within the area of study.

The survival of H. punctigera here was the subject of an extensive investigation and is reported in the next section.

CHAPTER 3 SURVIVAL OF HELIOTHIS PUNCTIGERA IN SOUTH AUSTRALIA

3.1 INTRODUCTION

In this section, the alternative hypothesis to the phenomenon of migration is considered i.e. the ability of Heliothis punctigera to survive in South Australia.

The only detailed study relevant to this hypothesis was presented by Cullen (1969) and has been summarised above (see Chapter 1). However, other workers have made observations or assessments of the importance of various mortality agents affecting Heliothis spp. in Australia e.g. virus epizootics (Teakle 1973a, b, 1974, Greenup (pers. comm.)) and parasites and predators (Lea 1928, Wallace 1941, Sloan 1942, Crosskey 1973, Michael 1973, Twine 1973, Bishop and Blood 1977, Room 1979).

While most emphasis in the present study was placed on an examination of survival in the larval stage, the other stages were also considered. A discussion of each is presented.

3.2. ADULTS

Aspects of the ecology of moths discussed here include their abundance, mortality prior to copulation and oviposition, and the fertility and fecundity of females.

3.2.1. Abundance

The relative abundance of many species of moths has been assessed by using light traps. Much of the work by Cullen was based on light trap catch data. While some of his conclusions are quite justified, the actual numbers may not reflect the fact that H. punctigera occupies a range of habitats within the study area. To examine whether sizes of light trap catches reflect the abundance of moths in the field, a comparison was made between estimates of moth abundance as obtained from the two light traps operated

during the present study and an alternative method of collection. This comparison was made at a time of year when the abundance of moths was known to be small and declining i.e. during autumn. Lucerne crops N_1 and N_6, approximately 40 km from each of the light traps (see Figure 3.1.), were chosen for this comparison. Moths were collected at approximately weekly intervals in the lucerne using a net with a 60 cm aperture. Each collection was made over a period of 30 minutes and at about the same time of day (mid-afternoon); moths were sexed and counted. The mean daily light trap catch for the appropriate week was determined, with the day on which the lucerne crops were sampled as mid-week.

The results (see Table 3.1) indicated that during autumn in the study area, light traps did not monitor the abundance of H. punctigera moths efficiently in areas distant from them. The timing of the pre-winter decline in moths was depicted reasonably well by both methods although it was less clear in the case of the light trap at Booborowie. In related studies on Heliothis spp., Hartstack et al. (1973) also considered that the location of food plants was an important consideration when interpreting light trap catch data.

The crops used for this study must be considered ideal for the production of large numbers of moths (irrigated, recycled fodder lucerne) and therefore somewhat atypical. However, crops of seed lucerne which are extensive in South Australia (see Figure 3.1) were examined at the same time and also found to be harbouring moths.

Even though the trap at M.E.S. was situated near lucerne, few moths were caught. This is indicative of the sparseness of nearby stands of lucerne compared with those near Booborowie and Jamestown during the trapping period (see Section 3.4.3).

FIGURE 3.1

The location of seed and fodder lucerne crops in the area of study; crops sampled during field surveys; light trap locations.

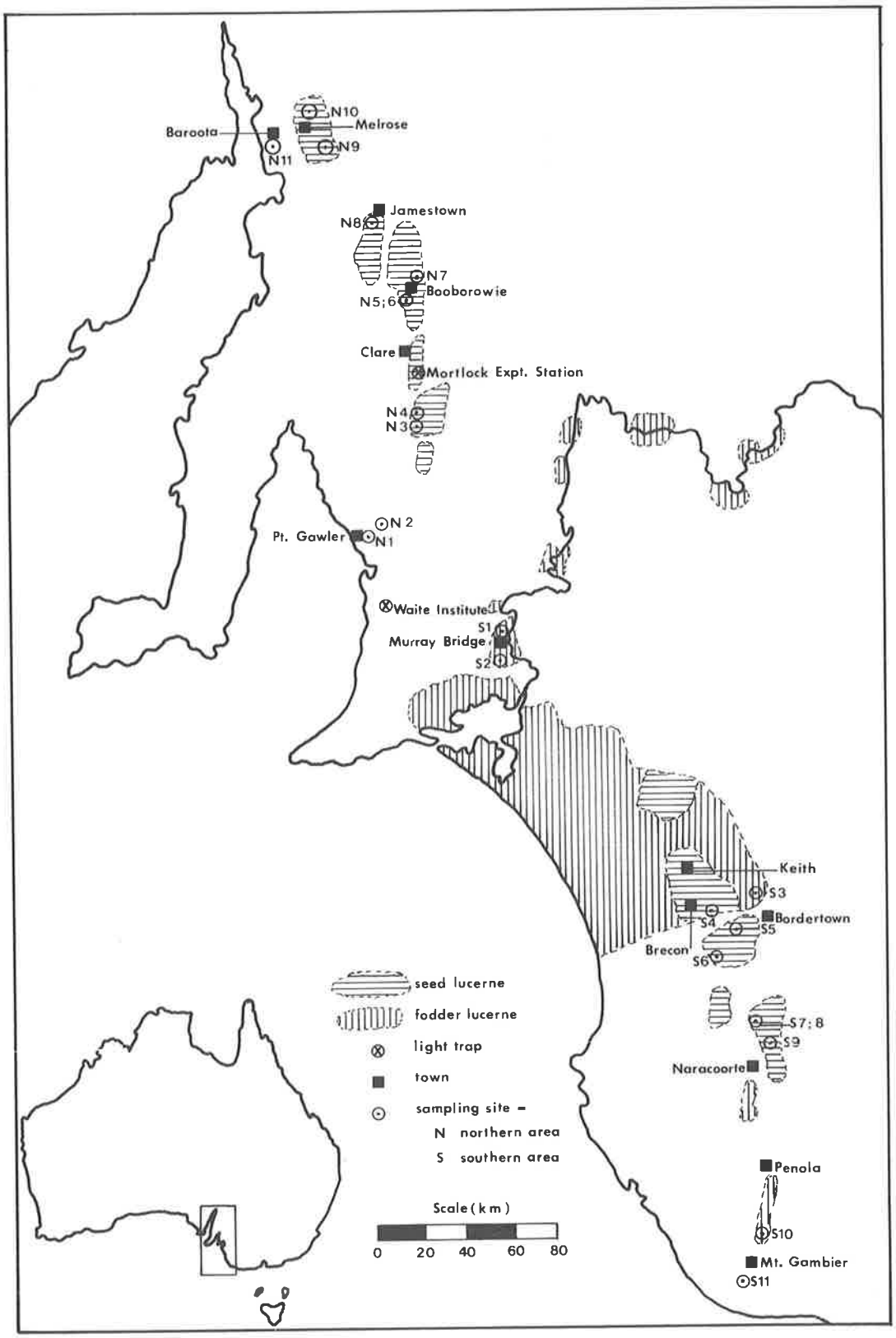

N10
Melrose
Baroota
N9
N11
Jamestown
N8
N7
Booborowie
N5;6
Clare
Mortlock Expt. Station
N4
N3
N 2
Pt. Gawler
N1
Waite Institute
S1
Murray Bridge
S2
Keith
S3
S4
S5
Bordertown
Brecon
S6
S7; 8
S9
Naracoorte
Penola
S10
Mt. Gambier
S11
seed lucerne
fodder lucerne
light trap
town
sampling site –
N northern area
S southern area
Scale (km)
0 20 40 60 80

TABLE 3.1 Comparison of estimates of moth abundance from lucerne crops and light traps.

(1) Pt. Gawler lucerne crop - 40 km from Waite Institute light trap.

Date	No. moths in lucerne (from sweep-net)				Light trap catch (7 day arithmetic mean)			
	male	female	total	% max. catch	male	female	total	% max. catch
15 Mar	12	6	18	100	0.3	0.6	0.9	100
22	4	10	14	78	0.3	0.3	0.6	67
29	3	3	6	33	0.5	0.3	0.8	89
12 Apr	7	10	17	94	0	0.1	0.1	11
23	3	5	8	44	0.1	0	0.1	11
7 May	0	4	4	22	0.1	0	0.1	11
21	0	0	0	0	0	0	0	0

(2) Booborowie lucerne crop - 40 km from Morlock light trap

Date	No. moths in lucerne (from sweep-net)				Light trap catch (7 day arithmetic mean)			
	male	female	total	% max. catch	male	female	total	% max. catch
16 Mar	-	-	36	100	0.5	0.7	1.2	22
23	-	-	36	100	0.5	0.1	0.6	11
30	11	18	29	81	0.6	0.6	1.2	22
6 Apr	9	9	18	50	0	0	0	0
13	21	5	26	72	0	0.1	0.1	2
20	4	1	5	14	2.3	3.1	5.4	100
27	0	0	0	0	0	0	0	0
4 May	0	0	0	0	0.1	0	0.1	2
11	0	0	0	0	0	0	0	0

If the efficiency of light traps is taken into account (Hollingsworth *et al.* 1968) together with estimates of their range of effectiveness (Hartstack *et al.* 1971), the seasonal trends in light trap catches could be interpreted in relation to the availability of larval and adult food plants nearby.

Because of the changing distribution of food plants during the season in South Australia, it is appropriate to compare light trap catches directly only between corresponding times in different years i.e. spring to spring etc. Comparisons between different seasons are not meaningful unless variations in the availability of food plants have been considered.

3.2.2. Survival

Cullen suggests that the availability of nectar is the most important determinant of survival of *H. punctigera* moths. In particular, the many moths which develop on annual food plant species during spring and emerge when these dry off, would have to find alternative sources of nectar.

The study area consists of two different regions with respect to the availability of nectar after early summer: the well-cleared cereal producing region to the North-east of Adelaide, where sources of nectar are confined to pockets of natural vegetation as well as lucerne crops which tend to be restricted to areas adjacent to seasonal creeks; and the region to the South-east of Adelaide where land clearing has not progressed to the same extent and lucerne occupies a greater proportion of the agricultural land. Nectar would be more readily found in the latter region.

The ability of *Heliothis* spp. to undertake continuous dispersal flight over a distance of about seventy kilometres has been reported by Haile *et al.* (1975). This indication of strong dispersive flight in the genus and that *H. punctigera* can obtain nectar from a wide variety of plants (e.g., lucerne, citrus and *Xanthorrea* - Birks pers. comm.) would confer some survival advantages on moths of this species and facilitate the location of suitable oviposition sites after early summer.

Swallows were the only predators of moths noted during the present study. Their effect on mortality would be limited because of the primarily nocturnal activity of moths.

3.2.3. Fertility and fecundity

From the study of moths caught in the light trap at the Waite Institute, Cullen noted a decline in the fertility (i.e. whether and how often successful mating occurred) with the onset of summer and proposed that a shortage of nectar was the cause. This conclusion must be examined in terms of the above discussion on the availability and distribution of nectar, where it was argued that the study area consists of a range of habitats, some of which are quite favourable with respect to availability of nectar from spring through early autumn.

In the study of light trap efficiency already mentioned, concurrent estimates of the abundance of larvae suggested that both moth fertility and fecundity were high (see section 3.4.3). This is in contrast to the findings of Cullen for the same time of year. Therefore, while the results of Cullen's study are not questioned, in as much as light trap catch data reflect the unsuitability of nearby areas for the survival of H. punctigera, the conclusions drawn may not be applied to the range of habitats occupied by this species in the area of study.

The fecundity of the genus Heliothis, and H. punctigera in particular, has been studied by Hardwick (1965) and Cullen (1969), respectively. Provided a suitable source of nectar is available and copulation occurs, fecundity of moths is high. From Cullen's data and observations in lucerne crops during the present study, fecundity of moths is quite variable throughout the area of study, especially in autumn.

3.3. EGGS

The causes of mortality of eggs of Heliothis spp. have been the subject of

much research. Those found to be the most important are weather, parasites and predators.

To quantify the importance of these factors on egg mortality in South Australia, locating large numbers of eggs in the field would be required. Because of the oviposition pattern of H. punctigera in lucerne, where eggs are laid on stems and leaves as well as flowers which occur throughout the crop canopy, the collection of sufficient numbers of eggs would have been far too time-consuming. The study was therefore confined to an identification of parasites that emerged from field-collected eggs and predators recorded during field sampling for larvae. The effect of weather has been discussed by Cullen (1969).

Two species of egg parasite were noted - Trichogramma ivelae and Telenomus sp. (identification by Carver, pers. comm.). Known predators of eggs of Heliothis spp. (Moore et al. 1974, Bryson and Schuster 1975) that occurred in lucerne crops included two species of Coccinellidae - Coccinella repanda (see Figures 3.6a, b for estimate of seasonal abundance) and Verania frenata - and two unidentified species of lacewings (from Fam. Chrysopidae and Fam. Hemerobiidae). The abundance of V. frenata and the lacewings was usually low.

Obviously, further studies are required to quantify the importance of these parasites and predators in terms of mortality in the egg stage of H. punctigera. However, observations made during the present study in lucerne and speculations by Cullen (1969), suggested that the survival of the larval stage may be a more important determinant of the survival of H. punctigera in South Australia. An extensive sampling programme was undertaken to study this.

3.4. LARVAE

3.4.1. Introduction

The initial size of an over-wintering population of H. punctigera pupae is determined by the abundance of larvae present in late autumn. Various factors affecting the abundance of larvae at this time of the year were studied in the field. Observations were also made in spring which was a time considered to have important implications for the survival of H. punctigera in the study area. This aspect is discussed first.

3.4.2 Food plants of larvae during spring

H. punctigera larvae feed on both indigenous and introduced food plants (Richards 1968). No indigenous species of food plants were found in South Australia during the course of the present study. During spring and early summer, larvae of H. punctigera were seen feeding on some introduced annual species, the most important being Echium spp. (mainly E. plantagineum, (Salvation Jane)), Oxalis pes-caprae (soursob), Carthamus lanatus (saffron thistle) and Arctotheca calendula (capeweed). Reichardia tingitana (Family Compositae) was recorded as a food plant but this species was not very abundant in the study area. The effect of some of these plants on the rate of larval development was examined in the laboratory.

Groups of twenty-five first instar larvae from laboratory reared moths were placed on flowers of Salvation Jane, capeweed, and soursob; a fourth group was placed on artificial diet (see App. 1), acting as a control. Larvae were reared singly and at a temperature of approximately 26°C. High mortality occurred in larvae placed on the flowers due to the necessity of frequent handling and no larvae reared on Salvation Jane survived. The length of larval and pupal stages and weight approximately four days after pupation, were recorded (see Table 3.2).

TABLE 3.2 Larval and pupal period and pupal weight of H. punctigera reared on different diets.

Diet	No. reaching adult stage 1.	Larval period (days)		Pupal period (days)		Pupal weight (g)	
		$\bar{x}$	2. $S\bar{x}(\%\bar{x})$	$\bar{x}$	$S\bar{x}(\%\bar{x})$	$\bar{x}$	$S\bar{x}(\%\bar{x})$
Art. diet	17	18.2	1.3	14.4	1.5	0.27	8.9
Soursob	7	19.1	1.8	13.9	1.9	0.21	5.1
Capeweed	8	17.9	2.0	13.3	2.3	0.25	15.5

1. Initially 25 first instar larvae on each diet.

2. $S\bar{x}$ = standard error of the mean and here is expressed as a percentage of the mean.

Compared with artificial diet, these annuals had no significant effect on the parameters measured. The fact that no larvae were successfully reared on Salvation Jane was ascribed to the frequent changes of food which probably resulted in puncturing of the larval cuticle by the spines of the plant. Mature larvae had been observed in almost pure strands of Salvation Jane in the field.

Too few moths emerged from each of the treatments to enable a comparison of fecundity to be made but pupal weights suggested that fecundity may be only slightly affected (Lozinskii 1961, Morris and Fulton 1970). To study this, a better method might be to collect mature larvae from isolated stands of the various food plants in the field.

Observations in the field, confirmed by the results of the above rearing experiment, demonstrate the suitability of some of the annual species that occur in spring as larval food plants.

Soursob, Salvation Jane and saffron thistle are especially important, as these species are widespread in the study area during spring. Also, saffron

thistle, more abundant in the areas to the N and N.E. of Adelaide, appears to be better adapted to the hot, dry summer - flowering plants were observed in late December when other species of annuals had long since dried off.

Considering the extensive distribution of these annuals, it is apparent that H. punctigera is able to disperse throughout the study area during spring. Moreover, these species flower before field peas and lucerne, the two cultivated species of which H. punctigera is most consistently a pest in South Australia. Once the annual species begin to dry off during summer, the population of adults is well-positioned to locate what has become a shortage of both nectar and suitable oviposition sites. Lucerne crops provide the most important source of these requirements at this time. The survival of larvae present in lucerne crops after early summer was the subject of an intensive study.

3.4.3. Survival of larvae in lucerne crops

3.4.3.1. Introduction

This aspect has been examined by Cullen (1969) in the study area, but his conclusions were based on a small number of collections of larvae from the field and the use of glass-sided cages. While statements made from this work should be viewed with caution, they highlight certain aspects of larval survival which deserve more attention e.g. the NPV epizootics mentioned above. Parasites and predators of Heliothis spp. may also be important mortality agents because many have been reported both in Australia (e.g. Lea 1928, Sloan 1942, Wallace 1941, Crosskey 1973, Michael 1973) and overseas (e.g. Danks 1975, Young and Price 1975, Smith et al. 1976).

With this background, sampling programmes were undertaken in an attempt to identify, and in some cases quantify, the more common mortality agents affecting larvae of H. punctigera. For reasons already given, (see Chapter 1) these studies were confined to lucerne crops after early summer.

Initially only lucerne at the Waite Institute's farm, Mortlock Experiment

Station (M.E.S.) was to be extensively sampled, but following a decline in the abundance of larvae there in late summer, it was necessary to find an alternative sampling site. A crop at Booborowie (approximately 40 km North of M.E.S.) was chosen. In addition to these studies, surveys of the major lucerne-producing areas of the state were undertaken.. The results of the sampling at M.E.S. and Booborowie, and the field surveys, are presented below.

3.4.3.2. Mortlock Experiment Station (M.E.S.)

The location of M.E.S. in the study area is shown in Figure 3.1 (see above). Since the station was established only in 1970, weather data for Clare located 15 km North-west of M.E.S. indicate the climate experienced on the station (see Table 1.1).

Sampling procedure

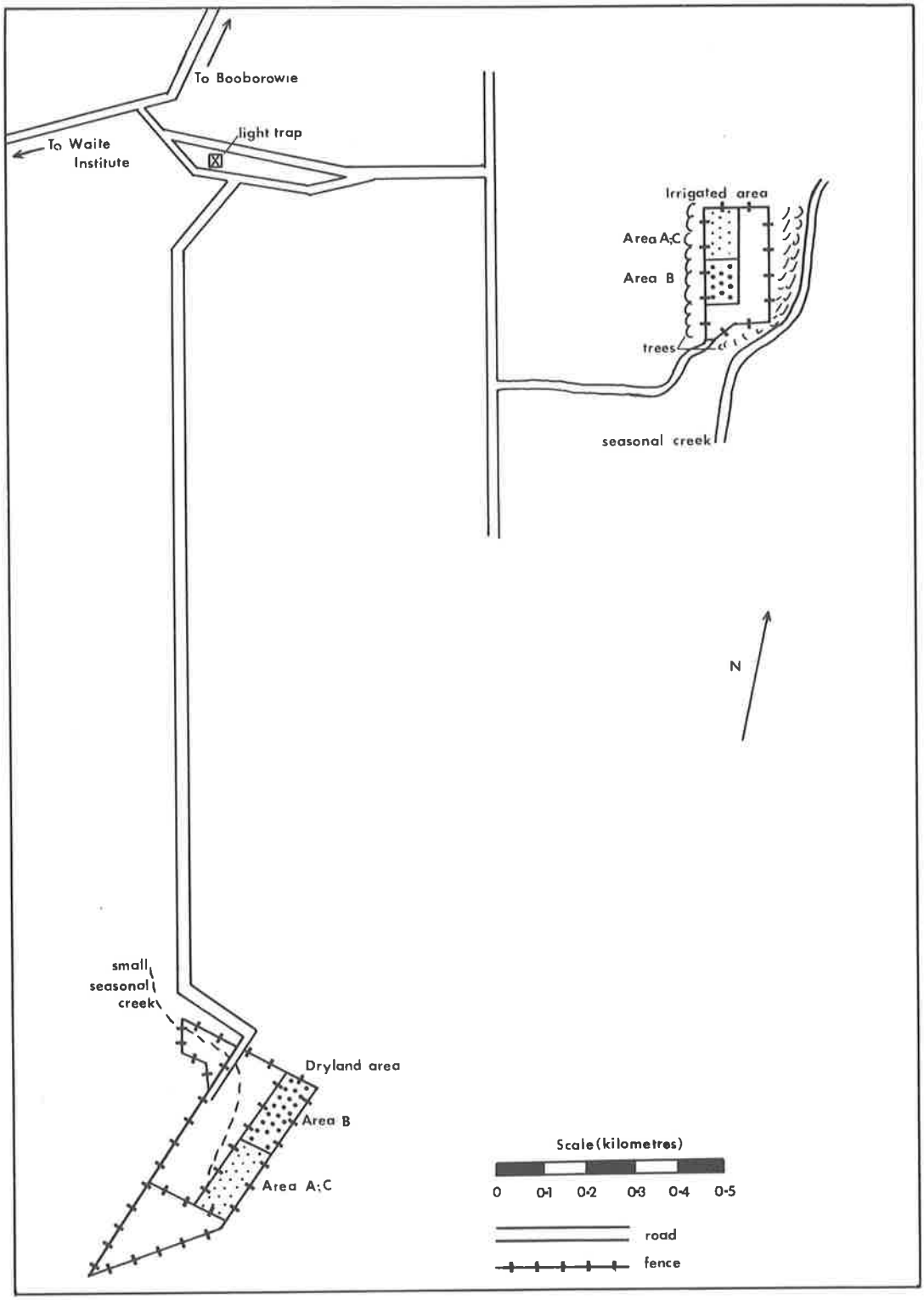
The occurrence of adults was monitored using a light trap of the same type used at the Waite Institute (see Appendix 7 for description of trap design and Figure 3.2 for location of trap). The trap was cleared daily and adults counted.

For the study of mortality of H. punctigera larvae, two crops of lucerne were used. One was adjacent to a small, seasonal creek in a depression surrounded by a gently undulating, treeless ground, with pasture on three sides and lucerne on the fourth. The other was adjacent to a larger, seasonal creek and bordered on three sides by trees. This crop was surrounded by pastures and had provision for flood irrigation. The location of these crops in relation to each other and to the light trap is also shown in Figure 3.2.

The management of lucerne requires that it be cut (or grazed) at flowering cycles until summer, at which time the crop may be shut-off to produce seed, or the procedure continued if it is a fodder crop (see Chapter 1). Each of the experimental lucerne crops was divided into equal-sized areas with

FIGURE 3.2

The location of lucerne crops and the light trap at Mortlock Experiment Station.


To Booborowie
To Waite Institute
light trap
Irrigated area
Area A;C
Area B
trees
seasonal creek
N
small seasonal creek
Dryland area
Area B
Area A;C
Scale (kilometres)
0 0·1 0·2 0·3 0·4 0·5
road
fence

alternate and complementary mowing, so that lucerne would be continuously in flower and hence available for oviposition throughout the season. The details of the timing of mowings decided upon for this purpose are given in Table 3.3.

TABLE 3.3 Preselected times of mowing lucerne crops at Mortlock Expt. Station.

Area	Mowing date	* Flowering period (approx.)	Management practice after peak in flowering
A	Sept 1	Oct 20 - Nov 28	Lucerne mown (becomes Area C).
B	Oct. 15	Nov 20 - Dec 30	Discontinue sampling after peak flowering.
C (A)	Dec. 2	Dec 20 - Jan 15	Lucerne "shut-off" to become a seed crop; sampling continued.

* The dates were estimates based on the mowing time, which was chosen to produce an overlap in flowering periods in adjacent blocks to attract ovipositing H. punctigera. See Fig. 3.5 for observed phenology of the lucerne areas.

In both dryland and irrigated crops, lucerne in one area only was sampled until it was due to be mown. At this time, both areas were sampled and thereafter sampling was continued in the second area only until late flowering. The decision on when to mow was based on commercial practice i.e. when all stems bore flowers.

Lucerne is a relatively homogeneous habitat for H. punctigera moths (i.e. a uniform distribution of feeding and oviposition sites), but to obtain representative samples from the area of lucerne being examined and to avoid double sampling, a stratified random sampling plan was used. The areas were divided into blocks and each block further subdivided into plots, each plot being one sampling station (see Figure 3.3 for layout). On each sampling date, two plots were randomly selected from each block (one plot in the

FIGURE 3.3a

Sampling stations of dryland lucerne at

Mortlock Experiment Station.

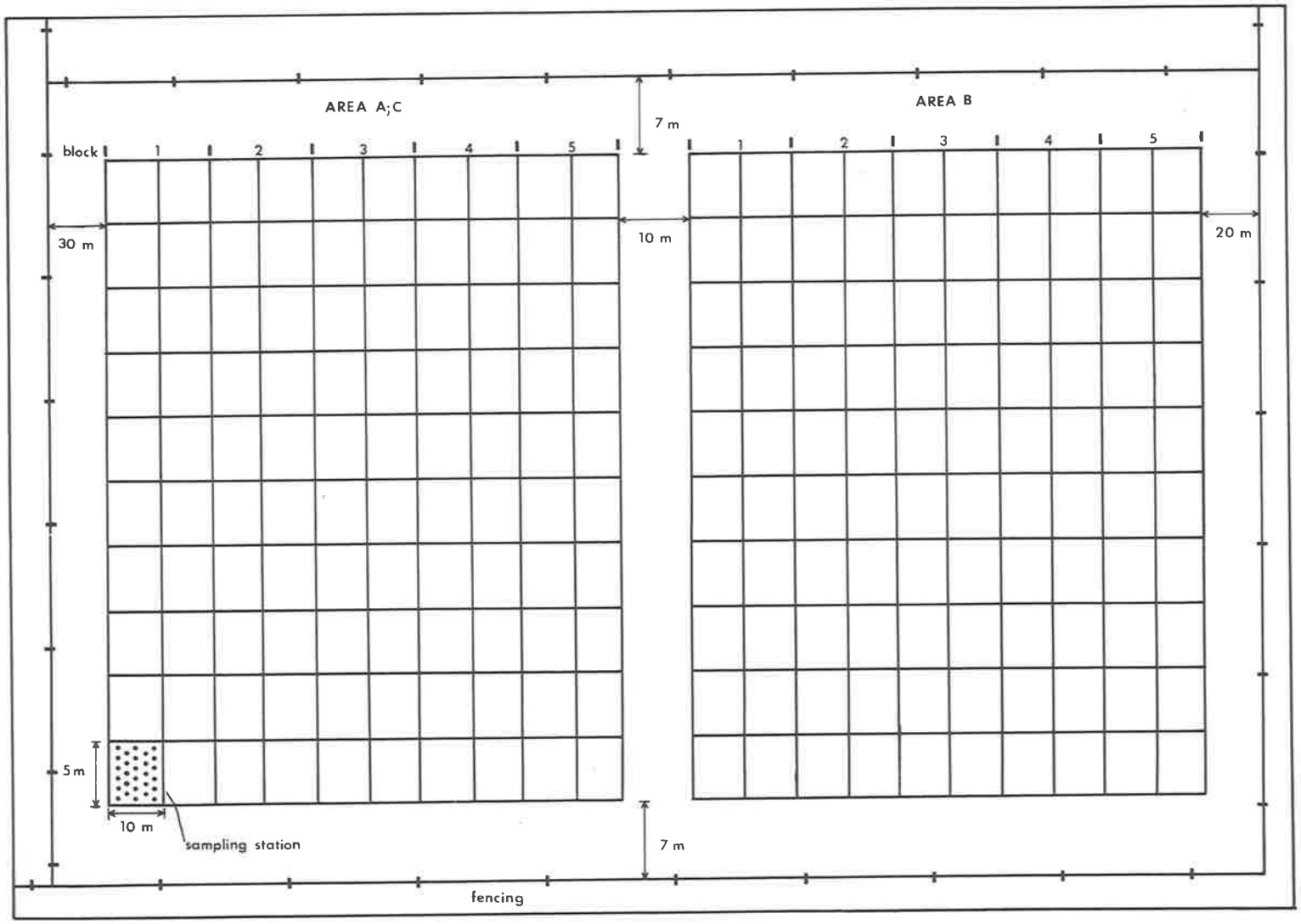
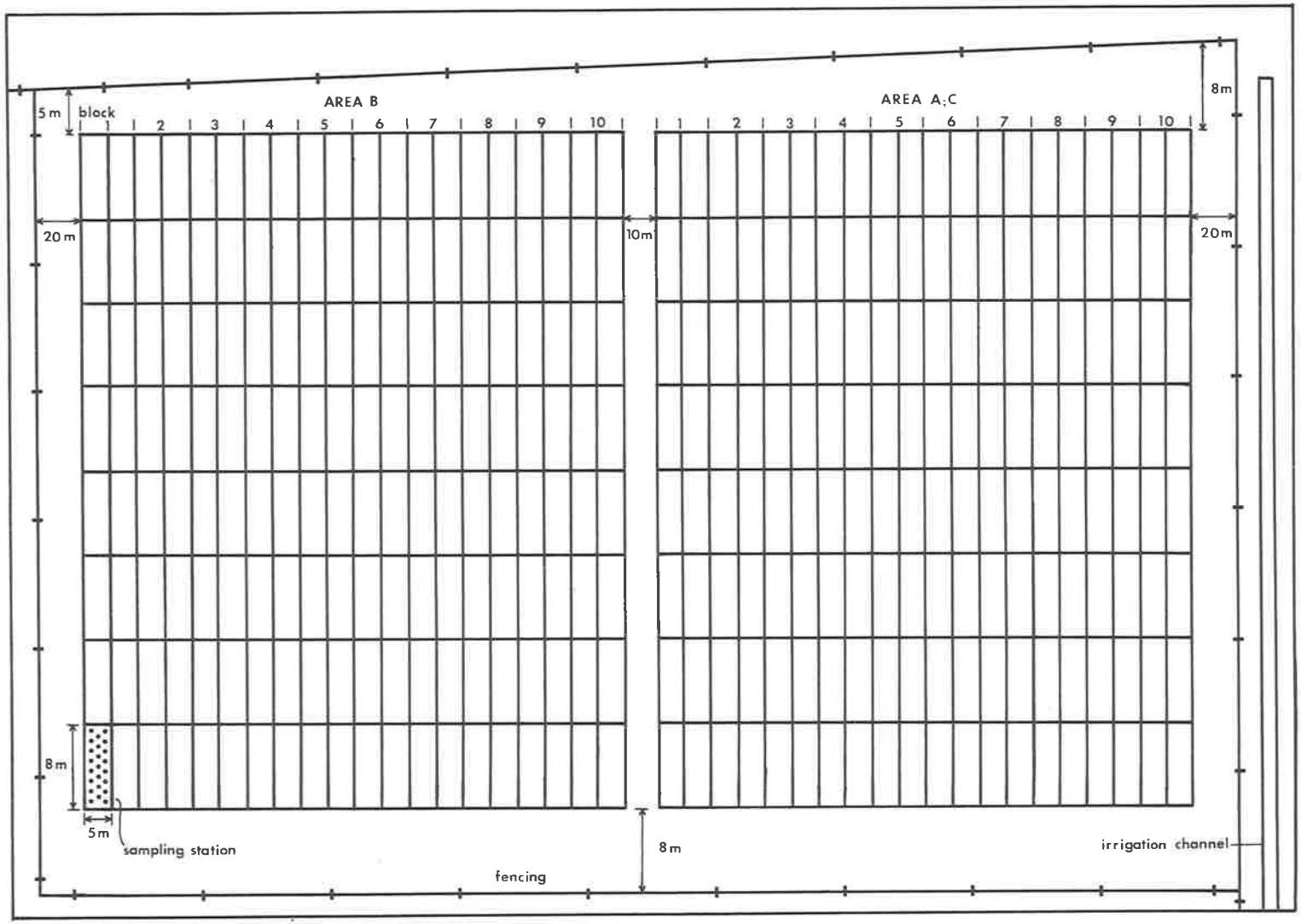

AREA A;C
AREA B
7 m
block
1
2
3
4
5
30 m
10 m
20 m
5 m
10 m
sampling station
7 m
fencing

FIGURE 3.3b

Sampling stations of irrigated lucerne at

Mortlock Experiment Station.


AREA B
AREA A;C
block
1
2
3
4
5
6
7
8
9
10
5 m
8m
20 m
10m
20m
8m
5m
sampling station
8m
fencing
irrigation channel

case of the irrigated lucerne) and these plots become unavailable for selection in subsequent sampling in that block until it had been mown and a second cycle of flowering produced. A sampling unit consisted of ten sweeps with a standard D-shaped sweep-net in each plot (see Appendix 8 for details). Larvae were removed from the calico bag of the net and placed in a glass vial containing Carnoy's fixative, left there for approximately 24 hours and then transferred to 70 per cent alcohol for storage. Larvae were subsequently aged (see Appendix 9), scored for parasitism (see Appendix 10) and counted. In addition to collecting larvae, it was noticed that the adult stage of known predators of eggs and larvae of H. punctigera were also collected in the sweep-net. With a view to obtaining an index of predator abundance, these adults were also retained and later identified and counted. The effect of NPV on larvae was estimated from observation of the characteristic cadavers (Teakle 1973a). The maturity and condition of the lucerne crops were noted. Proposed soil sampling for pupae to complement the study on mortality of larvae and to monitor diapause induction was abandoned following the decline in abundance of larvae in late summer.

Results

Light trap catch data are presented as daily catches and running seven day geometric means in Figures 3.4.a, b respectively. The timing of moth flights, and to a lesser extent their magnitude, were consistent with the situation at the Waite Institute (see Figure 3.4c). Prior to mid-November, few moths were caught in the light trap. This situation was reflected in the abundance of larvae in the field, which were readily found in soursobs, but only rarely in lucerne (vegetative stage) or field peas. The first major moth flight was recorded during late November - early December. It occurred over a relatively short interval and produced the greatest catch per night for the season. Subsequent flights were less well-defined, occurring over longer intervals (probably as a result of an extended oviposition period of moths from previous flights) and each consisting of progressively fewer moths.

FIGURE 3.4a

Number of *H. punctigera* moths caught per night in the light trap at Mortlock Experiment Station.

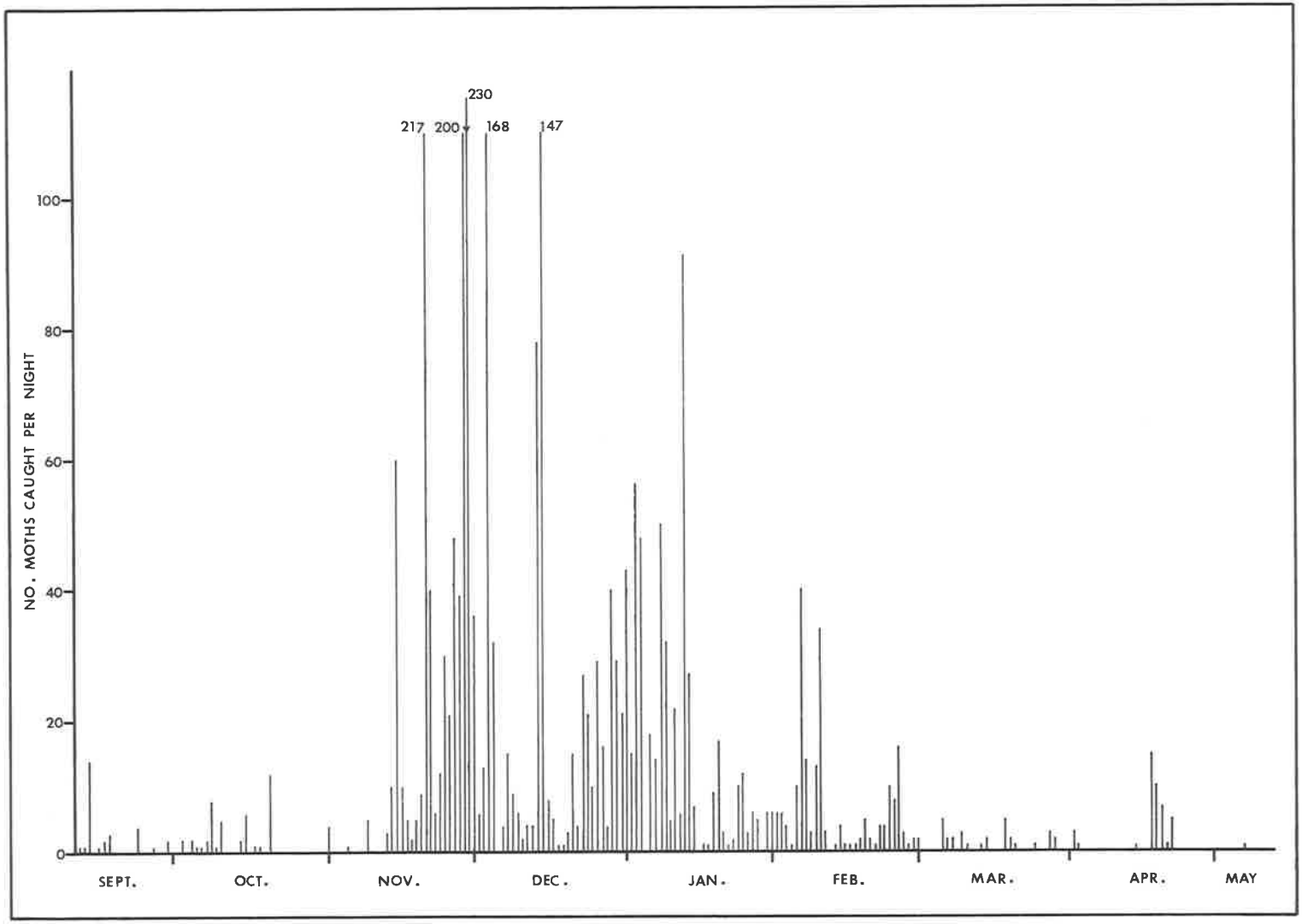

230
217
200
168
147
100
80
60
40
20
0
NO. MOTHS CAUGHT PER NIGHT
SEPT.
OCT.
NOV.
DEC.
JAN.
FEB.
MAR.
APR.
MAY

TABLE 3.4b

Running seven day geometric mean of the number of

H. punctigera moths caught per night in the light trap

at Mortlock Experiment Station.

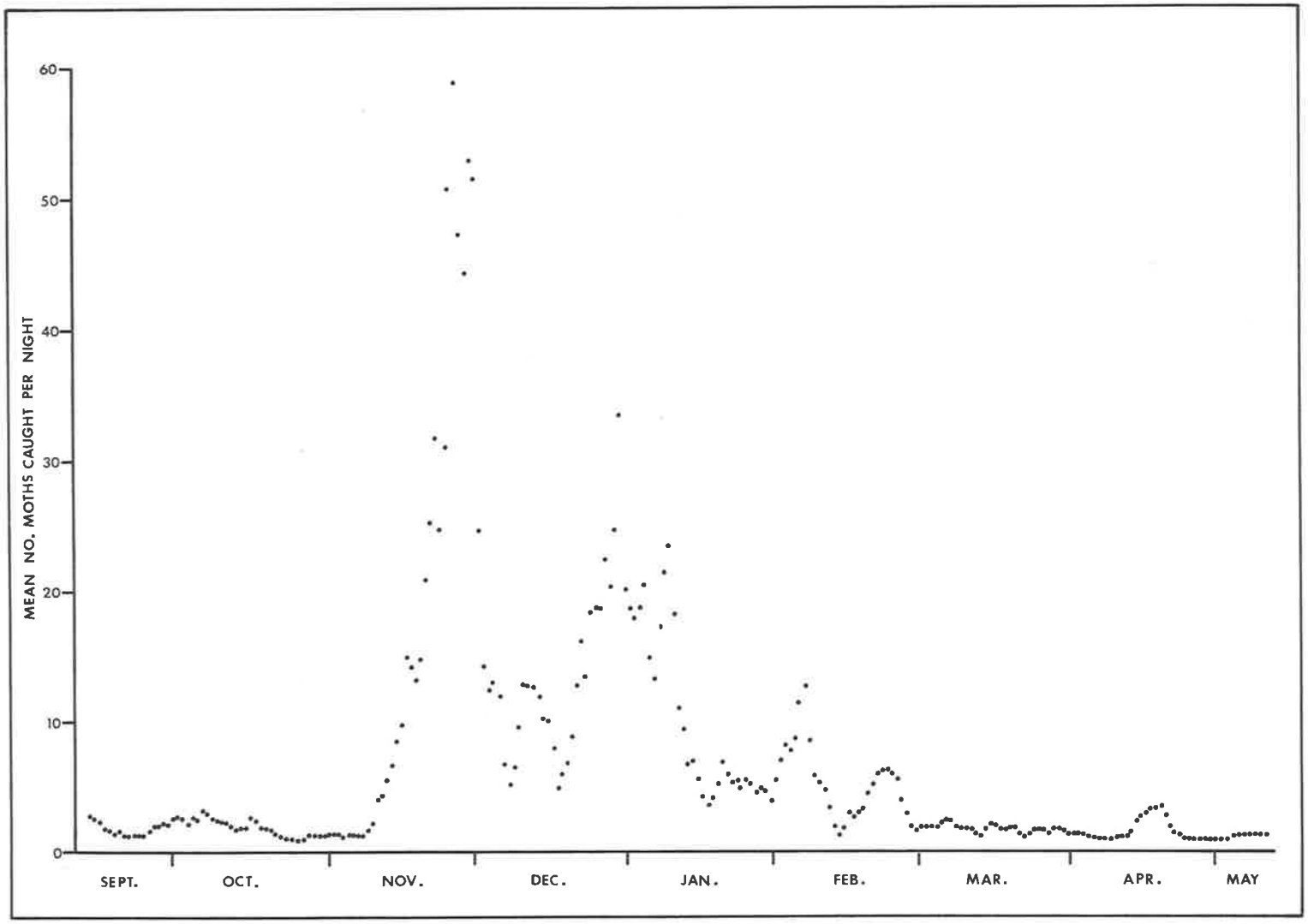
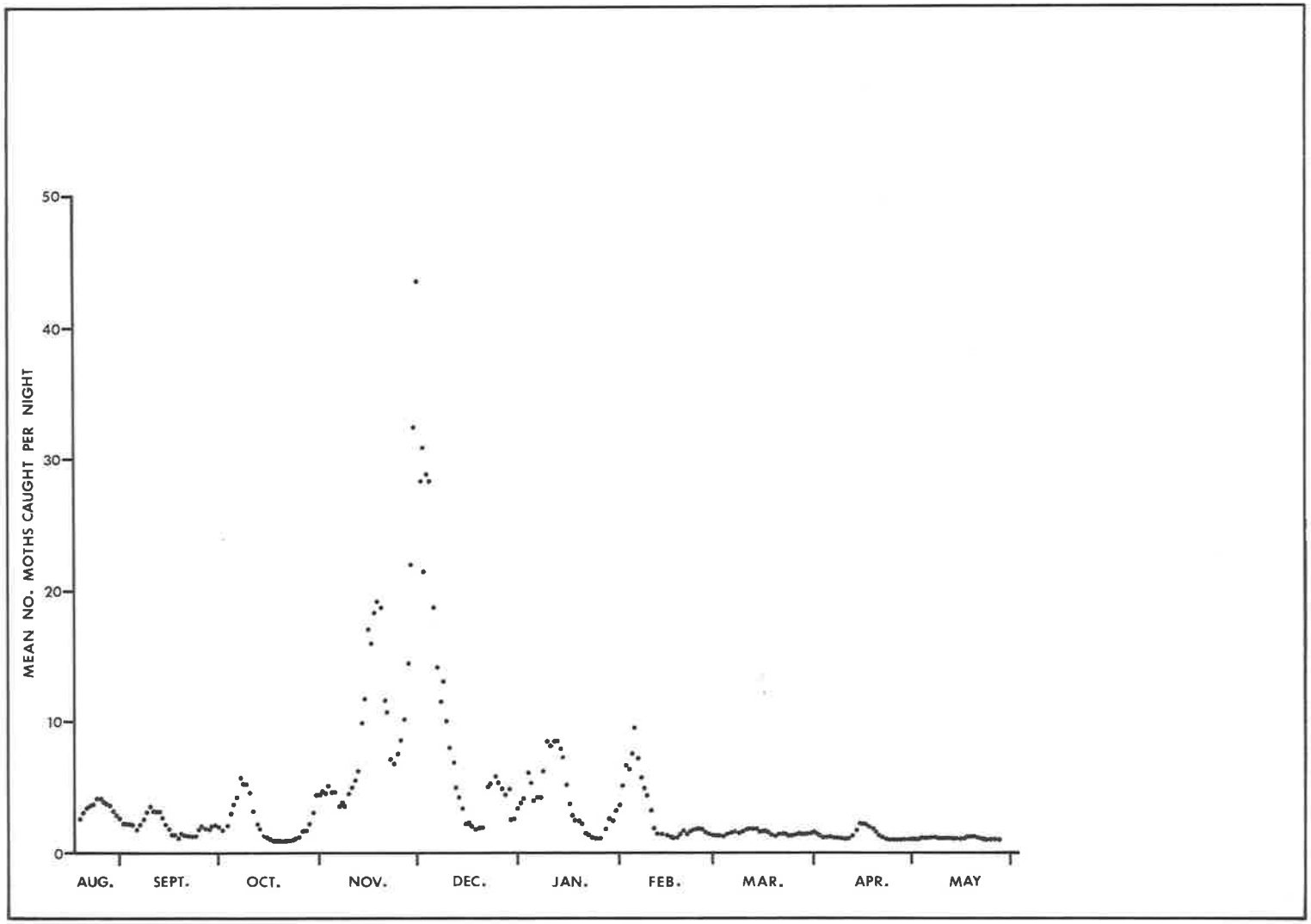

MEAN NO. MOTHS CAUGHT PER NIGHT
60
50
40
30
20
10
0
SEPT.
OCT.
NOV.
DEC.
JAN.
FEB.
MAR.
APR.
MAY

FIGURE 3.4c

Running seven day geometric mean of the number of *H. punctigera* moths caught per night in the light trap at Waite Institute.


MEAN NO. MOTHS CAUGHT PER NIGHT
50
40
30
20
10
0
AUG.
SEPT.
OCT.
NOV.
DEC.
JAN.
FEB.
MAR.
APR.
MAY

Flowering dates of the areas of lucerne differed from those expected (Table 3.3 cf. Figure 3.5); nevertheless an overlap of flowering periods between adjacent blocks within the two areas was obtained. The occurrence of peak flowering in dryland area B could not be easily delineated because damage to plants by Heliothis as well as other insects (e.g. Sitona sp. weevils and Chrysodeixis sp. larvae) was severe. Two cycles of flowering were recorded in area C in the irrigated area. The reason for this was not known. Nevertheless, for the purpose of monitoring the abundance of larvae, this management programme was considered reasonably successful.

The mean number of larvae per sampling unit collected from the lucerne is also presented in Figure 3.6. These data together with those for the individual instars and relevant standard errors are given in Appendix 8 (Table 1). The numbers of larvae of the first three instars were grouped (see Appendix 8).

Few larvae were collected until December (area B). A second peak occurred in the dryland lucerne in January (area C). The presence of mature larvae in this area at the commencement of sampling (30.xii - 6.i) was probably the result of oviposition by the minor flight of moths that occurred in mid-December (see Figure 3.4a above). Such an increase did not occur in the more mature lucerne of adjacent area B (see Figure 3.5) suggesting a relationship between crop maturity and attractiveness as an oviposition site.

This suggestion is further supported by the decline in the abundance of larvae as the lucerne matured. A further cut of lucerne might have enabled a continuation of sampling at M.E.S.

The level of parasitism of larvae is presented in Figure 3.6. The reasons for not assigning quantitative importance to the individual parasite species are given in Appendix 10. The rate of parasitism appeared to increase with time and with instar (see Appendix 10, Table 2).

Sweep-net sampling revealed that four species of predators were consistently

FIGURE 3.5

Phenology of lucerne crops sampled at

Mortlock Experiment Station.

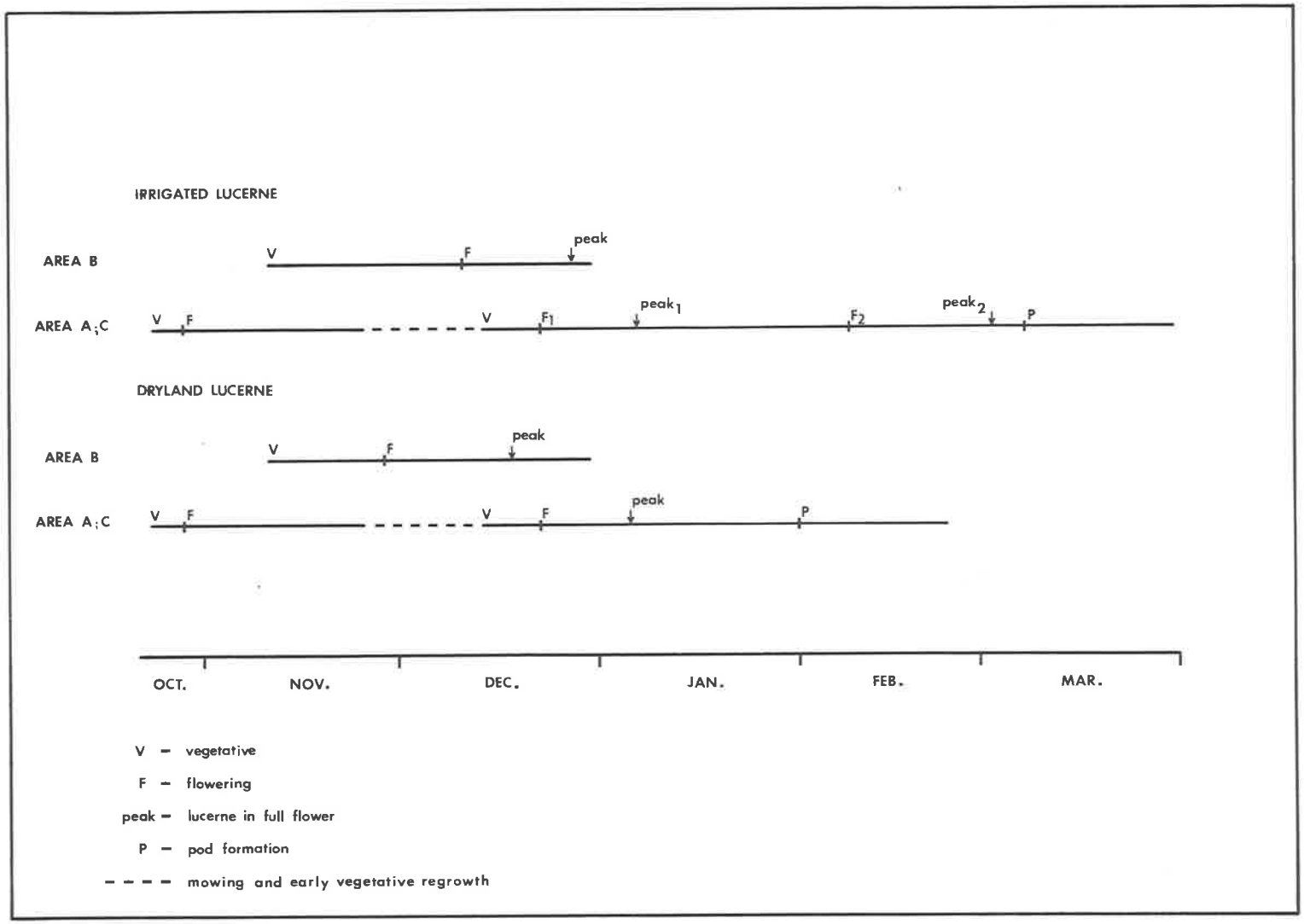

IRRIGATED LUCERNE
AREA B
V
F
peak
AREA A;C
V
F
V
F1
peak1
F2
peak2
P
DRYLAND LUCERNE
AREA B
V
F
peak
AREA A;C
V
F
V
F
peak
P
OCT.
NOV.
DEC.
JAN.
FEB.
MAR.
V – vegetative
F – flowering
peak – lucerne in full flower
P – pod formation
– – – – mowing and early vegetative regrowth

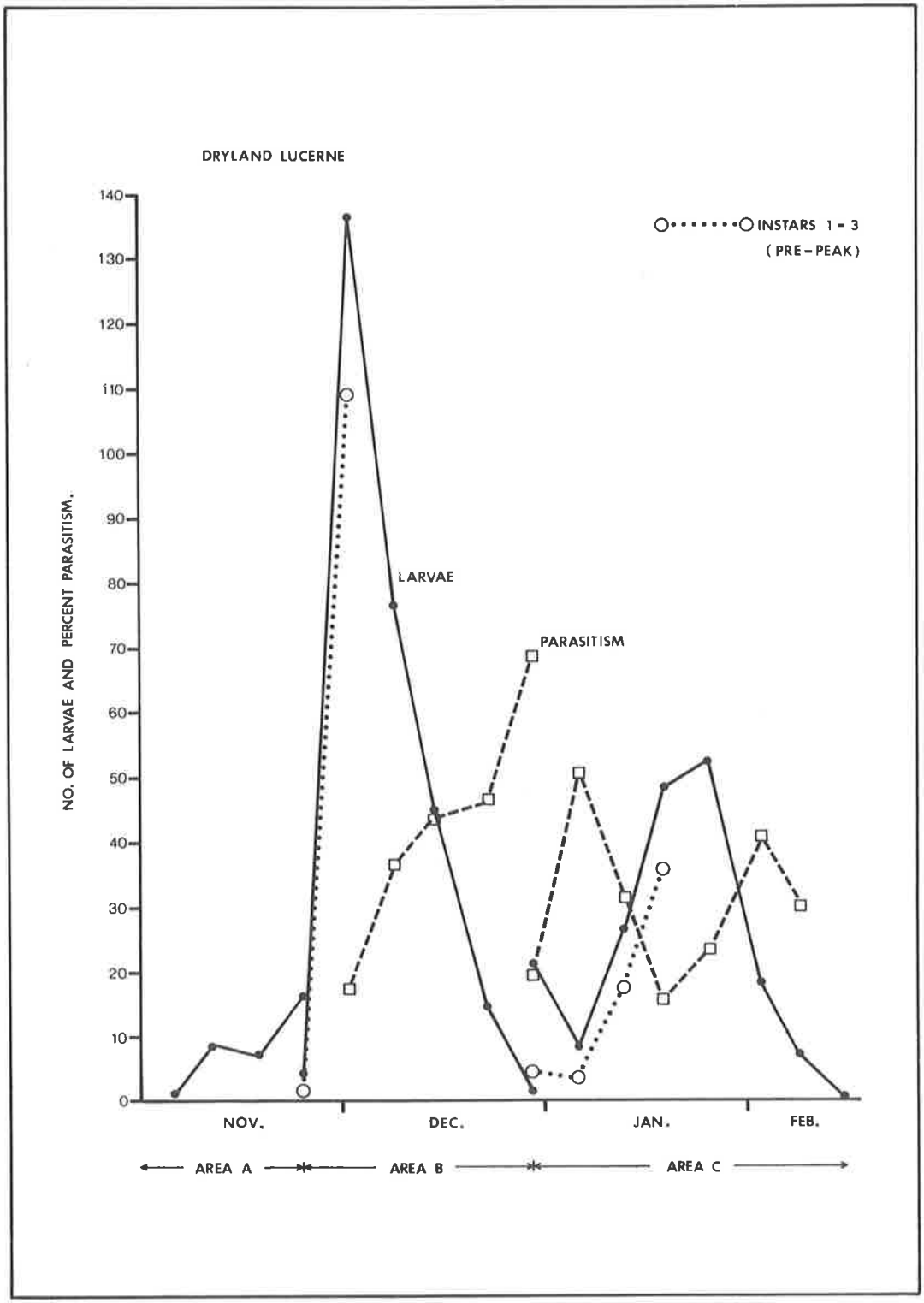
FIGURE 3.6

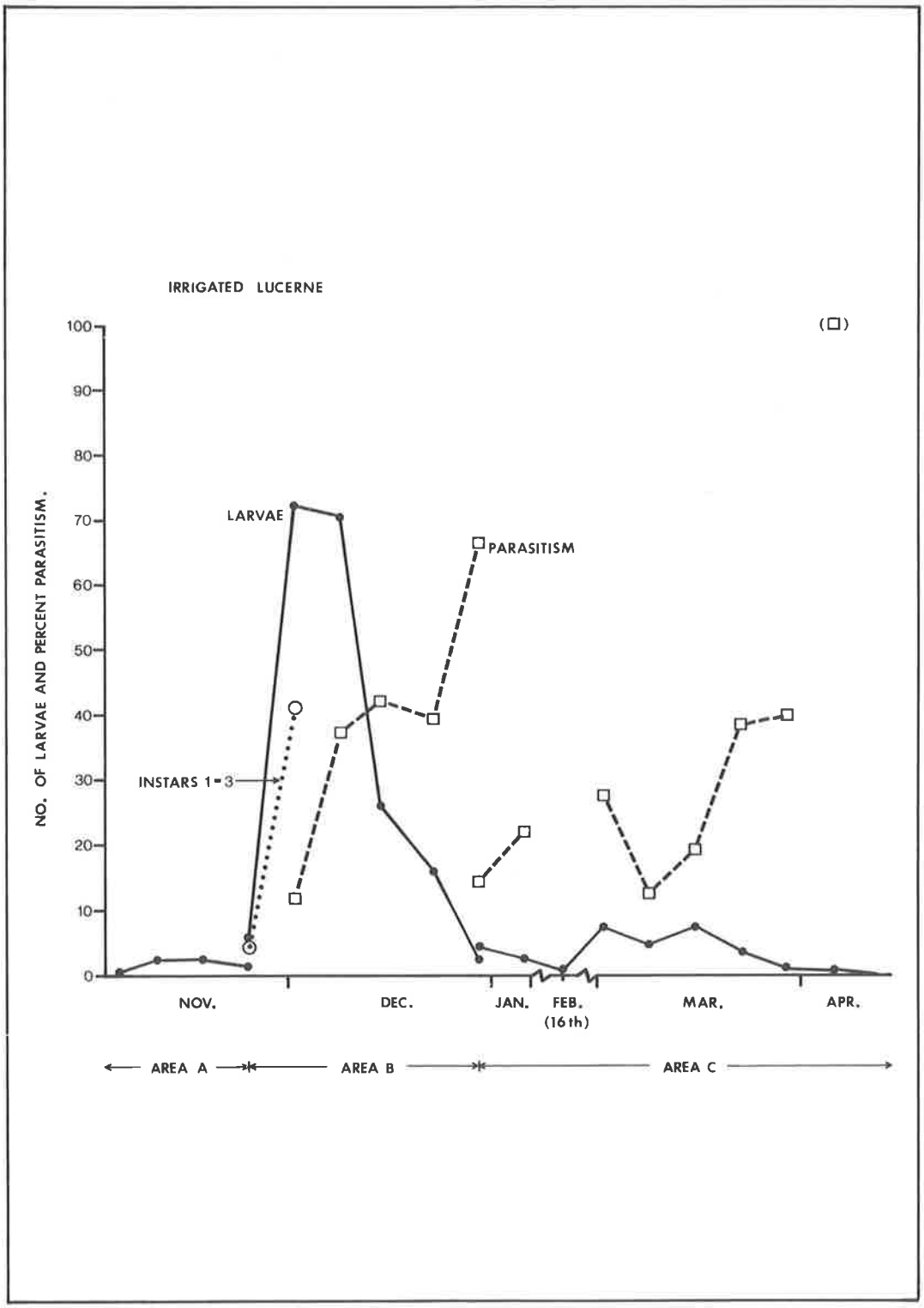
Sweep-net sampling in dryland and irrigated lucerne areas at Mortlock Experiment Station.

●——● mean number larvae (all instars) per sampling unit.

○·····○ mean number first-third instar larvae per sampling unit preceding the peak in larval abundance in dryland areas B, C and irrigated area B.

□-----□ mean percent parasitism of all larval instars.


DRYLAND LUCERNE
NO. OF LARVAE AND PERCENT PARASITISM.
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
INSTARS 1 - 3
(PRE-PEAK)
LARVAE
PARASITISM
NOV.
DEC.
JAN.
FEB.
AREA A
AREA B
AREA C


IRRIGATED LUCERNE
(□)
NO. OF LARVAE AND PERCENT PARASITISM.
100
90
80
70
60
50
40
30
20
10
0
LARVAE
PARASITISM
INSTARS 1-3
NOV.
DEC.
JAN.
FEB.
(16th)
MAR.
APR.
AREA A
AREA B
AREA C

present - C. repanda, Nabis kinbergii, Cermatulus nasalis and Oechalia schellinbergii (see Figure 3.7). From observations and records in the literature, these predators consume the egg, early larval instars, and (the last two) all but the last two larval instars of H. punctigera respectively. Two species of Neuropterans were also collected in the net (see Section 3.3 above). Their abundance was very low (confirmed by field observation).

C. repanda and N. kinbergii were consistently the most abundant predators, which suggests that mortality may be high in the egg and early larval instar stages of H. punctigera. Without a knowledge of their importance, estimates of overall mortality would be difficult (see discussion below).

No epizootics of the larval disease NPV occurred at M.E.S., but characteristic cadavers (Teakle 1973a) were observed. The first was noted on 15.xii and by the next sampling date (23.xii), it was visually estimated that 10 per cent. of larvae had succumbed. On 30.xii, little evidence of NPV was seen and no cadavers were observed during February. The abundance of larvae in both crops declined subsequently (not necessarily from disease since few cadavers were seen).

Discussion

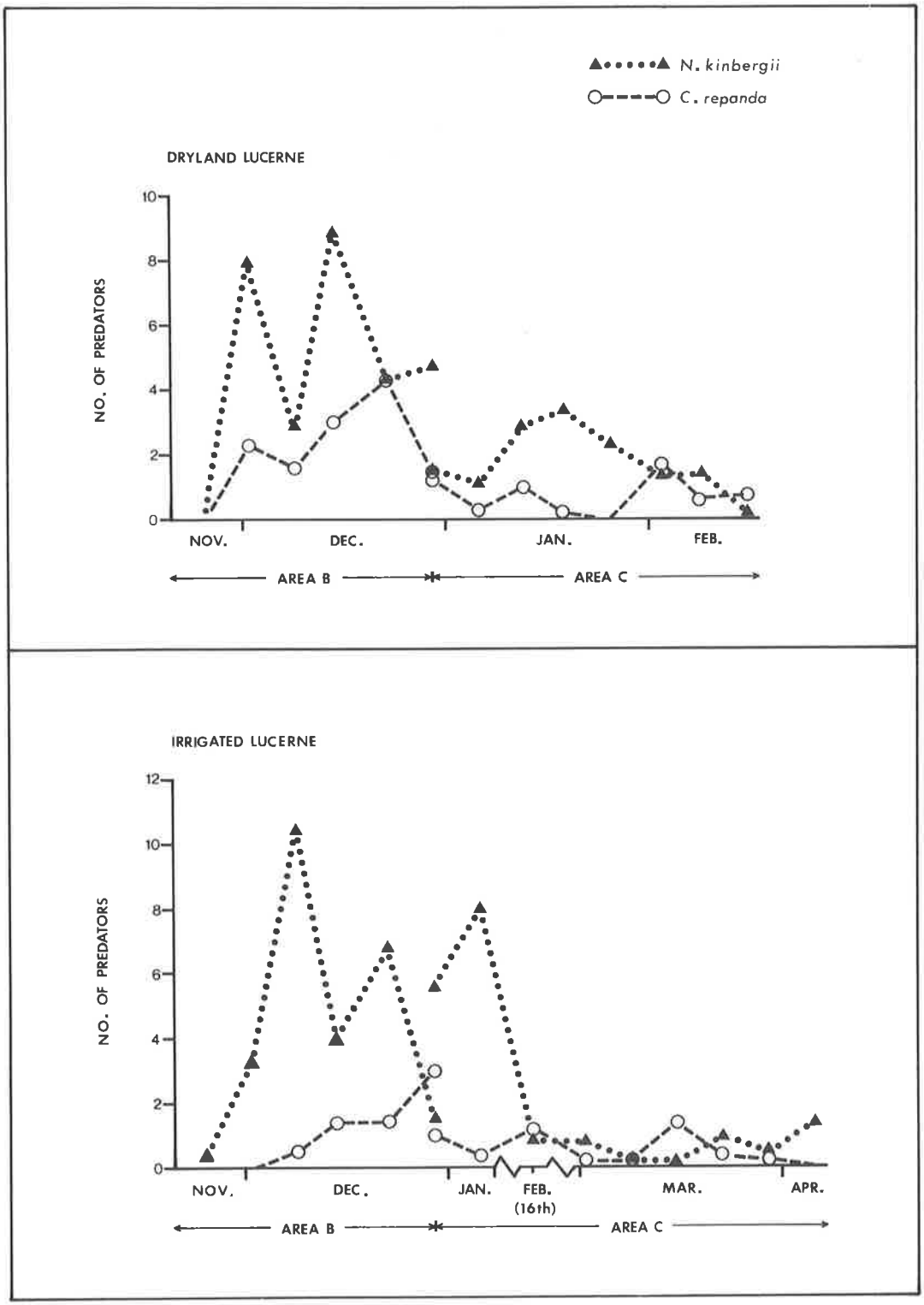
Even though the main objective of the programme undertaken at M.E.S. was not realised because of the decline in abundance of larvae after mid-February, it was considered worthwhile to attempt to draw some conclusions about the survival of H. punctigera larvae based on the data collected there. (Obviously, it is feasible to consider dryland areas B, C and irrigated area B only).

For an insect population where each stage has a well-marked peak in abundance and the overall population mortality rate is approximately constant for the duration of the stages, a method has been developed to determine the number of individuals entering each stage (Richards and Waloff 1954).

FIGURE 3.7

Mean number of predators per sampling unit from sweep-net sampling of dryland and irrigated lucerne crops at Mortlock Experiment Station.

(No. of sampling units examined is given in Tables 1a and b of Appendix 8 and the standard error of the mean density is indicated in Figure 4 of Appendix 8).


▲•••••▲ N. kinbergii
○----○ C. repanda
DRYLAND LUCERNE
NO. OF PREDATORS
0
2
4
6
8
10
NOV.
DEC.
JAN.
FEB.
AREA B
AREA C
IRRIGATED LUCERNE
NO. OF PREDATORS
0
2
4
6
8
10
12
NOV.
DEC.
JAN.
FEB.
(16th)
MAR.
APR.
AREA B
AREA C

It is apparent that there was a peak abundance for each instar at M.E.S. (see Appendix 8, Table 1). A survivorship curve of the total number of larvae plotted against time may be used to determine whether the mortality rate is constant throughout the stages (Deevey 1947, Slobodkin (in Southwood 1966)). If so, a straight line relationship should be obtained between the logarithm of the total number of larvae and time. This was the case for the present data (see Figure 3.8 below - post-peak cumulative totals). Associated r^2 values for these lines (see Table 3.4 below) indicate the appropriateness of the linear relationship. The M.E.S. data were therefore considered to fulfill the requirements outlined above.

The technique of Richards and Waloff was tested by Dempster (1956) using a laboratory population of insects. He concluded that for many species the assumption of steady mortality after hatching is not valid for the whole of the life cycle and proposed that a better estimate of the number of insects entering a particular stage would be obtained if it were based on accumulated totals between the peak and the end of that stage rather than on the complete life cycle (or larval stage of the life cycle as was the case for the present study).

This proposed modification was suitable for the comparatively long instar periods of grasshoppers. However, it was thought to be inappropriate for the short duration instars of H. punctigera and since few samples were collected during a larval generation at M.E.S., the modification proposed by Dempster was not adopted.

This may not necessarily introduce large errors because even in Dempster's work, the suggested use of single instar portions of curves resulted in a reduction in error of only 3.6 per cent. compared with the use of the entire nymphal portion. The technique of Richards and Waloff was therefore followed without modification.

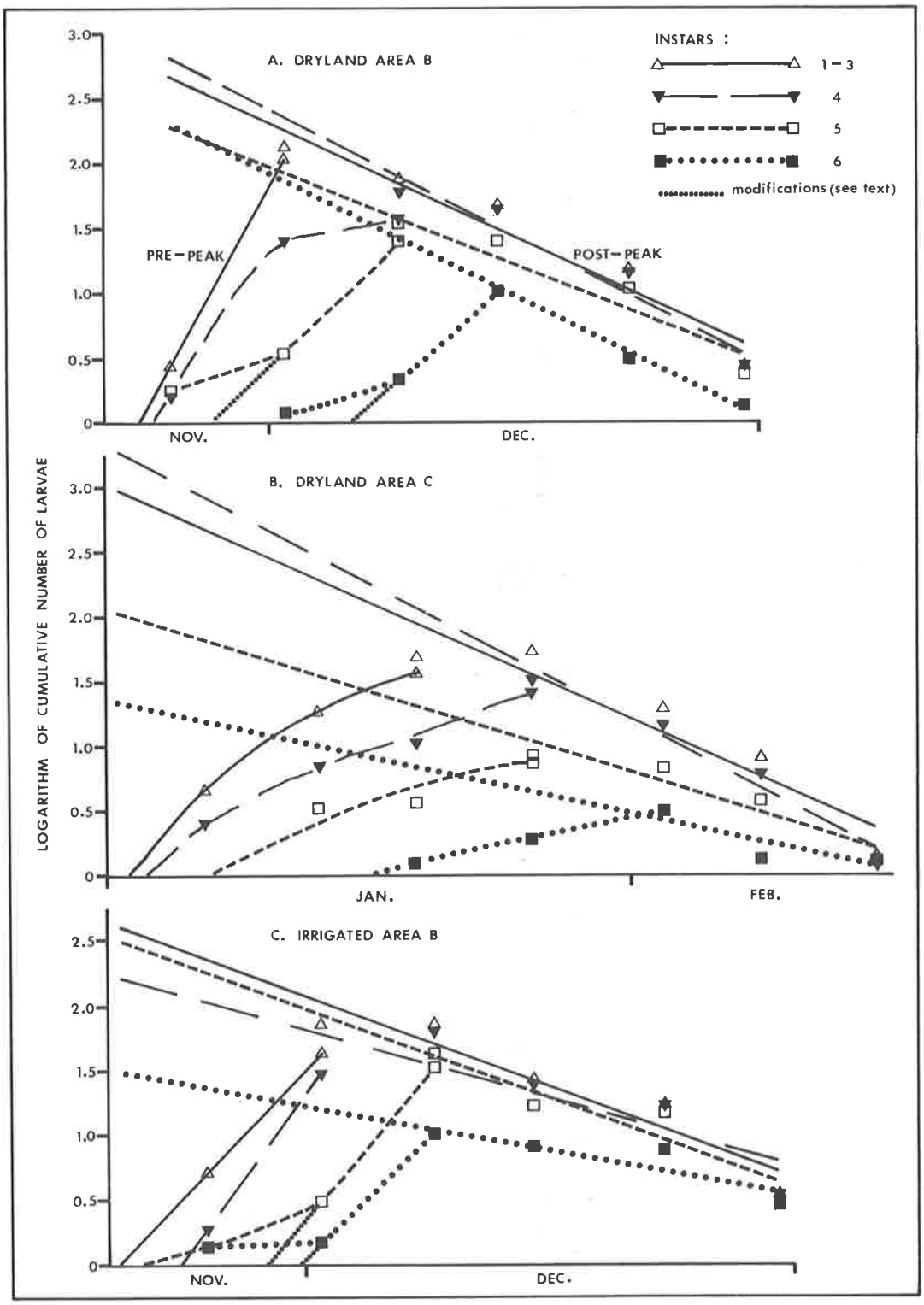
The procedure is to plot the accumulated totals of insects (expressed in

FIGURE 3.8

Abundance of H. punctigera larvae at Mortlock Experiment Station dryland areas B, C and irrigated area B plotted against date of sampling:

(i) lines of negative slope represent linear regressions of the logarithm of cumulative number of larvae after peak occurrence of larvae;

(ii) curves drawn by eye of the logarithm of the pre-peak cumulative number of larvae to obtain times of first occurrence of instars. (See text re modifications in Figs. A, C).


A. DRYLAND AREA B
INSTARS :
1 – 3
4
5
6
modifications (see text)
PRE – PEAK
POST – PEAK
NOV.
DEC.
B. DRYLAND AREA C
JAN.
FEB.
C. IRRIGATED AREA B
NOV.
DEC.
LOGARITHM OF CUMULATIVE NUMBER OF LARVAE
3.0
2.5
2.0
1.5
1.0
0.5
0

logarithms) after the peak of a particular stage, against time. (For a given instar, on each sampling occasion, the number of larvae includes that instar and later ones). The regression line through these points is determined and the number entering a particular stage is given by that value on the regression line corresponding to the time at which the stage first appeared. The regression coefficient for these lines is the logarithm of the average (and supposedly constant) survival rate.

The data collected from the M.E.S. study have inherent shortcomings necessitating some modifications before the above method could be used. Low numbers of larvae (less than one per sampling unit) meant that it would be more convenient to express the data in the form log (N + 1) rather than log (N), where N is the cumulative total of the mean number for each larval instar (or grouping) collected on any sampling occasion. Further, there was one occasion on which the peak abundance of larvae, as suggested from sampling, would have underestimated that in the field viz. the peak in fourth instar larvae in the irrigated crops (Figure 3.6, 2.xii) (more frequent sampling would have overcome this problem). Therefore, an additional modification to the above technique was made - for the fourth instar larvae in the irrigated crop, the peak occurrence was taken to occur on 9.xii. Regressions for post-peak accumulated totals are presented in Figure 3.8. Associated r^2 values and regression coefficients are given in Table 3.4a.

The next step, to determine the time at which each stage first appeared (i.e. log (N + 1) = 0), was performed somewhat subjectively. Where the numbers of larvae were decreasing (as in dryland area C) or the initial population appeared to belong to another flight (e.g. fifth and sixth instars in dryland and irrigated area B), data were ignored. Curves of the logarithm of pre-peak accumulated totals of individual instars were drawn by eye and extrapolated to the X-axis to obtain the time at which a particular stage appeared. The number of larvae entering that stage was

calculated using the appropriate regression equation for the post-peak accumulated totals and this time. Results for the present data are given in Table 3.4b.

The impossible situation of having a greater number of larvae entering the fourth instar than the third is an obvious shortcoming of the data - no doubt caused by a combination of low sweep-net efficiency in collecting early instars and too long an interval between successive sampling dates. In addition to this, it is apparent that the subjective method used for determining the time at which a stage first appears may lead to considerable error because a logarithmic ordinate is used.

TABLE 3.4a Values of r^2 and regression coefficients in linear regressions of the number of each instar and time of sampling, for curves in Fig. 3.8.

(i) r^2

Lucerne area	Instar			
	1 - 3	4	5	6
Dryland B	0.94	0.94	0.87	0.99
Dryland C	0.88	0.97	0.90	0.71
Irrigated B	0.90	0.72	0.86	0.78

(ii) +Regression coefficients

Lucerne area	Instar								Aver. % surv.
	1 - 3		4		5		6		
	+log	+%	log	%	log	%	log	%	
Dryland B	-0.060	11.5	-0.065	11.6	-0.051	11.3	-0.064	11.6	11.5
Dryland C	-0.057	11.4	-0.066	11.6	-0.039	10.9	-0.028	10.7	11.2
Irrigated B	-0.047	11.1	-0.036	10.9	-0.047	11.1	-0.023	10.5	10.9

\+ Expressed as a logarithm and as % survival

TABLE 3.4b Number of larvae entering each stage, from Fig. 3.8.

Lucerne area	Instar and initial no. of larvae			
	1 - 3	4	5	6
Dryland B	660	758	150	40
Dryland C	911	1150	62	8
Irrigated B	388	203	75	16

On closer examination of the pre-peak abundance of first to third instar larvae (see Figure 3.6), the time at which this group first appeared could be reasonably obtained by extrapolation to the X-axis. Since most of the larvae collected in this category consisted of third instars, it was proposed to consider this date as the time at which third instar larvae first appeared. Using this date, the temperature experienced by the larvae, and a knowledge of the rate of larval development, it would be possible to calculate the times of first appearance of the other instars. The subjective method used previously for determining this time would be dispensed with and a more critical and therefore possibly more realistic estimate would be obtained. This procedure was adopted for the present data using daily air temperatures recorded at M.E.S. and the rate of development of the larval instars was calculated using the method described in Appendix 9.

Hence, theoretical times of first appearance for all instars were calculated (see T_0 values in Figure 3.9). Because of the inefficiency of the sweep-net in collecting the early instars, only these times for fourth, fifth, and sixth instars could be compared with the field data (Appendix 8, Table 1), and it can be seen that the calculated times compare favourably with what might be considered reasonable estimates if this information only was used.

The independent time-axis could now be measured in either of two units - days or per cent. larval development. The latter unit was selected because per cent. larval development provides a more meaningful measure of the

interval between sampling dates and it was more convenient to use in delineating the occurrence of particular instars. The data were thus plotted with the origin of the X-axis coinciding with a convenient date chosen in such a way that the initial occurrence of first instar larvae was after "time zero". The regression lines for the final three instars are presented in Figure 3.9 and the associated r^2 values and regression coefficients are given in Table 3.5a.

Using the method described above, the numbers of larvae entering each of the final three instars were determined (see Table 3.5b).

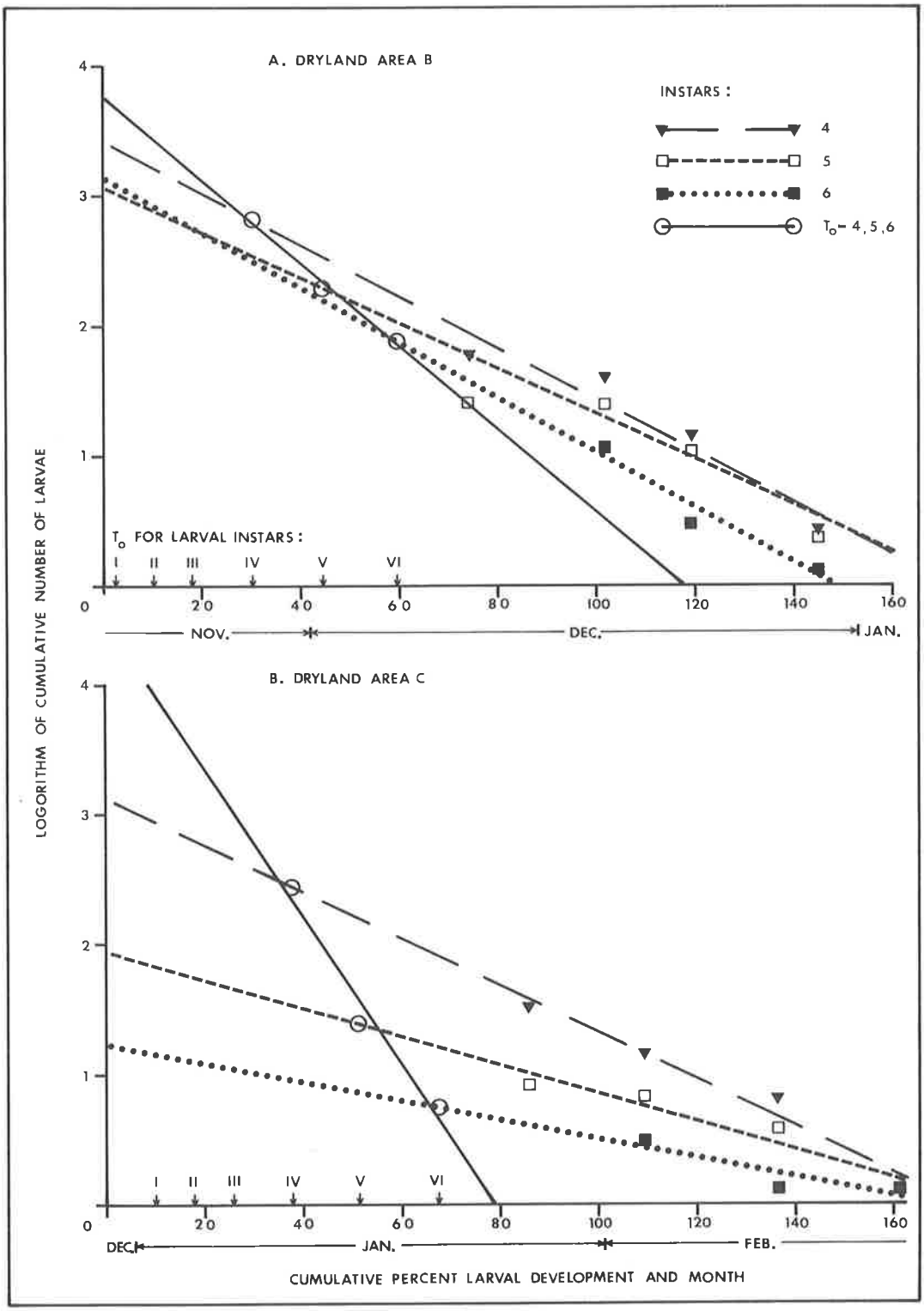
On the assumption that mortality is roughly constant throughout the larval stages (see above), the number of larvae entering the first three instars can be calculated from the regression equation for the number of larvae entering the fourth to sixth instars. The appropriate lines were determined (see Figure 3.9) and numbers of larvae entering all instars calculated (see Table 3.6a). The corresponding r^2 values and regression coefficients for the equations for the regression lines in Figure 3.9 are given in Table 3.6b.

Naturally, there is a slight deviation in the numbers of larvae entering the final three instars obtained in the latter calculations - Table 3.5b cf. Table 3.6b. For consistency, calculations below are based on data in Table 3.6b only.

Using these estimates, intra-instar mortality was calculated (see Table 3.7, theoretical mortality). For comparison, estimates of field mortalities based on estimates of parasitism and the effect of NPV were also determined.

FIGURE 3.9

Linear regressions of the logarithm of the cumulative number of fourth, fifth, and sixth instar larvae of *H. punctigera* after their peak occurrence and linear regression of the total number of fourth to sixth instar larvae at the time of first occurrence (T_o) plotted against cumulative percent larval development for sampling at Mortlock Experiment Station.


A. DRYLAND AREA B
INSTARS :
4
5
6
T_o = 4, 5, 6
T_o FOR LARVAL INSTARS :
I II III IV V VI
0 20 40 60 80 100 120 140 160
NOV. DEC. JAN.
B. DRYLAND AREA C
I II III IV V VI
0 20 40 60 80 100 120 140 160
DEC. JAN. FEB.
LOGORITHM OF CUMULATIVE NUMBER OF LARVAE
CUMULATIVE PERCENT LARVAL DEVELOPMENT AND MONTH

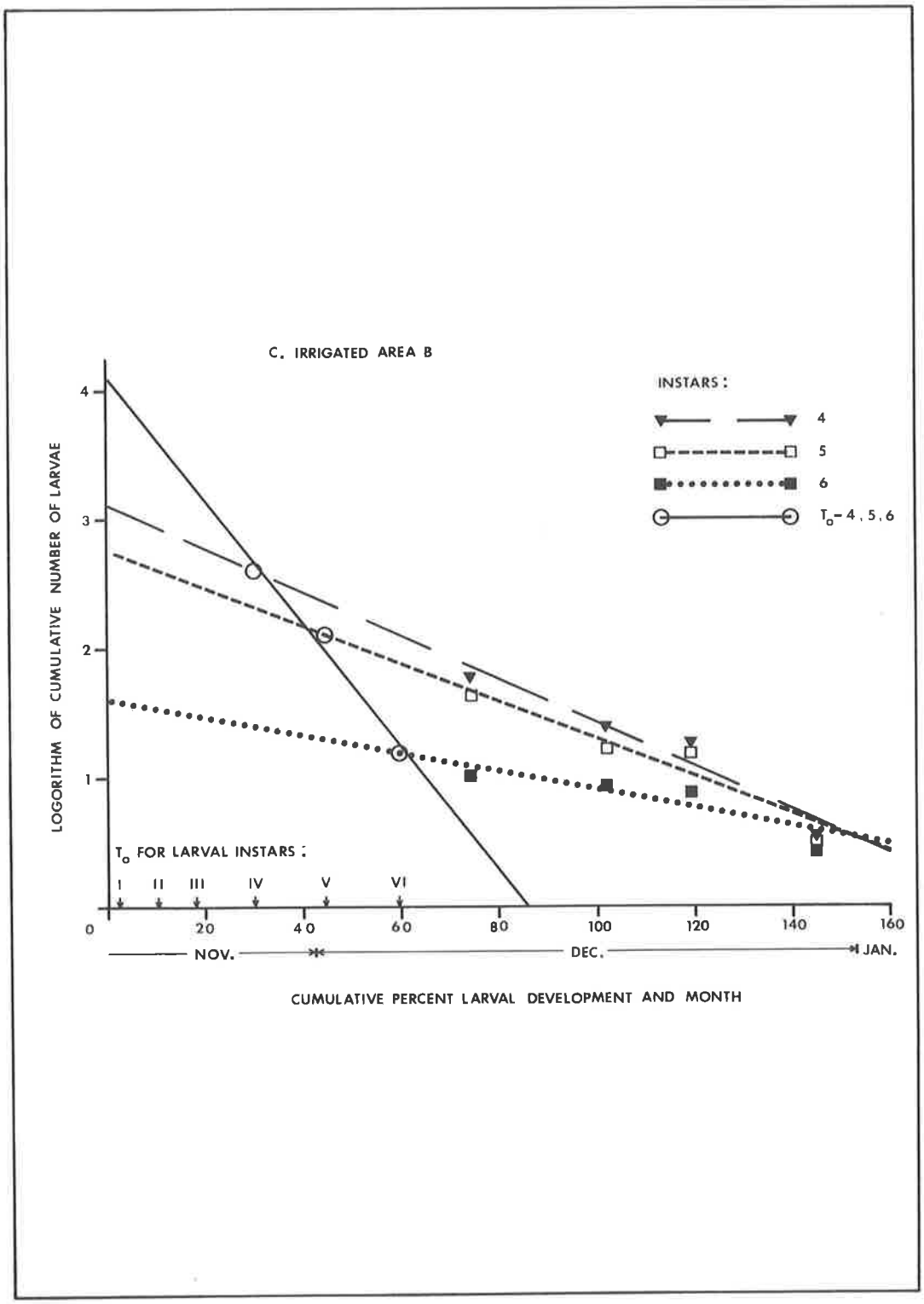

C. IRRIGATED AREA B
INSTARS :
4
5
6
T_o - 4, 5, 6
LOGORITHM OF CUMULATIVE NUMBER OF LARVAE
4
3
2
1
0
T_o FOR LARVAL INSTARS :
I
II
III
IV
V
VI
20
40
60
80
100
120
140
160
NOV.
DEC.
JAN.
CUMULATIVE PERCENT LARVAL DEVELOPMENT AND MONTH

TABLE 3.5a Values of r^2 and regression coefficients in linear regressions of the number of each instar and time of sampling, for curves in Fig. 3.9.

(i) r^2

Lucerne area	Instar 4	5	6
Dryland B	0.92	0.84	0.95
Dryland C	0.98	0.92	0.77
Irrigated B	0.95	0.91	0.80

(ii) Regression coefficients

Lucerne area	Instar 4		5		6		Aver. % surv.
	+log	+%	log	%	log	%	
Dryland B	-0.020	10.4	-0.018	10.4	-0.021	10.5	10.4
Dryland C	-0.018	10.4	-0.011	10.3	-0.007	10.2	10.3
Irrigated B	-0.017	10.4	-0.015	10.4	-0.007	10.2	10.3

\+ Expressed as a logarithm and as % survival

TABLE 3.5b Number of larvae entering the final three instars, from Fig. 3.9.

Lucerne area	Instar and initial number 4	5	6
Dryland B	656	200	74
Dryland C	267	23	5
Irrigated B	390	127	14

TABLE 3.6a Number of larvae entering each stage, from Fig. 3.9.

Lucerne area	Instar and initial number of larvae					
	1	2	3	4	5	6
Dryland B	4762	2692	1525	624	220	70
Dryland C	8220	2974	1081	220	34	4
Irrigated B	9751	4123	1750	455	94	16

TABLE 3.6b Values of r^2 and regression coefficients of initial number of larvae of the last three instars and time of sampling, for curves in Fig. 3.9.

Lucerne area	r^2	Regression coefficient	
		log	% survival
Dryland B	0.99	-0.032	10.8
Dryland C	0.97	-0.057	11.4
Irrigated B	0.98	-0.048	11.2

From the data on predator abundance (Figure 3.7), it may be reasonably assumed that predation was a minor source of mortality and it is assumed to be zero*. The highest visual estimate of NPV will be used i.e. 10 per cent. It is also assumed that this level is constant among instars and appropriate for either parasitised or healthy larvae (see Section 3.4.3.3, results). The level of parasitism was calculated for each instar from the sweep-net

* Footnote:-

N. kinbergii primarily feeds on the eggs and first two instars of H. punctigera (Awan 1981) and therefore can be ignored when considering the mortality of third to sixth instars. The other larval predators, O. schellenbergii and C. nasalis, consume all larval instars except the last two. These bugs were consistently present in significant numbers only in the irrigated lucerne area B (Figure 3.7). This indicates a weakness in the above assumption of zero predation, but without any information available on the quantitative importance of these predators, it was thought best to adhere to the above assumption. Nevertheless, it is necessary to qualify any conclusions made.

collections made over the entire sampling period for each area. The data on first to third instars are assumed to apply to third instar larvae only. Apart from being the most prevalent in the grouping, this instar was usually the only one that could be determined as being parasitised or not, based on the methods used (see Appendix 10).

Since 10 per cent. of parasitised larvae succumbed to virus (assumption above), total mortality was determined as:-

$$\text{Total instar mortality (\%)} = \left(M_V + M_P \frac{(100 - M_V)}{100}\right) \%$$

$$= (10 + 0.9M_P) \%$$

Where M_V is % mortality due to virus and M_P is % mortality due to parasites.

Results of these calculations are presented in Table 3.7, along with corresponding levels of mortality obtained from the theoretical consideration already described.

Obviously, a re-examination of the procedures used is necessary because of the large differences obtained from the two methods. In particular, the assumption concerning predation and the validity of the constant mortality rate for each instar need to be questioned. Also the effect of NPV was not critically determined. The level of 10 per cent. used here may have been an under-estimate of the field situation. However, the level of parasitism may have provided a reasonable indication of the field situation and, if anything, probably represents an over-estimate of mortality from this cause (see Appendix 10).

The differences between estimates of larval mortality based on sweep-net collections (Table 3.7) and theoretical determinations, decrease with later instars. Therefore estimates of sixth-instar mortality from the sweep-net data may be used with some confidence. These estimates, in conjunction with field abundance of sixth-instars (see Table 3.6b), were used to calculate the probable abundance of pupae (see Table 3.8).

TABLE 3.7 Estimates of the level of parasitism and total mortality of third to sixth instars.

Lucerne area	Mortality	Instar and % mortality 3	4	5	6
Dryland B	+ Parasitism (P)	15	25	54	78
	+ P + NPV	24	33	59	81
	x Theoretical (Th)	59	65	68	*
Dryland C	P	12	31	53	60
	P + NPV	21	38	57	64
	Th	80	85	88	*
Irrigated B	P	8	16	49	60
	P + NPV	18	25	54	64
	Th	74	79	83	*

\+ Estimates based on sweep-net data

x Estimates based on data in Table 3.6b

* Information on abundance of pupae not available to obtain this estimate.

Moth fecundity and egg mortality data obtained by Cullen (1969) and the results of the above calculations were used to determine the changes in the size of generations of H. punctigera at M.E.S. (see Table 3.8). Pupal mortality was assumed to be zero.

Naturally the data for generation 2 in dryland area B (i.e. calculated as above using area B data only) should be indicative of the situation for generation 1 in dryland area C (calculated from area C data). The calculated increase in size of generation 2 from dryland area B is vindicated by the larger number of eggs in generation 1 dryland area C compared with that for area B. However, the extent of the increase differed by 2 per cent. using area B data only, and 73 per cent. for areas B and C.

TABLE 3.8 Estimation of the change in size of generations of H. punctigera at Mortlock Experiment Station.

Lucerne area	Generation 1					Generation 2	Gen 1 to Gen 2
	Stage and $^{+}N_o$			% mortality Instar VI	No. healthy pupae.	* No. eggs	% change, egg_1 to egg_2 within each area
	Instar I	^{x}No. eggs	Instar VI				
Dryland B	4762	9524	70	81	13.57	9750	+ 2
Dryland C	8220	16440	4	64	1.44	1035	-94
Irrigated B	9751	19502	16	64	5.72	4110	-80

$+N_o$ = Number of individuals entering a stage

* = Moth fecundity of 1437 eggs is used (Cullen 1969) and adult sex ratio of 1:1

x = Egg mortality of 50% is used (Cullen 1969)

Undoubtedly, changes in moth fecundity and egg mortality could lead to such variation. Also the extent to which immigration occurred was not examined, but considering the situation in the irrigated area, it may not have been great.

The decline in the size of generation 2 predicted from the irrigated lucerne from area B data, is consistent with the low numbers of larvae found there after December.

Conclusions

Light trap catch data were found to provide a reliable indication of the occurrence and abundance of field populations of H. punctigera in a nearby food plant.

The main causes of larval mortality, investigated for the later instars only, were parasites and probably NPV, although the effect of the latter agent was not critically examined. Predators of late instars were not very abundant and hence were neglected as a major mortality factor; however, the predator N. kinbergii may play a major role in early instar mortality. This was by far the most abundant predator at M.E.S. and occurs throughout the study area (see Section 3.4.3.4).

Because the sweep-net sampling interval was long in relation to larval instar development time, an attempt to use these data to calculate net larval mortality was not completely successful. Nevertheless, the trends indicated by such calculations were consistent with the subsequent abundance of H. punctigera.

After February, the number of larvae in lucerne at M.E.S. declined to such a low level that to continue the study of larval mortality, it was necessary to locate an alternative site where the species was more abundant. Such a site was found at Booborowie. The results of a sampling programme undertaken there are presented below.

3.4.3.3 Booborowie

An irrigated lucerne crop on the property of Collinsville near Booborowie was selected for sampling H. punctigera larvae after late summer (see Section 3.4.3.4, Figure 3.1, N6). Four roughly equal-sized paddocks of sprinkler irrigated lucerne were cultivated in staggered rotation such that one of them was always in flower (and hence available for grazing by sheep). This area was therefore considered suitable to continue the work begun at M.E.S.

Sampling procedure

Because of the management system used for the lucerne, only one of the four paddocks was suitable for sampling at any time. The lucerne was in the late vegetative stage when sampling commenced and discontinued when sheep were introduced at peak flowering - equivalent to the stage at which fodder lucerne would be cut for hay production. By this time, the next paddock in rotation was at an appropriate stage to commence sampling. Only two of the paddocks were suitable for sampling prior to the late autumn decline in abundance of larvae - these are designated Bay 1 and Bay 2.

As in the study at M.E.S., estimates of larval abundance, level of parasitism and predator abundance were obtained using a sweep-net. The sampling unit was unchanged (i.e. 10 sweeps) but fewer units were collected on each sampling occasion (see Appendix 8). The positions of sampling stations were defined with the aid of fence posts and other suitable landmarks to avoid double sampling. The importance of NPV in mortality of H. punctigera at this time of year was also studied.

Earlier in the season, an investigation was carried out in a lucerne crop at Pt. Gawler (Figure 3.1, N1) to determine an appropriate rearing regime to use in the study of NPV at Booborowie.

The lucerne at Pt. Gawler was managed in a similar way to that at

Collinsville, but used for hay production. Sixth instar larvae that appeared to be healthy, though not necessarily unparasitised, were collected at random. A section of the lucerne on which the larvae was feeding was cut and both were placed in a perspex tube, with only one larva in each tube. Larvae were returned to the laboratory and divided into two groups, for rearing at either 19°C or 25°C. Each of these groups was further subdivided for rearing on fresh lucerne sprigs collected from the crop, standard artificial diet (Appendix 1), or artificial diet without formaldehyde. Thirty larvae were used for each treatment; they were examined daily and the cause of death of larvae suspected of dying from disease was determined using phase contract microscopy (Cooper, pers. comm.).

Of the 180 larvae collected at Pt. Gawler for the study of pathogens, 138 (77%) died of NPV, 5 (3%) of granulosis virus (GV), 1 (1%) of the fungal pathogen Beauvaria bassiana, and 10 (6%) of septicaemia*.

NPV was therefore confirmed as the most important pathogen. Teakle (1974) recorded a level of 10 per cent. mortality in larvae of H. punctigera in lucerne during a GV epizootic, but this level is low compared to the spectacular NPV epizootics.

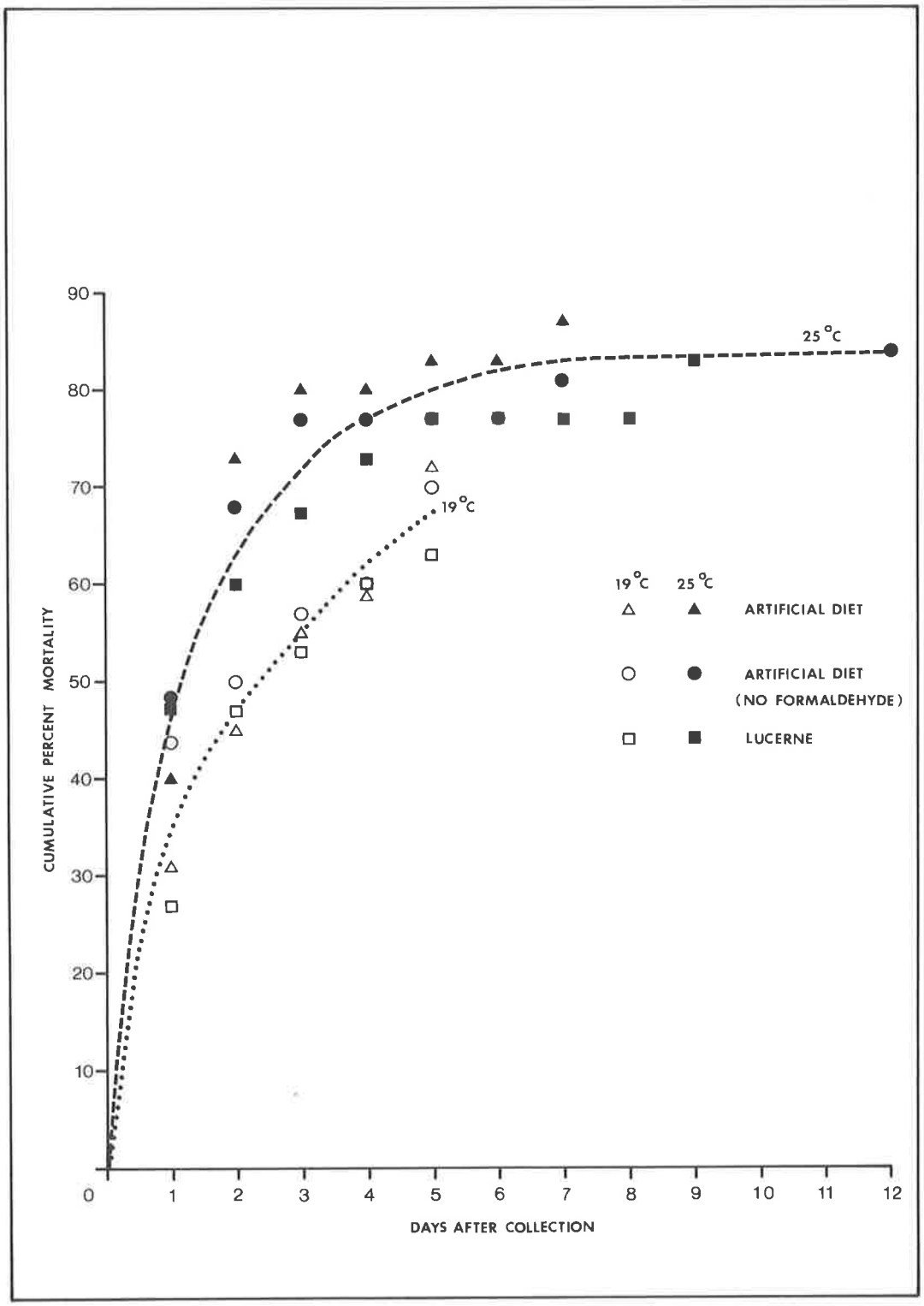
The daily cumulative mortality of larvae from NPV is indicated in Fig. 3.10. The data for each rearing temperature (diets combined) were reasonably well described using asymptotic regression (i.e. Mitscherlich's curves) passing through the origin (a necessity since field collections consisted of live larvae). A discussion of the goodness of fit of such a model and other parameters relating to the curves is given in Appendix 10.

* Footnote:-

The cause of the septicaemia could have been either a primary bacterial pathogen (e.g. as with a Bacillus infection; B. thuringiensis had been recorded from a diseased H. punctigera pupa collected under lucerne at Brecon, see Section 3.5) or a stress during handling (physical damage to the gut wall) or rearing (perhaps because of the temperature and/or humidity change). The incidence of septicaemia for the various diet/rearing temperature combinations was similar.

FIGURE 3.10

Cumulative percentage mortality caused by NPV of sixth instar H. punctigera larvae collected from lucerne at Pt. Gawler and reared in the laboratory. (Curves drawn by eye).


90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
CUMULATIVE PERCENT MORTALITY
DAYS AFTER COLLECTION
25 °C
19 °C
19 °C 25 °C
ARTIFICIAL DIET
ARTIFICIAL DIET
(NO FORMALDEHYDE)
LUCERNE

Results of the NPV - related mortality levels of the larvae collected at Pt. Gawler are presented in Table 3.9.

TABLE 3.9 Effect of rearing conditions on mortality caused by NPV in field collected larvae.

Incubation temp. (°C)	Diet and *% mortality			
	Lucerne	Artificial Diet	+Artificial Diet	All Diets
19	63	70	72	69
25	83	84	87	85
Both temps.	73	77	80	77

* 30 larvae used for diet/rearing temperature combination.

\+ without formaldehyde.

Tests of interaction between both diet and temperature, and mortality were performed (see Appendix 11). The only significant difference obtained was for the diet of lucerne when rearing temperatures were compared. Because of this variation, lucerne was rejected as a suitable diet. Also, despite the absence of a significant difference between rearing temperatures for each of the other diets or all diets combined (Appendix 11 i, v, vi), the consistently higher mortality at 25°C suggested a real difference - either the occurrence of a stress or 25°C was more suitable for the development of NPV. To avoid either possibility, 19°C was preferred. Mortalities of larvae feeding on the two artificial diets were not significantly different, but it was desirable to use the diet without formaldehyde to avoid possible side-effects of this virus sterilant. Therefore, larvae collected for the study at Collinsville were reared at 19°C on artificial diet without formaldehyde, and the occurrence of NPV was noted as described above.

In addition to the study at Collinsville, sweep-net sampling and collections

of larvae for NPV determination were undertaken during late autumn in a dryland seed lucerne crop at North Bungaree, located approximately five kilometres West of Collinsville.

Results

The estimate of larval abundance from the sweep-net collections is given in Figure 3.11 (see also Appendix 8, Table 2 for the number of sampling units examined and standard errors). It was apparent that larvae were more abundant here than at M.E.S. (Figure 3.6) and that timing of moth oviposition (as reflected in the presence of larvae) was related to the stage of growth of the lucerne rather than the timing of moth flights.

Parasitism of larvae was lower at Collinsville and Nth. Bungaree compared with M.E.S. (Figure 3.11 cf. Figure 3.6). There were no consistent trends with time, but again, parasitism was highest in sixth instar larvae.

In contrast to the situation at M.E.S., O. schellenbergii was the most numerous predator (Figure 3.12) and an increase in its abundance with time was recorded. N. kinbergii and C. repanda were also recorded. C. nasalis was not recorded during this period (but see Figure 3.14 below). This situation at Collinsville suggested that predators may be more important mortality agents of later instars, in contrast to the situation at M.E.S.

Collection details, per cent. NPV mortality and abundance of sixth instar larvae from sweep-net collections at Booborowie are given in Table 3.10 (estimates of mortality from the small number of larvae comprising the first and last samples in Bay 2 are not reliable). On March 30 a second group of 50 larvae selected as being healthy and not obviously parasitised, succumbed to the virus to the same extent as did randomly collected larvae (collection 1-3-1, Table 3.10).

Observations in the lucerne crops were consistent with the level of NPV mortality obtained from the laboratory incubations. In each bay sampled, the

FIGURE 3.11

Number of H. punctigera adults caught by hand-net, and mean number of larvae per sampling unit and percent parasitism of larvae from sweep-net sampling at Booborowie.

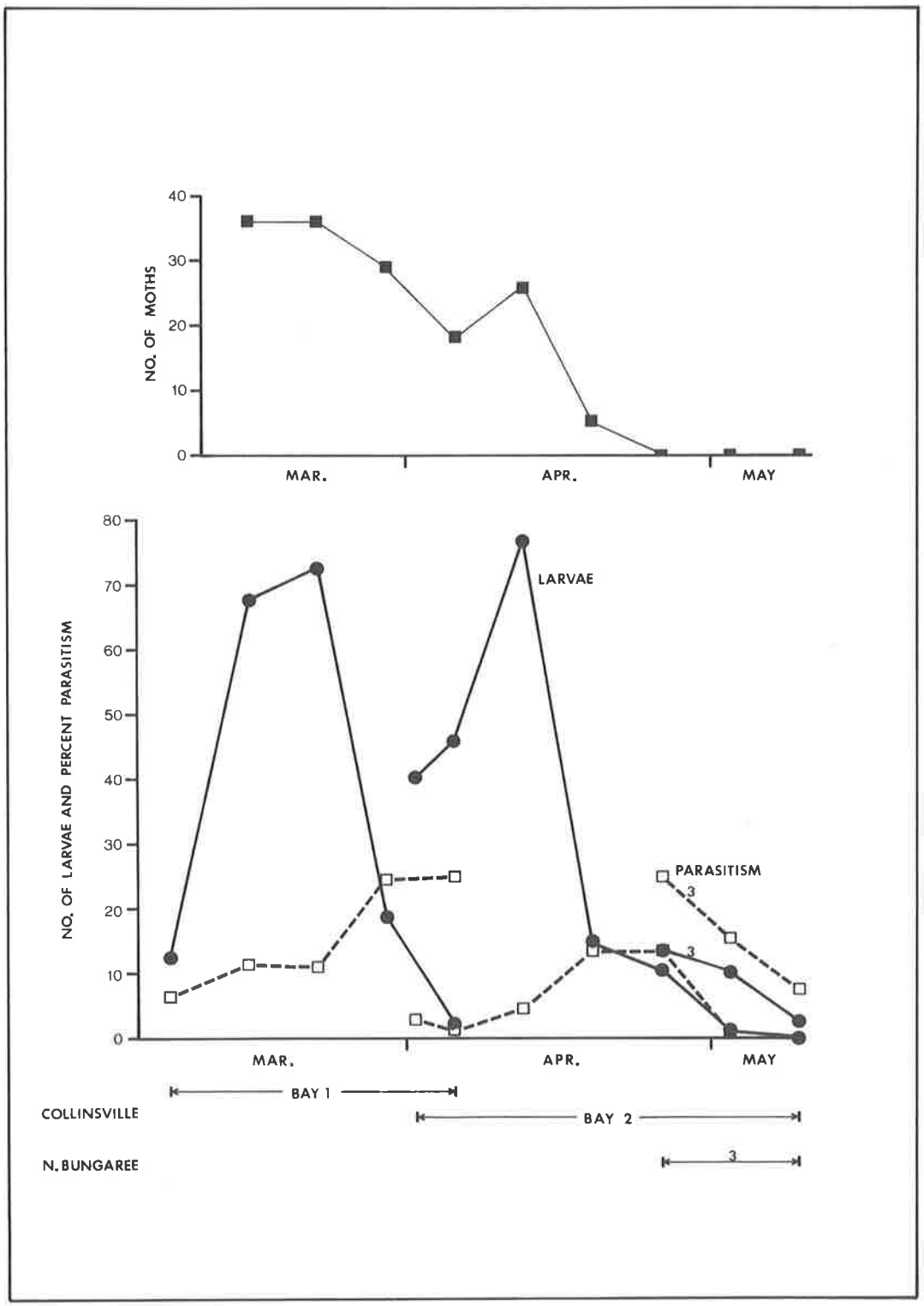

NO. OF MOTHS
40
30
20
10
0
MAR.
APR.
MAY
NO. OF LARVAE AND PERCENT PARASITISM
80
70
60
50
40
30
20
10
0
LARVAE
PARASITISM
3
3
3
MAR.
APR.
MAY
BAY 1
BAY 2
COLLINSVILLE
N. BUNGAREE

FIGURE 3.12

Mean number of predators per sampling unit from sweep-net sampling at Booborowie.

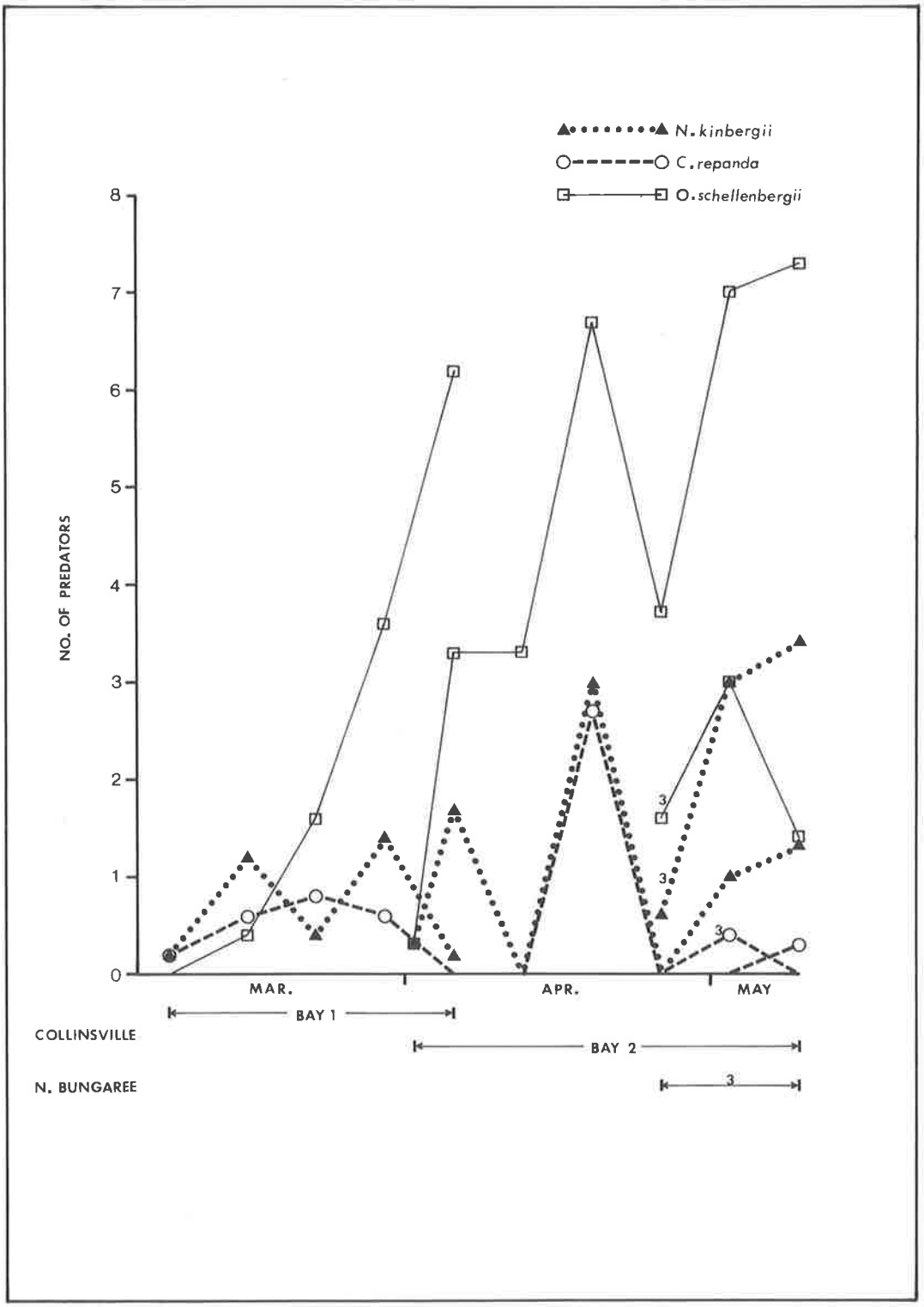

N. kinbergii
C. repanda
O. schellenbergii
NO. OF PREDATORS
0
1
2
3
4
5
6
7
8
MAR.
APR.
MAY
BAY 1
BAY 2
3
COLLINSVILLE
N. BUNGAREE

TABLE 3.10 Mortality due to NPV of sixth instar larvae collected at Booborowie.

*Bay-collection number	Collection date	No. collected	% mortality (NPV)	+Abundance of sixth instars
1 - 1	16.iii.76	63	5	10.6
1 - 2	23	95	35	32.4
1 - 3	30	100	77	13.2
1 - 3 - 1	30	50	78	
2 - 1	2.iv	20	25	1.0
2 - 2	6	68	16	1.7
2 - 3	13	100	30	8.7
2 - 4	20	96	81	2.3
2 - 5	27	19	58	1.7
3 - 1	27.iv	43	23	6.2
3 - 2	4.v	49	37	5.6
3 - 3	11	x n.c.	n.c.	1.0

* = Bays 1, 2 - Collinsville; Bay 3 - North Bungaree

+ = Data obtained from sweep net collections; mean no./sampling unit

x n.c. = no collection made

same trend in mortality was found - epizootics among sixth instars were characterised by a gradual increase in mortality, reaching its highest level after the larvae had reached maximum abundance. This has important implications for the survival of H. punctigera in the study area (see below).

As well as the total mortality of larvae from NPV, the cumulative daily mortality was recorded for each collection *(Figure 3.13). The proportion of larvae succumbing in the first day or two of incubation increased as the peak of the epizootic was reached. A more gradual cumulative mortality occurred in larvae collected earlier. Curves for the Pt. Gawler collection (Figure 3.10) are consistent with those for the collections made at Collinsville at the time of peak NPV mortality.

Discussion

The nature of the curves on larval abundance at Collinsville (Figure 3.11) suggested that mortality throughout the stage was not constant (see discussion in Section 3.4.3.1). Also, the sampling interval was so long, that peaks in the abundance of instars were poorly defined. Indications of the time of first appearance of each instar were even less clear. These characteristics precluded the use of Richards and Waloff's (1954) technique to estimate total larval mortality. Nevertheless, the graphs may be used to indicate trends e.g. the area under a curve of animal abundance versus time is related to the total abundance of that animal (Southwood 1966). On examination of Figure 3.11, there may have been a slight decline in the abundance of H. punctigera larvae from bay 1 to bay 2 (i.e. from March to April) possibly because of the greater abundance of O. schellenbergii (see Figure 3.12). Therefore although the level of parasitism of larvae was lower in bay 2 (see Figure 3.11) a smaller population of healthy pupae would have been expected there (see Table 3.13 below).

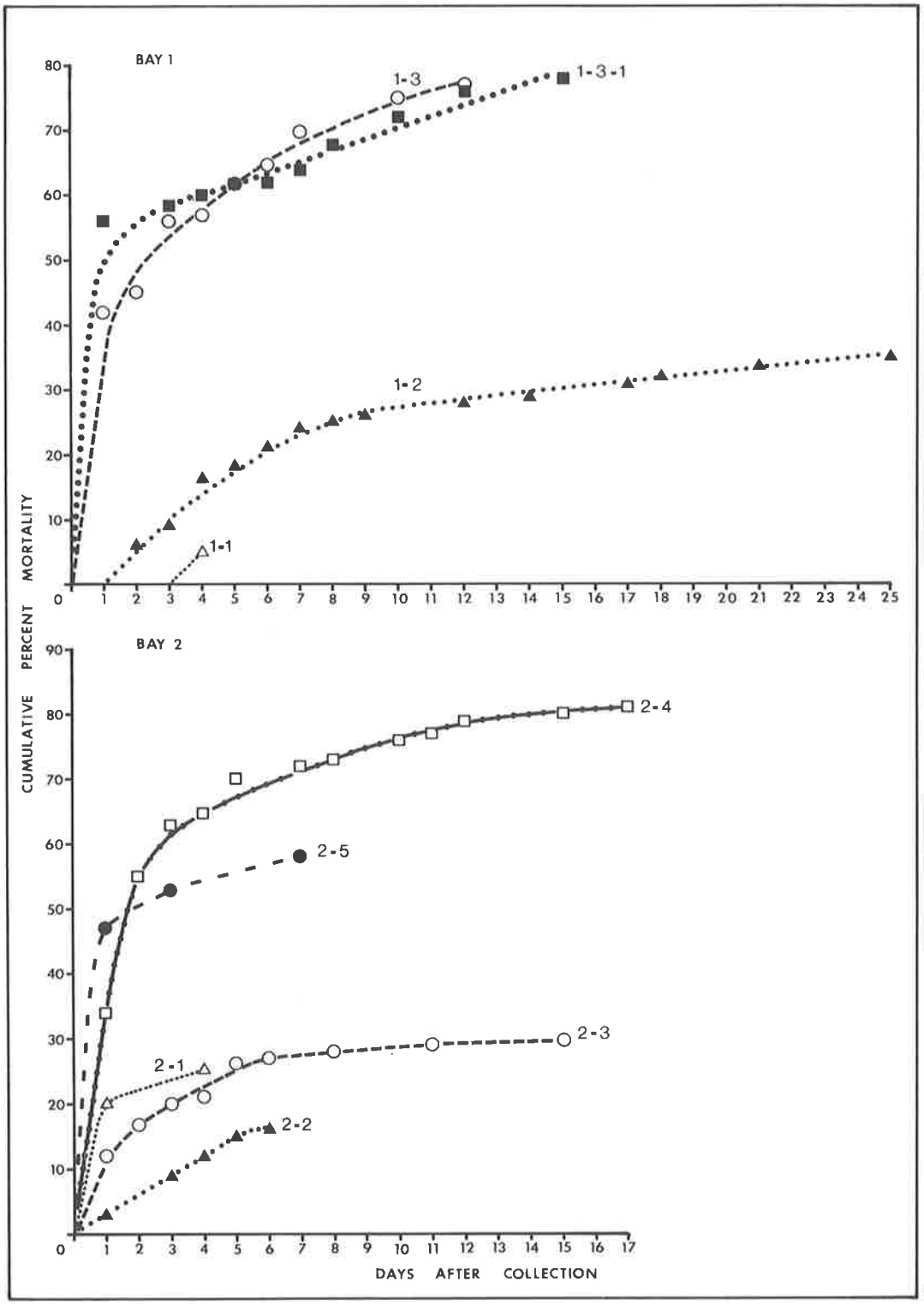
Of greatest interest with respect to larval mortality was the effect of NPV. The origin of epizootics has been the subject of investigation by many workers (e.g. Bird 1961, Jacques 1962, David and Gardiner 1965, Stairs 1966,

* Footnote:-

Asymptotic regressions were again fitted, where data were sufficient. However, this model was less appropriate than was the case for the data from the Pt. Gawler study (see Appendix 10 for discussion on goodness of fit).

FIGURE 3.13

Cumulative percentage mortality caused by NPV of sixth instar *H. punctigera* larvae collected from lucerne in bay 1 and bay 2 at Booborowie (see Table 3.10 for collection details).


BAY 1
1-3
1-3-1
1-2
1-1
BAY 2
2-4
2-5
2-3
2-1
2-2
CUMULATIVE PERCENT MORTALITY
DAYS AFTER COLLECTION

Doane 1975) but the factors involved in the initiation of such disease outbreaks remain unclear. For H. punctigera in South Australia, the collection of larvae at Collinsville and subsequent identification of pathogens represent an attempt to describe an epizootic and its net effect on the survival of larvae without a detailed knowledge of the agents causing it.

Although larvae were reared in a tube for these investigations and this may have constituted a source of stress, an attempt was made to minimise it by rearing larvae individually. In any case, the same technique was used for each sampling occasion and differences in mortality levels for each occasion could reasonably be attributed to differences in the level of NPV infection in the field.

The results of the study at Collinsville indicate that high larval mortality occurs at the peak of the epizootic, but only after the larvae have reached maximum abundance. Further, net larval mortality from NPV, using sweep-net data on larval abundance and disease levels, was calculated to be 39 per cent. for both bays at Collinsville. This figure is much lower than the level expected (Cullen 1969).

Detailed examinations of NPV epizootics of H. punctigera in South Australia were subsequently made by Cooper (1979). Although he recorded the level of NPV mortality for all larval instars combined, the trend in mortality with time and the timing of the peak mortality level are in general agreement with the findings of the present study. Also a level of 100% mortality (in April) was obtained only at the conclusion of one of the epizootics; for three others examined, peak levels were 61.4% (in December), 14.9% (in February) and 38.7% (in April). Since epizootics were recorded as occurring over about three weeks, the net mortality rate would be lower than these levels.

Conclusions

Variability of the more important mortality agents of H. punctigera larvae

was demonstrated as a result of the studies at M.E.S. and Booborowie. Results of a detailed study of the effect of NPV during an epizootic suggested that this disease is less important than previously considered.

3.4.3.4. Field surveys of lucerne crops from mid-summer to autumn 1976.

To substantiate some of the conclusions in sections 3.4.3.2 and 3.4.3.3 above, a survey of lucerne crops in the study area was undertaken during late January - late February 1976. Also, observations were made on the abundance of moths and larvae of H. punctigera in some of these crops and others later in the season.

The location of crops sampled during the survey in January - February is presented in Figure 3.1. Larvae and predators of H. punctigera were collected using the sweep-net (see Appendix 8 for sampling details). Larvae were aged, scored for parasitism and counted; predators were identified and counted. Each crop sampled was described by visual assessment in terms of a density index (on a scale of 1 to 5, with increasing density), crop height, maturity, whether it was irrigated and intended use. Lucerne crops N3, N6 and N8 had been sprayed at least four weeks prior to the time of sampling. Results of the sweep-net collections and crop descriptions are presented in Figure 3.14. The level of parasitism of larvae is given in Table 3.11.

Inconsistencies in insect abundance and the age structure of the larval population between crops in a given crop type are apparent in Figure 3.14 and may be explained by short-comings of using a visual assessment method in describing crop type.

Nevertheless, after taking into account the types of crops sampled in this survey, it was inferred that the abundance of larvae and predators*, and the

* Footnote:-

Two species of predator not recorded at M.E.S. but noted during the survey were Coranus sp. (Fam. Reduviidae) and Calosoma schayeri (Fam. Carabidae). They were found in low numbers and infrequently during the survey.

FIGURE 3.14

Results of a survey of lucerne crops in the area of study in mid-summer - descriptions of crops sampled and insects collected.

CROP DETAILS

location (refer Fig. 3.1)	M.E.S. 19·i·76	N 1	N 2	N 3	N 4	N 5	N 6	N 7	N 8	N 9	N 10	N 11	M.E.S. 26·i·76	S 1	S 2	S 3	S 4	S 5	S 6	S 7	S 8	S 9	S10	S 11	M.E.S. 3·ii·76
height (cm)	60	65	40	50	50	40	80	50	100	30	40	80	60	100	50	60	50	80	80	60	60	60	50	60	60
density (1 = sparse ; 5 = very dense)	4	4	3	3	4	3	5	4	3	2	2	5	4	5	4	4	1	3	4	5	5	2	3	2	4
flowering (P = peak ; 100 percent)	P	> P	P	> P	> P	> P	< P	> P	> P	> P	> P	P	> P	P	P	> P	P	> P	P	> P	> P	> P	P	> P	> P
stage of pod formation ①	V	V	V	M	V	E	V	E	E	V	E	V	V	V	V	E	V	M	V	M	E	E	V	E	E
crop type (S = seed ; F = fodder)	S	F	F	S	S	F	F	S	S	S	S	F	S	F	F	S	F	S	F	F	F	S	F	S	S
irrigation	–	+	+	–	–	–	+	–	–	–	–	+	–	+	+	+	–	+	+	+	+	–	–	–	–

① V = no pods visible ; E = early pod formation ; M = advanced stage of pod formation

INSECTS

Predators (mean no. / sampling unit)

N. kinbergii	3·3	0	0·6	2·3	0·3	1·8	0·3	0	0·2	0	0	0·2	2·3	1·8	0·2	2·6	0	1·5	1·5	2·4	3·2	1·7	5·0	0·5	1·3
C. repanda	0·2	0·2	2·3	0	0·2	0·3	0·3	0·3	0·5	1·0	0·8	0·2	0	0	0	4·0	0·2	1·0	0·3	1·8	1·2	3·7	0·5	0·7	1·7
O. schellenbergii	0·1	0	0	0·1	0	0·2	0	0	0	0	0·4	0	0·1	0	0	0·2	0	0	0	0	0	0·1	0	0	0·1
C. nasalis	0·2	0	0	0	0	0	0·1	0	0	0	0·1	0	0	0 2	0	0	0	0·2	0·2	0	0·4	0	0·6	0	0·3

H. punctiger larvae

mean no. / sampling unit	48·3	20·9	6·3	13·9	20·3	38·8	46·1	40·6	24·5	24·8	6·3	36·2	52·9	31·4	76·4	48·4	40·8	9·2	33·8	26·2	27·0	16·3	8·9	7·0	18·3
no. sampling units	10	10	7	10	10	6	10	4	10	5	10	5	10	5	5	5	5	10	6	5	5	10	10	6	10

frequency of instars :

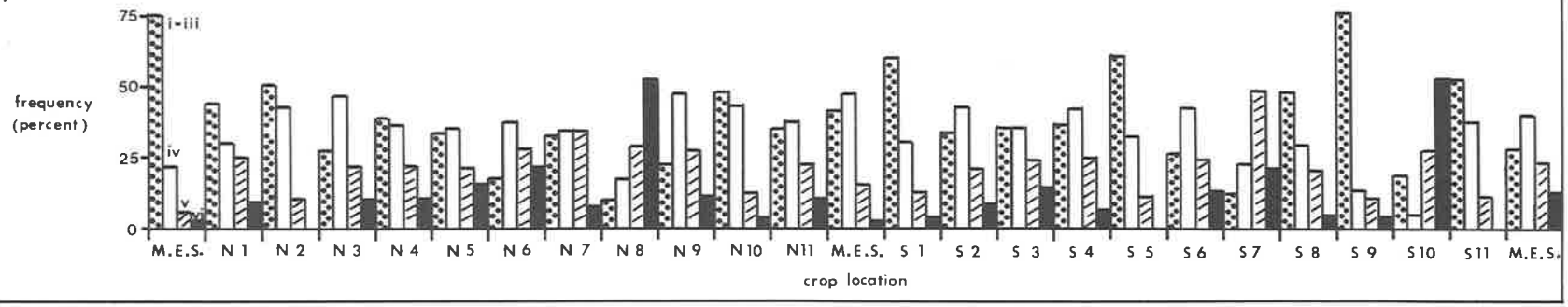


TABLE 3.11 Rate of parasitism of H. punctigera larvae collected during a survey of lucerne crops in mid-summer.

Crop	Instar and +Percent parasitism				
	1 - 3	4	5	6	All instars
M.E.S. 19.i.76	8.9	31.6	44.4	33.3	15.5
North 1	0	11.7	24.5	29.4	11.5
2	0	22.2	0	* -	9.1
3	8.1	20.6	10.7	9.1	14.4
4	10.5	16.7	12.5	20.0	13.8
5	0	0	0	8.6	1.3
6	0	7.3	10.4	9.2	7.4
7	2.2	3.9	0	0	1.9
8	5.0	7.9	16.7	33.1	22.4
9	14.8	36.4	29.0	18.2	28.2
10	0	23.1	14.3	0	11.1
11	3.3	4.6	10.0	25.0	7.2
M.E.S. 26.i.76	10.3	29.0	43.2	12.5	23.1
South 1	4.3	25.0	29.4	33.3	13.4
2	3.3	9.7	11.5	23.1	8.9
3	0	2.6	10.7	46.9	9.5
4	0	0	0	-	0
5	18.5	46.4	50.0	-	30.4
6	2.0	9.5	17.0	17.4	10.3
7	0	11.1	30.6	33.3	23.7
8	1.6	12.8	7.4	60.0	8.1
9	0	5.0	5.9	0	1.2
10	0	25.0	37.5	45.7	34.8
11	11.1	23.1	75.0	-	22.9
M.E.S. 3.ii.76	14.0	40.5	57.9	71.4	20.4
Av. M.E.S.	9.8	31.7	47.7	53.1	22.7
Av. North	3.5	11.9	11.9	20.0	11.0
Av. South	3.2	10.8	17.1	33.7	11.5
Av. N + S	3.3	11.4	14.2	24.2	11.2

+ Data on larval abundance is given in Fig. 3.14.

* - = no larvae of this instar present.

age distribution of larvae in the M.E.S. crop were not atypical of the situation in other crops in the study area.

The influence of crop maturity on age structure of larvae was apparent in a comparison between crops S.7 and S.8. Crop S.7. was in early pod formation and well in flower while crop S.8., 50m away, was more advanced with many pods and few flowers (Figure 3.14). The latter was infested by more mature larvae with few moths present compared with S.7 where moths were obvious.

The level of parasitism at M.E.S. was high, but not inconsistent with levels recorded in other individual crops (Table 3.11). The trend towards a greater level of parasitism with increasing larval maturity was demonstrated in most of the crops surveyed (see also Appendix 10).

In addition to these surveys in mid-summer, observations on the abundance of larvae were made later to corroborate the approximate time of the pre-winter decline noted at Booborowie (and related to the timing of diapause induction caused by shortening daylength (Cullen & Browning 1978)). In the South-east, larvae were easily found in flowering lucerne during April but by late May, none were found. At Pt. Gawler, larvae were present though declining in abundance from April to late May. By early June, no larvae were found, despite the presence of flowering lucerne.

These observations indicated general agreement with the situation at Booborowie (see Table 3.1 and Figure 3.11) and the light trap catches (Figure 3.4). Thus, an indication of the timing of diapause induction was obtained.

The above studies provided much information as to the nature and effects of the various mortality agents effecting H. punctigera larvae in the field. While most of these had been known to Cullen (1969), very little information was available on those agents important during the pupal stage. Investigations were undertaken to define these.

3.5. PUPAE

3.5.1. Introduction

Few reports were found in the literature on the mortality factors affecting pupae of Heliothis spp. Phillips and Barber (1929) discussed the effect of soil type and rainfall on the successful emergence of imagos from the soil. Barber and Dicke (1937) found that soil moisture at the time larvae burrow affects mortality and burrow destruction by rain becomes less important as the depth at which pupation takes place decreases. In the same study, it was suggested that soils with a high humus content provided conditions favourable to the development of the parasitic fungus, Sorosporella urella. It was necessary to take precautions against moles consuming pupae in a study on diapause by Ditman and Cory (1931), but there was no indication of the effect of this predator on a field population.

During the present study, attempts were made to obtain some information on the survival of the pupal stage of H. punctigera. Of greatest interest was the effect of mortality agents on diapausing pupae through winter. Soil sampling was conducted under lucerne crops near Brecon and on Collinsville (see Figure 3.1).

3.5.2. Brecon

An irrigated seed lucerne crop near Brecon was chosen. Lucerne was cultivated here in a series of bays each approximately 35m wide and 100m long with 0.5m high banks on three sides. The bays were flood-irrigated through a sluice gate in the bank on the short side, along which ran an irrigation channel. The soil was of a light sandy type which made searching for pupae relatively easy under all soil moisture conditions. The objective of the first visit to the farm (on 20.iv.75) was merely to verify the occurrence of pupae, but later a systematic sampling plan was adopted. Transects on each long side of selected bays were followed, and at every ten paces a sampling unit of soil was examined. A sampling unit consisted of

five consecutive spadefuls of soil, each 20 cm long, 14 cm wide and approximately 7 cm deep - pupae were usually found closer to the surface (see Figure 3.15). The number of healthy pupae, parasitised pupae and pupal exuvia were recorded for each sampling unit.

The sampling dates and abundance of pupae are given in Table 3.12. On the first sampling date, searching was confined to lucerne that had flowered late as a result of earlier damage by a vehicle used to apply insecticide . Moths were probably attracted to the flowers which would have been free of insecticide at the time. The recorded density of pupae there is atypical of the seed lucerne in general but perhaps representative of areas where insecticides are not used and where lucerne flowers later in the season. The density of pupae recorded on subsequent sampling dates would be more representative of seed lucerne crops. Because the time taken for exuvia to decompose in the soil is not known, the data on their abundance are of limited value.

On the first sampling occasion, approximately one third of the pupae collected were found to be infested with nematodes. These were subsequently determined as a new species of a primary nematode parasite Heterorhabditis bacteriophora (Poinar 1975) for which a new family, Heterorhabditidae, has been erected. Over all sampling dates, the nematode was found to have parasitised 32 per cent. of pupae (excluding the data on exuvia). While the work by Poinar (1975) demonstrates the nematode would probably infect H. punctigera larvae, it was not known whether the pupal stage was also capable of being infected. An attempt to confirm this by seeding one of the lucerne bays at Brecon with diapausing pupae was inconclusive. If a better understanding of the infection process were obtained, the distribution of the nematode in the field could be studied by seeding an area with the appropriate stage of H. punctigera and examining subsequent levels of infection.

FIGURE 3.15

Pupa of *H. punctigera* under seed lucerne

at Brecon (coin is approx. 28 mm in diameter).


20

TABLE 3.12 Abundance of pupae of H. punctigera under a seed lucerne crop near Brecon.

Sampling date	No. pupae collected				No. sampling units examined	Density of pupae (no./m^2)	
	Healthy	Parasitized		Exuvia		Healthy	Healthy and parasitized
		Nematode	Wasps				
20 - 21.iv.75	26	15	5	not rec.	+ 60	3.10	5.48
6 - 7. v.75	4	2	0	12	160	0.18	0.27
16 - 17.vi.75	6	6	0	3	80	0.54	1.07
6 - 7.vii.75	6	6	0	2	80	0.54	1.07
12-13.viii.75	6	0	0	0	90	0.48	0.48
27-28.viii.75	6	0	0	0	60	0.71	0.71
16 - 17.ix.75	3	0	0	0	86	0.25	0.25
						Av.*0.45	0.64

+ No. of sampling units not recorded for this date; estimate based on time of searching.

* Data from first sampling not included in average (see text).

The effect of wasps on pupal mortality was found to be small - 5.5 per cent. It was interesting to note that one of the species of parasites, Heteropelma scaposum (Morley) emerged from a pupa collected in diapause after an incubation period in the laboratory of 53 days at 26 to 28°C. Although parasites emerge from the pupal stage, it is arguable whether they should be considered as larval or pupal parasites (see Appendix 10 for discussion).

Beetle larvae (Fam. Elateridae) that were also found at Brecon and suspected of consuming pupae, were returned to the laboratory in an attempt to rear them. Although their predatory nature on H. punctigera pupae was confirmed, no adults were obtained and the beetle remains unidentified.

In the course of the study, Bacillus thuringiensis was identified from one of the dead pupae (D. Cooper, pers. comm.).

All pupae found under the seed lucerne at Brecon were in diapause (see Section 2.2.2). Therefore, a decline in the density of healthy pupae during winter would indicate the activity of mortality agents. Excluding the first sampling date where the abundance and mortality rate of pupae were biased, no such trend was apparent suggesting a very low mortality rate through winter.

3.5.3. Collinsville

The procedure for sampling pupae at Brecon was also used for this study. The same two bays from which larvae were sampled in late summer and autumn (see 3.4.3.3) were selected for sampling. Unlike soil at Brecon, that at Collinsville is an alluvial type with a high clay content which made both digging and searching more difficult.

A predacious beetle larva also occurred at Collinsville and its abundance was recorded. Identification of the larva is required to confirm whether it is the same species as that found at Brecon.

To indicate the distribution of the beetle larva, the results of the soil sampling are presented for each transect (see Table 3.13).

The variability in the occurrence of H. punctigera is well demonstrated. The fact that no healthy pupae were found on the earlier sampling date makes it impossible to draw any conclusions about the survival rate of pupae here.

3.5.4. Discussion and conclusions - pupal survival

Groups of biological agents causing pupal mortality of H. punctigera identified during this study were (i) parasites that attack H. punctigera in the larval stage and emerge during the pupal stage, (ii) a nematode which is apparently parasitic on the pupal stage only and (iii) a predacious beetle larva which is soil dwelling and hence predatory on both prepupae and pupae.

Without exception, species of wasp and fly parasites recorded from H. punctigera pupae were found to attack the insect in the larval stage. However, the level of parasitism of sixth instar larvae represents an over-estimate of pupal mortality because some of the parasites emerge prior to pupation (see Appendix 10).

The nematode parasite was more abundant in the light sandy soils near Brecon and a mortality level of approximately 32 per cent. was recorded on one farm. This relatively high level of parasitism and the fact that lucerne is a perennial crop, irrigated in some cases, and regularly infested by H. punctigera, suggest that the nematode has potential for use in an integrated control programme.

The effect of weather on survival of pupae was not examined. However, healthy pupae in diapause were found throughout the winter months at Brecon demonstrating the ability of the species to survive the low temperatures of the South Australian winter. The method of irrigating lucerne by flooding, as is the practice in the South-east, was considered a possible source of

TABLE 3.13 Abundance of H. punctigera pupae and a predaceous beetle larva under fodder lucerne at Collinsville.

Sampling date and location	No. pupae collected			Density of pupae (no./m^2)		Beetle larvae		No. sampling units examined in each transect
	Healthy	Parasitized	Exuvia	Healthy	Healthy and parasitized	No.	Density (no./m^2)	
3.vi.76								
Bay 1	0	6	1			2		5
	0	2	0			2		5
Total	0	8	1	0	5.71	4	2.86	10
Bay 2	0	0	4			1		5
	0	1	1			1		5
Total	0	1	5	0	0.71	2	1.43	10
12.viii.76								
Bay 1	1	3	1			2		10
	3	1	0			1		10
	0	0	0			0		10
	0	1	0			7		10
	1	2	0			1		10
	1	2	0			2		10
	0	0	0			1		10
	0	0	0			1		10
	0	0	0			0		10
Total	6	9	1	0.48	1.19	15	1.19	90

pupal mortality. Free water stands over the soil for no more than two days during irrigation. It was observed in the laboratory that this period of immersion caused no mortality in young pupae removed from their pupal cells. Undisturbed pupae in a field situation should be better protected. The effect of irrigation by flooding or overhead sprinklers on the structure of the emergence tunnel was not examined. In the sandy soils in the South-east this may not impede imagos from emerging but the situation for soil with a high clay content such as that at Collinsville may be different. The effect of two days immersion on fully-formed pharate adults in their pupal cells was not examined.

The low density of pupae found under lucerne and the resultant large variation in estimates of density suggest that a great deal of time needs to be spent in examining soil or a mechanical means of removing pupae from soil must be developed to obtain meaningful data.

While firm quantitative data on the effect of the above mortality agents on the survival of H. punctigera pupae have not been obtained, it was demonstrated that some pupae survived the winter in South Australia in a state of diapause.

This study on the mortality of the species within South Australia completes the examination of the two hypotheses proposed in the introductory chapter. It now remains for a consideration of the results in combination.

CHAPTER 4 GENERAL DISCUSSION

Neither of the investigations provided firm evidence to indicate the origin of spring flights of *H. punctigera* in South Australia, but the presence of diapausing pupae in the field during winter was confirmed.

The attempt to develop a model on the timing of spring flights to determine whether they were consistent with local weather conditions was hampered by assuming the diapause development-temperature relationship was a simple (single phase) one and by relying on laboratory data exclusively (although unsuccessful attempts were made with field cages and an outdoor screenhouse to obtain such data). From subsequent studies on *Heliothis* spp. (Holtzer *et al.* 1976, Wilson *et al.* 1979), it seems likely that diapause termination in *H. punctigera* consists of at least two stages, each with a different relationship between rate of development and temperature. Also, rather than leading to a marked synchronisation of moth emergence, the diapause probably results in a more protracted emergence (but possibly over a shorter interval than the duration of pupation). This was indicated here (section 2.2.2) and has been demonstrated by others (see above references and Kay 1982). In areas like South Australia with a mild winter (by Northern Hemisphere standards) the diapause of *H. punctigera* would appear to enable the species to remain inactive during this period of a shortage of food and less favourable temperatures. However, with the long growing season here, the diapause need not result in a highly synchronised emergence of moths in spring.

Therefore, the assessment as to whether the timing of flights is related to local conditions (mainly temperature) becomes more complex. It may not be appropriate to look at a period over which moths emerge, but rather a pattern of emergence. Such patterns have been described mathematically for *H. zea* (Logan *et al.* 1979) and *H. armigera* (Cunningham *et al.* 1981), and are yet to be demonstrated and defined for *H. punctigera* (though work is currently underway (Wilson 1983)).

Once this relationship is understood, it should be a relatively simple matter to incorporate post-diapause pupal development (see Section 2.2.3), so that a model on the timing and pattern of spring emergence is complete.

Use of field emergence cages to test predictive models will be mandatory. To assess such models over a range of habitats (i.e. different temperature regimes), less labour intensive methods such as traps for moths, will have to be considered.

The use of light traps to define the timing of spring emergence should be re-examined. A synthetic pheromone of H. punctigera has been developed and, after limited field experience, has proved successful in monitoring adults (Rothschild et al. 1982). Considering the reported greater efficiency of pheromone traps compared to light traps during spring for other species of Heliothis (Roach 1975, Hartstack and Witz 1981) and the advantages of catching the target species only, they may have an important role in defining spring flights of H. punctigera.

Detailed studies on the influence of certain aspects of weather on light trap catches (Persson 1976, Morton et al. 1981) indicate a need to correct such data. Such corrections would be likely to remove some of the artifacts of trends suggested by raw data alone. Even a cursory examination of the Turretfield trap catch data and air temperatures indicated a strong association e.g. sudden catches of large numbers of moths on nights when temperatures were markedly higher. Similar considerations would also be appropriate for the interpretation of pheromone trap catch data. The importance of trap location was discussed in section 3.2.1.

Much remains to be done to follow up the tentative negative conclusion regarding migration (section 2.4). H. punctigera is regarded as a mobile species (Wardhaugh et al. 1980, Morton et al. 1981) and evidence by other workers of its migratory ability was presented in section 2.4. Further studies on synoptic weather analysis in more detail e.g. examination of

weather and habitat conditions at proposed departure and arrival locations for specific flights, may result in a different conclusion concerning the area of study. Also, the absence of H. armigera from South Australia need not be an obstacle in considering potential source areas where this species occurs with H. punctigera. In such areas (e.g. eastern Australia) not only is H. armigera considered to be less mobile, but also moths are temporally absent in early spring (see above references and Wilson 1983).

As well as an examination of synoptic weather patterns and more direct methods such as radar and detailed analysis of moth flight behaviour, novel techniques associated with genetic markers may also be useful in studies on migration (Bartlett and Raulston 1982, Daly and Gregg 1985).

Field studies on the survival of the different life cycle stages of H. punctigera in the area of study demonstrated its ability to survive during summer. In particular, the mortality level of larvae caused by the disease NPV, was found to be considerably less than that suggested by Cullen (1969). A similar conclusion was reached by Cooper (1979) who further argued that NPV probably evolved to cause somewhat less than total mortality. It was also suggested by Cooper that, as the growing season progresses NPV epizootics would be less likely to occur in areas such as lucerne seed crops and extensive (i.e. dryland) forage lucerne crops where larvae would be present at lower densities.

It was therefore concluded that the apparent decline in abundance of H. punctigera, as was experienced at Mortlock during the present study, was related not so much to NPV epizootics, as to other factors such as the condition of lucerne (the main food plant at this time), and the effects of other larval mortality agents such as parasites and predators.

Considering the lower level of NPV-related mortality, it was no surprise to find pupae of H. punctigera during winter in the area of study, which were invariably in a state of diapause (though light trap data indicate a

simultaneous non-diapausing population). However, the present study failed to confirm the timing of diapause induction in the field as proposed by Cullen to corroborate, in an empirical way, the origin of such an overwintering population. Further, it was not demonstrated here that, what had become a very contracted distribution of a potential overwintering population, accounted for the widespread distribution of moths in the following spring. The latter shortcoming is less significant when the situation in spring is considered - moths that had spent the winter as diapausing pupae under lucerne would not find a ready supply of nectar nearby (unless the crop was heavily infested with weeds), necessitating dispersive flights (Hackett and Gatehouse 1982). Given the reputation of the genus for strong flight and the mobility of H. punctigera in particular, it is a simple matter to visualize the reinvasion of what have become favourable (and well-distributed) habitats. Traps could be used to monitor such dispersal of moths either by indicating changes in relative abundance (e.g. Hartstack and Witz 1981) or the direction of moth flights (in Southwood 1978).

An understanding of the timing and origin of spring flights is important in providing a basis for pest prediction studies and the broader holistic approach to pest control as discussed by Reed and Pawar (1982) and Knipling and Stadelbacher (1983). However, it is also relevant to pest management when considering the feasibility of applying control measures to a pest species when present on non-economic plants, in order to alleviate or remove the need for subsequent (and more expensive) control measures on crops (Mueller et al. 1984). This principle would not be applicable to H. punctigera in the area of study during spring, but, should an hypothesis of local survival accounting for spring flights be proved, would be appropriate during autumn when the distribution of the species is more restricted.

Obviously, much more information on the ecology of H. punctigera in the area of study would be required before an assessment of the feasibility of such

an approach was even contemplated. The timing of diapause induction and the distribution of food plants in autumn would need to be more accurately defined. Also, the proportion and fate of pupae that do not enter diapause in autumn is not known, but from studies here (section 2.2.4), some may emerge in early spring, perhaps augmenting the numbers of moths that spent the winter months as diapausing pupae. The ecological significance (size and emergence pattern) of this non-diapausing population would have to be determined.

While this study has asked more questions than it answered, it is hoped that it will be of some assistance to others who may also seek to unravel the mysteries of the ecology of H. punctigera.

APPENDICES

APPENDIX 1 REARING HELIOTHIS PUNCTIGERA IN THE LABORATORY

The method used in this study to hold, mate and collect fertilised eggs from moths of Heliothis punctigera was the same as that developed by Cullen (1969).

It was not found necessary to surface sterilise eggs. The strips of felt or paper towelling used as oviposition substrates in the mating canister were removed daily and held in glass petri dishes prior to hatching.

A fine camel-hair brush was used to transfer first instar larvae to Duranol® perspex vials (50 or 85 mm high, 36 mm diameter) with plastic screw or clip on caps (see Fig. A.1.1.a). About six larvae were placed in each vial with about 1 cm^3 plug of artificial diet (Shorey and Hale 1965). High survival rates of larvae were obtained with the diet but it was necessary to provide some circulation of air and the lids were therefore punctured using a mounted needle. Also, an early problem of excess moisture in the diet was overcome by ensuring the agar component was well mixed (by mixing the agar powder with water at room temperature and bringing this mixture to the boil) and using the minimum amount of water when blending the peas and dry ingredients. It was also important to allow newly-made diet to set and cool at room temperature, prior to storing in a refrigerator.

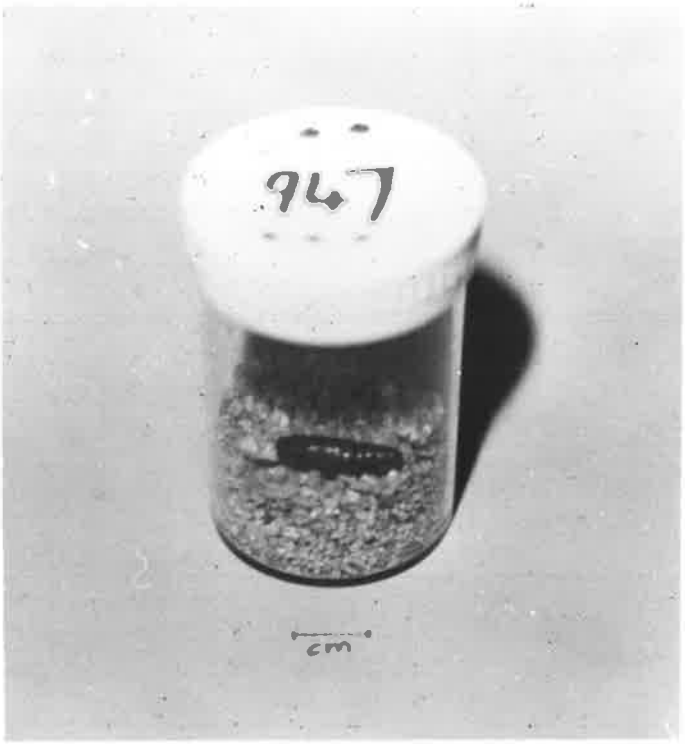
For convenience, rearing vials were held in perforated sheet metal trays (sheet metal - 20 gauge with 6 mm diameter holes at 8 mm centre giving an open area of 69%). Trays were held in shelving constructed using angle-iron (see Fig. A.1.1.b).

After reaching the third or fourth instar, larvae were reared separately to avoid cannibalism. At the sixth instar stage, about 1 cm of vermiculite was placed in each vial for larvae to burrow in and pupate. While larvae pupated readily in frass and unconsumed diet, there was usually considerable fungal growth in some vials by this time. Also, excess moisture in unserviced vials sometimes resulted in malformation of pupae. Use of

FIGURE A.1.1

a. Rearing vial and pupa (removed from its pupal cell).

b. Angle-iron shelving set up for darkened conditions and perforated sheet-metal trays used to hold rearing vials.


947
cm

vermiculite enabled the vials to be cleaned and kept dry, thus increasing the survival rate of pupae.

Burrowing larvae were placed in darkness (shelving was covered with cardboard and black plastic; see Fig. A.1.1.b). This procedure, to simulate the darkness of a pupation site was considered necessary to avoid any effect of light on pupal development. The cardboard and plastic did not cause any undue temperature variations in the constant temperature rooms where the experiments on diapause termination were conducted.

The depth of vermiculite in the vials was found to be such that larvae would include the base of the vial as part of the pupal cell wall. Therefore, it was possible to observe each larva and record date of pupation without disturbing it. In order to facilitate ageing of pupae (see Appendix 4), they were removed from their pupal cells after the cuticle had hardened and placed on top of fresh vermiculite in the rearing vial.

APPENDIX 2 PROCEDURE FOR RANDOM ALLOCATION OF PUPAE IN THE EXPERIMENTS ON DIAPAUSE TERMINATION

The history of moths used to produce pupae in the experiments on diapause termination (see section 2.2.2) varied from a laboratory strain reared under a non-diapause-inducing regime to field-collected diapausing pupae. While Cullen (1969) demonstrated a relatively minor effect of parental history on the proportion of their progeny that entered diapause under certain laboratory rearing regimes, the existence of different strains of insects within species that have a variable response to diapause-inducing conditions has been well documented (e.g. Rabb 1969, Benschoter 1970, Prevett 1971, Rabb et al. 1975, Herzog and Phillips 1976). Hence, for each pupae used in the present experiment, the history of each parent was recorded. It was then necessary to ensure that pupae from each of the different mating combinations were spread amongst the various treatments in equal proportions to counter any possible effect of parental history on the properties of the artificially induced diapause and therefore, the effect of the various treatments on the termination of diapause.

An additional unknown incorporated into the randomisation procedure, was the day of oviposition in relation to the complete oviposition period of the moth. Therefore, each pupa was classified according to the day of oviposition as well as the history of its parents.

For each mating combination of parents and oviposition time grouping, a random order of numbers was assigned, equivalent to the number of the treatments to be examined. As larvae in each group pupated, they were allocated to the treatments in the predetermined random order.

Since pupation times were recorded on a daily basis, pupae were also allocated to the treatments on a daily basis. The sequence of random order for each treatment was maintained for consecutive days i.e. without returning to the first treatment in the random order on each day, but continuing with the next number in the order for the first pupae to be allocated the next day.

APPENDIX 3 THE METHOD USED TO WOUND PUPAE

The reasons for wounding pupae have already been described (see section 2.2.2.).

Pupae were wounded as soon as practicable after pupation (Andrewartha et al. 1974) : as soon as the pupal cuticle had hardened.

It was necessary to anaesthetise the pupae prior to wounding to prevent excessive bleeding. Carbon dioxide was used for this purpose and it was found to successfully reduce the blood pressure to a very low level and little loss of blood occurred. The wound was made using what was called a micro scalpel - a fragment of razor embedded in small diameter glass tubing using a resin. The position of the wound was such that no vital organs would be damaged nor so deep that the alimentary system was penetrated, which in practice meant plunging the needle to a depth of approx. 1 to 2 mm beneath a small incision made laterally in the cuticle where the adult wing would later form. A low melting point wax was used to seal the wound.

Mortality rates of wounded pupae were the highest for any of the treatments, ranging from 10-23 percent (see Table 2.2). Nevertheless, sufficient pupae survived the surgery to enable assessment of its effect on diapause intensity (see Chapter 2).

APPENDIX 4 THE PUPAL STAGE AND DIAPAUSE OF HELIOTHIS PUNCTIGERA

i) The pupal stage

The duration of the pupal stage of insects in relation to morphological changes, has been discussed by Hinton (1976). While the pupal stage of H. punctigera also begins prior to the larval-pupal ecdysis (as a pharate pupa) and ends well before adult emergence (as a pharate adult) (Browning 1979), for this study, that part of the life cycle during which the insect is enclosed by pupal cuticle shall be referred to as the pupal stage i.e. from the larval-pupal ecdysis to the pupal-adult ecdysis.

Morphological changes which occur during the pupal stage of H. punctigera can be monitored through the transparent cuticle and a series of sub-stages had been defined by Cullen (1969). A redefinition of sub-stages was made for the purposes of this study and it was found that the duration between each sub-stage and adult emergence was reasonably constant for a given temperature (see Table A.4.1).

ii) Diapause

The occurrence of what is referred to as a pupal diapause in the life cycle of members of the genus Heliothis has been well documented (e.g. see Hardwick 1965). Other studies (Komarova 1959, Phillips and Newson 1966, Cullen 1969) have shown that the extended duration of the pupal stage during diapause occurs after the completion of the larval-pupal ecdysis and before the migration of the *stemmata (i.e. during stage A - see Fig. A.4.1).

It was further demonstrated by Cullen (1969) that once the stemmata migrated, diapause was completed and normal development resumes. This post-diapause pupal development procedes at a rate equivalent to that of

* Footnote:-

By describing the "eye-spots" of a stage A pupae as "stemmata" it should not be understood to infer they are functional in the pupa. It is possible they are merely non-functional vestiges of the larval stemmata.

TABLE A.4.1. Description of sub-stages within the pupal stage of H. punctigera and the duration between each sub-stage and adult emergence at three constant temperatures.

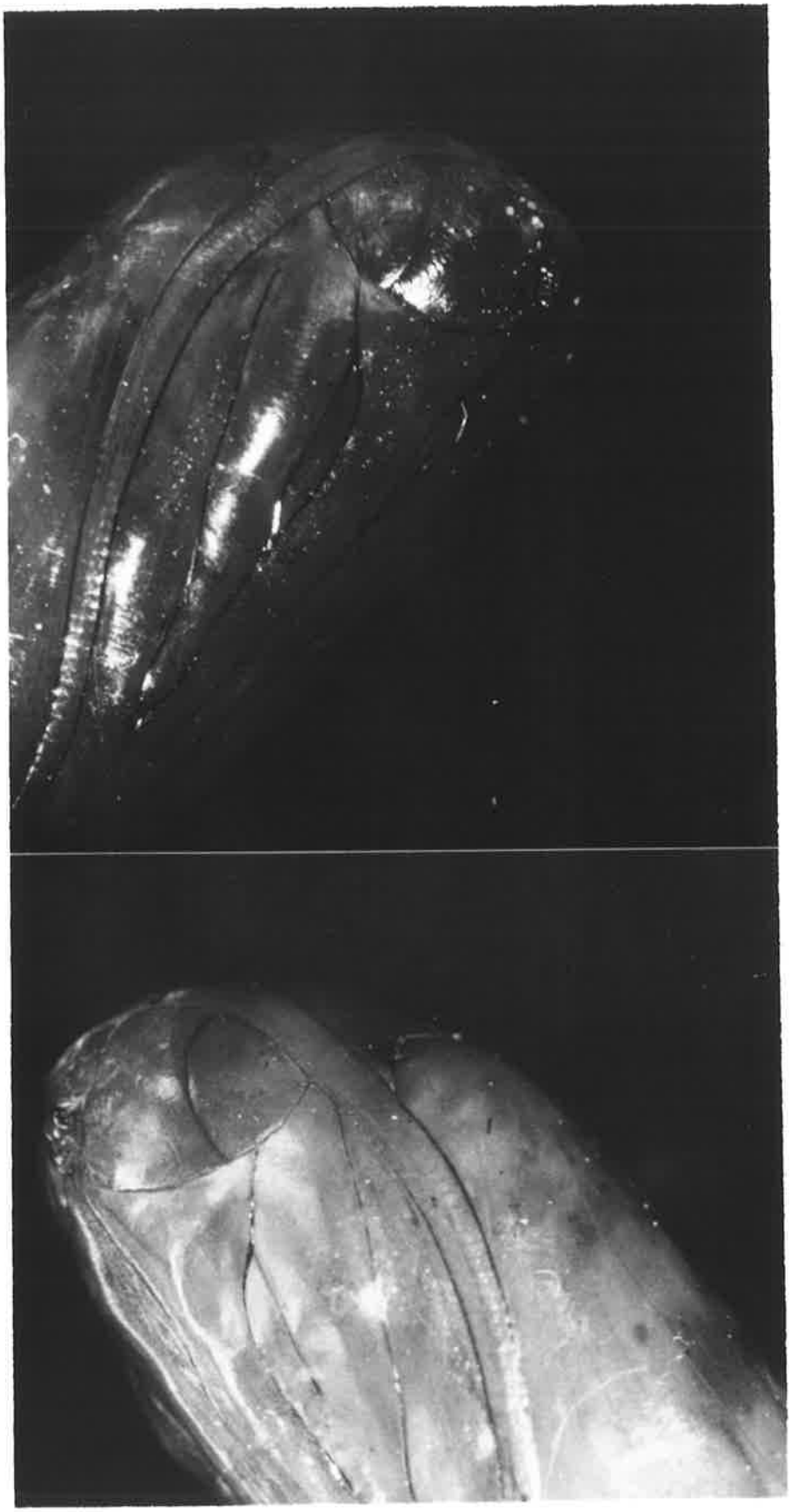
Sub-stage and Description	Temperature (°C) and duration from sub-stage to adult emergence (days).		
	19	25	29
B. Migration of stemmata to edge of "adult eye"; stemmata remain circular.	28	15	10
C. Stemmata lengthen to slits at the margin of what becomes the adult eye; centre two stemmata coalesce and all stemmata migrate anteriorally somewhat and fade; margins of adult eye just discernable by the end of the stage.	25	13	9
D. Stemmata no longer visible; pupa darkens slightly, adult eye obvious (chocolate brown colour)	20	11	7
E. Adult eye continues to darken and precursors of ommatidia apparent as rows of circles; outline of appendages becomes discernable; scale development begins, most noticaebly on the appendages so that "trachae" in the legs are no longer visible; scales on wings appear as a sheen but development not complete and "trachae" still obvious.	16	9	6
F - early. Hexagonal outline of ommatidia distinct; outline of appendages and scales on legs much more distinct; scales on wings well-developed and obscure the "trachae"; spines on legs are now obvious with a pearly lustre; scale development on abdomen just noticeable.	13	7	5
F - mid. Ommatidia very distinct and dark; scales are well-formed and becoming darker; segments of antennae are discernable; spines on legs are distinct and darkening.	6	5	3
F - late. Pupa very dark; appendages and scales appear fully formed with final colouring.	3	3	2
Pre-emergence. Pupal cuticle very soft; haustellum sometimes vibrated; fully formed adult.	2	2	1

FIGURE A.4.1.

Stemmata of H. punctigera pupae.

1. Stage A - stemmata circular and are anterior to the lateral margin (suture on pupal cuticle) at the base of the maxilla.

2. Stage B - stemmata circular and begin to migrate towards the post-genal region; migration has commenced when stemmata are in a straight line continuous with the lateral margin (suture) at the base of the maxilla.



non-diapausing pupae and the duration for the various sub-stages already mentioned was approximately the same i.e. at a given temperature, pupae that had been in diapause but had subsequently resumed normal development could be aged. In this way it was possible to reduce the number of observations required to determine the timing of diapause termination of individual pupae in the relevant experiments conducted during the present study.

In developing the model on the duration of post-diapause pupal development in field situations (see section 2.2.3), it was necessary to define the amount of normal development that must be completed prior to moth emergence.

As stated above, once the pupal stemmata migrate, normal development resumes and the interval between this event and moth emergence occupies approximately 86% of the pupal period of both non-diapausing and diapausing pupae that have resumed normal development (see Table A.4.2). Of the remaining 14% of normal pupal development (i.e. stage A of non-diapausing pupae), it is not known what proportion (if any) needs to be completed within stage A after diapause termination and before migration of the stemmata.

TABLE A.4.2. The duration of the pupal stage from migration of the stemmata to emergence as a proportion of the total pupal period at 19 and 25°C.

Temp. (°C)	Duration of stages (hrs)						Proportion of pupal period from stemmatal migration to emergence (%)		
	Stemmata migration to emergence			Pupation to emergence					
	male	female	both sexes	male	female	both sexes	male	female	both sexes
19	634	588	611	753	687	720	84.2	85.6	84.9
25	390	350	370	446	401	424	87.4	87.3	87.3
									Av. 86.1

If the rate of normal stage A development (in post-diapause pupae) were different from that for diapause development, the solution to this problem concerning the timing of diapause termination would be in hand. An attempt to measure these rates was made by Cullen who weighed pupae that were in diapause or developing normally, but he found that the rate of weight loss during stage A (and therefore rate of development) was similar for the two groups. Further, the rate of weight loss in a pupa that had completed diapause was the same for the entire duration of Stage A. Therefore, using this technique it was not possible to determine the exact time of diapause termination. Nevertheless, use of more rigorous techniques to quantify these development rates might provide definitive information e.g. measurement of oxygen consumption (Phillips and Newsom 1966).

In the absence of conclusive data, it is assumed here that after diapause had been completed, development associated with half the normal stage A period has yet to be completed prior to the migration of the stemmata, and that the rate of this development is equivalent to that of normal non-diapausing pupae (the first half of stage A development would then have been completed either during or before diapause - see Figure A.4.2 (ii)).

Therefore, after diapause is complete, pupae must complete a period of normal development which begins with half the amount of normal stage A development to complete prior to the migration of the stemmata. In quantitative terms the percentage of normal development left to complete is:

$$\left[\text{(proportion of normal development from the migration of the stemmata to adult emergence, } D_s) + \frac{(100 - D_s)}{2} \right] \%$$

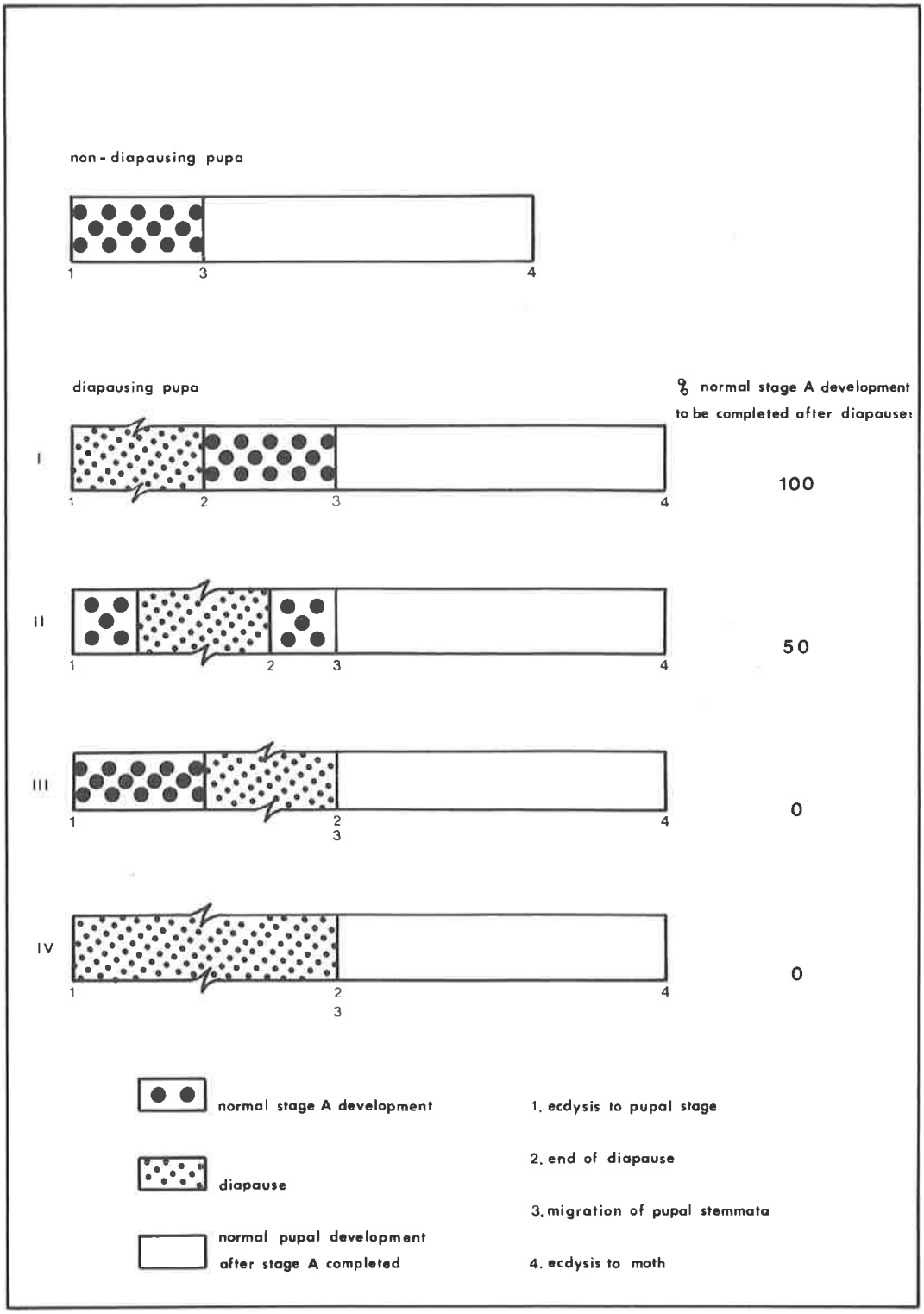
$$= \left[86 + \frac{100 - 86}{2} \right] \%$$

$$= 93\%$$

The way in which this value is used to determine the timing of moth emergence in the field is described in section 2.2.3.

FIGURE A.4.2

Diagrammatic representations of the duration of non-diapause and diapause pupal stages of H. punctigera to indicate possible variations in the physiological (or morphological) age of pupae at which diapause occurs.


non-diapausing pupa
1
3
4
diapausing pupa
% normal stage A development to be completed after diapause:
I
1
2
3
4
100
II
1
2
3
4
50
III
1
2
3
4
0
IV
1
2
3
4
0
normal stage A development
diapause
normal pupal development after stage A completed
1. ecdysis to pupal stage
2. end of diapause
3. migration of pupal stemmata
4. ecdysis to moth

Use of the above value is associated with an error no greater than 7%, since it is possible that all or none of normal stage A development is completed prior to diapause termination (see Fig. A.4.2 (i), iii). (Naturally it would provide a useful marker if the former were true, as diapause termination would coincide exactly with the migration of the stemmata). Another possibility is that development associated with stage A is entirely completed during diapause (see Fig. A.4.2. (iv)).

APPENDIX 5 DATA FROM THE EXPERIMENTS ON DIAPAUSE TERMINATION

The durations of the pupal stage spent in diapause for the experiments described in section 2.2.2 are listed in the tables below. The tables are in a two-way format for ease of data presentation - the units value of the duration is read across the top of each table while the tens component is read down the left-hand side. The durations are time in days spent at the final rearing temperature prior to diapause termination (see App. 4 for definition of this). The numbers represent the frequency of pupae completing diapause in that time. At the conclusion of the experiments, some pupae were still in diapause (refer Table 2.3).

Experiment 1 Treatment 1

	0	1	2	3	4	5	6	7	8	9	Total every ten days
0							1				1
1		2		2	2				1		7
2	1					1		1		1	4
3				1		1	1				3
4			4			2		1		1	8
5	1		1		1	6	1	4			14
6	1		2	1	2		3	2	1	1	13
7	2	3	3	3	2	3	2	1		1	20
8	1	2					1	2	1	3	10
9	1		2		2	1	1	1	1	1	10
10	2		1	2	2	2					9
11		2				2		1	1	2	8
12		5		1	3	4	2			1	16
13			1						1	1	3
14			1		2						3
15				1				1			2
17	1						1				2
18	1			1		2					4
19							1			1	2
20					1						1
22					1						1

Experiment 1 Treatment 2

	0	1	2	3	4	5	6	7	8	9	Total every ten days
4								2			2
5									1		1
6			2				1	1		1	5
7		1				2				1	4
8		3	3	1	1	2			2		12
9			2	1		3	1		1		8
10		1	2					2			5
11	1										1
12				1							1
15	1								1		2
22									1		1
23		1		2	2		1	2	1	2	11
24	2	1	1			1	3	4			12
25		2	1		3	1				2	9
26			1		1	1	1			2	6
27	1	1		1		1			1		5
28	1	2						2	2		7
29	1			1	1					1	4
30					1				1	1	3
31	1			1		2	1	2		1	8
32				1	1		1				3
33			1	1							2
34		1	1	1							3
35						1				1	2
37	1										1
39						1					1
45	1										1
55	1										1
58	1										1

Experiment 1 *Treatment 3-2

	0	1	2	3	4	5	6	7	8	9	Total every ten days
1			1		2	2	2	1	2		10
2	2					1		1			4
3	1			1			1				3
4	4		2		1						7
6						1					1

Treatment 3-3

0			1			2	1	2		2	8
1	4			2		1	2	1		1	11
2		1	3	1			1	1		2	9
3	1										1

Treatment 3-4

0			3	4	1	7	2	1	4	5	27
1			1			1					2

* For treatment 3-2, six pupae were still in diapause at conclusion of experiment; see Table 2.2

Experiment 1 Treatment 4

	0	1	2	3	4	5	6	7	8	9	Total every ten days
0				1	1	8	3	2	3	1	19
1	2	2	1	2	1	2	3	2	4	1	20
2	3	3	2	4	2	1	2	1	2	2	22
3	2	2	1	4	3	2	2	3	2	3	24
4		2	1		1	2	2	1	1	2	12
5		2			1	2	1				6
6								1	1	1	3
7		1	3		2	2		1			9
8				1		1	1			1	4
9		2		1			1	1			5
10			1		1						2
11		1		1				1			3
12			1	1	1		1	1		1	6
13			1		1						2
14	1										1
16								1			1

Experiment 1 Treatment 5

	0	1	2	3	4	5	6	7	8	9	Total every ten days
0		5	1	6	3	5	3	7	4	5	39
1	5	3	6	2	1	1	2	3	2	2	27
2	3	1	1	4	1			2		2	14
3						1	3	1			5
4	1	1		1	1			1	1	1	7
5					1		2	1	1		5
6	1	2			2	1				1	7
7	1		1		2	1					5
8		1				1		2			4
9					1	1	1				3
10	1						2				3
11		1							1		2
12	1										1
14			1								1

Experiment 1 Treatment 6

	0	1	2	3	4	5	6	7	8	9	Total every ten days
0		1		1	5	4	3	4	6	2	26
1	3	3	3	3	1	3	3	1	1	2	23
2	3	3			2	1	1	2	2	1	15
3	1	2			1		1	2			7
4	1		3		1	1	1	1			8
5	2	2	2	2			1	1	3		13
6		1	1		4	1			1		8
7	2	1		1				2	1	1	8
8	1	1	1		1			1		1	6
9			1			2	1		1	1	6
10			1	2	1						4

Experiment 1 Treatment 7

	0	1	2	3	4	5	6	7	8	9	Total every ten days
0	1			5	2	2	3	7	2		22
1	3	2	1	2	1		1	5	2	2	19
2		3	2	4		3	1		1	1	15
3	5	2	3		2	2	2		3	1	20
4	3	3	2	1	1	2	2	4	1	1	20
5	2	1			2			2	3		10
6	1	2		1							4
7	2	1	1			1					5
8	1			1			1				3
9	2				2						4
10		2			1	1			1		5
12										1	1
13		1	2					1			4
14			1								1
15						1					1
16				1							1
17		2	1								3
20							1				1
21								1			1

Experiment 1 Treatment 8

	0	1	2	3	4	5	6	7	8	9	Total every ten days
0	8	9	8	5	7	5	4	3	6	2	57
1	7	3	3	2	3	2	5	2	2	2	31
2	4	3	4				1	2	1		15
3	2	1		2		1			1	1	8
4	1	3				1				2	7
5					1						1
6				1							1
8				1							1
9	1							1			2
11			2								2
15			1								1

Experiment 1 Treatment 9

	0	1	2	3	4	5	6	7	8	9	Total every ten days
0	2		2	2	2	2	4	1	1	1	17
1	3	3	1	1	3	4	1		2	1	19
2	1	1	2		1			1	1		7
3			1			1	2	3			7
4			3			1		1	1		6
5		1	1	1		2			2		7
6	1	1		1			1	1	1	1	7
7	1	3	1	3	3	1		1		2	15
8	5		3		6	1	2		2	1	20
9		3	3	3			1	1	1	1	13
10	1				1	1			2		5
11		1	2			1					4

Experiment 1 Treatment 10

	0	1	2	3	4	5	6	7	8	9	Total every ten days
0	1		1	2	6	6	1	5	4	6	32
1	3	7	4	3	4	3	4	3	2	3	36
2		3	2	2	1			1			9
3		1			1	2					4
4	1	1	1	1		1			1		6
5		1		1		3	2		1		8
6		3	1	2	1	1	1				9
7	1	1		1	1	2			1		7
8	1		1	1					3		6
9						1		1			2
10				1			1				2

Experiment 2 Treatment 11

	0	1	2	3	4	5	6	7	8	9	Total every ten days
0										1	1
1			1			1	1	1	1		5
2	1							1	2		4
3						1					1
4		1	2		1						4
5	1				1		1				3
6	1										1
7		1			1	1		1		2	6
8		1									1
9								1	1		2
10			1	1							2
11								1			1
12	1			1	1	1			1	1	6
13						1					1
15		1		1	1	1			1		5
18						1					1
20				1							1

Experiment 2 Treatment 12

	0	1	2	3	4	5	6	7	8	9	Total every ten days
0				2	1						3
1			1				1	1		1	4
2	1	1	1				1	1			5
3			1				2		1	1	5
4		1		2	1			1	2	5	12
5	1		1	1	2		1		2		8
6				1			1			1	3
7		1				1					2
9					1		1	1	1	1	5
10		1		1		1	1			1	5
11					1						1
12						1	1		1		3
13		1		1			1			1	4
14	1	2		1	1			1			6
15			1	1	1		1		1		5
17							1				1
19		1								1	2
20			1								1

Experiment 2 Treatment 13

	0	1	2	3	4	5	6	7	8	9	Total every ten days
0							1		1	1	3
1	2	1	1	2	1		2	1			10
2	1					1					2
3	1							2			3
4			1	1	2	1					5
5		1	1		4	1					7
6		1	2	4	1	1					9
7	1		1	2		1		1		1	7
8										1	1
9								2			2
10					1	2		1			4
11	1										1
12								1			1
13						1	1				2
14						1					1
16					1						1
17						2					2

Experiment 2 Treatment 14

	0	1	2	3	4	5	6	7	8	9	Total every ten days
0							1				1
1	2				1			3			6
2										1	1
3							2			1	3
4		1	1	1			1	2			6
5				1	1						2
6			1	1			1		1		4
7				1						1	2
8	1			1					1		3
9	1	1									2
10					1			1	1		3
11						1					1
12								1			1
13	1										1
15			1						1		2
17									1		1
18					1						1
20									1		1

Experiment 2 Treatment 15

	0	1	2	3	4	5	6	7	8	9	Total every ten days
0	3	1	3	5	5	8	4	7	2	2	40
1	4	3	1	1	2	1	2		1	1	16
2		1	1	2		1			3	1	9
3	1			1		1		1			4
5									1		1
8	1		1								2

Experiment 3 Treatment 16

	0	1	2	3	4	5	6	7	8	9	Total every ten days
1		1	2		2			1		2	8
2	3	2	3	1	1		1		1		12
3		1			2				2		5
4	1	1	1			1		1		1	6
5	3	1	1	1		1		1			8
6	1	1		1		1					4
7		1	1				1				3
11			1				1				2
13								1			1
14			1								1

Experiment 3 Treatment 17

	0	1	2	3	4	5	6	7	8	9	Total every ten days
0										1	1
1				1	2				2		5
2		1			1	2		3			7
3			2			1	1		1		5
4						1	2	2	1	1	7
5		1		3		2			1		7
6	3	1	1		1						6
7	1			1	1						3
8							1			1	2
9					1			1			1
10			1	1	1					1	4
11								1			1
12									1		1
13							1				1
14			1					1			2
15			1					1			2

Experiment 3 Treatment 18

	0	1	2	3	4	5	6	7	8	9	Total every ten days
0					2	2	2	4	1	3	14
1	4	6	2	3	1	2	1				19
2			1		1					1	3
3	1	1	1	2	1	1					7
4		1			1		1	1		1	5
5				1	1	3		1	2		8
6		1			1		1	1	1	1	6
7	1								1		2
8		1			1						2

Experiment 3 Treatment 19

	0	1	2	3	4	5	6	7	8	9	Total every ten days
0		7	1	2	2	4	2	2		3	23
1	6	1	4	1	2	1	2	2	1		20
2	1	1					1	1		2	6
3			1					1			2
4		1								2	3
5	1			1							2
6										1	1
7	1										1

APPENDIX 6 OTHER FACTORS AFFECTING THE DURATION OF THE PUPAL DIAPAUSE OF HELIOTHIS PUNCTIGERA

An analyis was carried out to determine whether there was any effect of the factors parental history, day of oviposition within the cycle of the parent or sex of the pupa on diapause intensity for pupae in treatments 1, 4, 5, 6, 7, 8 of the experiment discussed on Chapter 2.

The diapause history of each parent was recorded. Six parental crosses were made in this experiment but they represent only four different combinations of origins (see Table A.6.1).

TABLE A.6.1 Origins of moths used in the experiments on diapause termination.

Crossing no.	Origin no.	Sex and Origin of parent	
		male	female
1	1	lab.	lab.
2	2	lab.	field
3	2	lab.	field
4	3	field	lab.
5	3	field	lab.
6	4	field	field

Origins of adults were:-

(a) Lab. strain from culture reared under diapause averting conditions.

(b) Field strain collected as diapausing pupae (see section 2.2.1., Fig. 2.1).

The oviposition cycle was divided into seven time intervals - the first six days of oviposition were considered as separate categories with the seventh category including all eggs laid thereafter.

The mean duration of diapause and the number of observations for each of the four parent-cross categories/day of oviposition/sex of pupa combination for the above treatments are given in Table A.6.2. Part 1. The analysis of variance of the data was carried out by means of regression for each of these factors (i.e. origins of parents, day in the oviposition cycle, and sex) and for interactions among factors. Initially a maximal model of all factors was first fitted and non-significant terms were dropped out for successive analyses.

For all treatments, origins of parents and day in the oviposition cycle were non-significant for any effect on diapause intensity. The factor sex was significant at the 5% level in treatments 1, 4 and 7 only (see Table A.6.2. Part 2). All interaction effects among the factors were non-significant.

TABLE A.6.2. The mean duration of diapause and number of pupae for various parent origins/day of oviposition/sex of pupae of selected treatments in the experiment on diapause termination.

PART 1

Origin of parents.	Day of oviposition Interval	Treatment no. and sex of pupae. Mean duration of pupal diapause in days (no. pupae)											
		1		4		5		6		7		8	
		male	female	male	female	male	female	male	female	male	female	male	female
1	1	-	-	-	-	-	-	-	-	-	-	8(1)	-
	2	109(2)	114(3)	48(2)	54(2)	16(3)	10(2)	-	51(4)	40(5)	43(3)	12(2)	9(3)
	3	-	96(1)	30(1)	-	10(1)	-	26(1)	-	11(1)	132(1)	22(1)	-
	4	-	59(2)	11(1)	63(2)	-	5(1)	-	5(2)	-	91(2)	10(3)	-
	5	199(1)	-	14(1)	-	41(2)	-	37(2)	8(1)	-	25(1)	18(1)	-
	6	78(2)	45(1)	20(2)	6(1)	-	1(1)	16(2)	-	-	64(3)	21(1)	14(1)
	7	-	-	31(1)	-	-	-	-	-	-	-	-	-
2	1	73(5)	99(11)	43(12)	41(4)	22(7)	43(4)	34(5)	16(5)	25(9)	92(6)	11(7)	2(7)
	2	72(8)	126(9)	31(10)	67(6)	35(6)	24(8)	19(9)	52(6)	41(9)	37(8)	22(3)	23(10)
	3	80(7)	100(7)	49(11)	22(3)	37(5)	50(7)	42(6)	25(7)	32(7)	90(7)	15(7)	34(7)
	4	83(5)	75(2)	44(3)	64(4)	29(4)	52(2)	56(2)	61(4)	46(4)	101(3)	27(3)	4(1)
	5	11(1)	-	36(2)	-	23(1)	6(2)	-	71(1)	-	17(1)	14(2)	-
	6	42(1)	73(1)	-	5(1)	-	-	-	6(1)	-	-	-	2(1)
	7	-	-	-	-	-	-	-	-	-	-	-	-
3	1	89(4)	82(3)	71(5)	42(1)	40(4)	30(3)	44(1)	53(5)	34(3)	54(4)	20(4)	62(3)
	2	93(9)	94(7)	25(9)	55(5)	23(9)	44(5)	31(5)	38(8)	48(9)	39(8)	13(4)	25(11)
	3	79(2)	-	140(1)	8(1)	14(1)	95(1)	21(1)	13(1)	23(1)	17(1)	21(1)	-
	4	85(2)	42(1)	-	6(1)	8(1)	62(1)	-	64(1)	31(2)	22(3)	17(2)	13(1)
	5	-	158(1)	-	147(2)	7(1)	62(2)	-	29(2)	101(1)	-	13(1)	1(1)
	6	-	67(2)	32(1)	-	-	111(1)	-	45(1)	3(1)	42(1)	-	2(1)
	7	74(4)	72(4)	34(7)	83(2)	18(5)	53(2)	42(3)	40(6)	48(3)	39(4)	4(1)	8(4)
4	1	57(2)	121(1)	18(3)	68(1)	45(3)	-	43(2)	31(2)	30(1)	30(2)	3(1)	8(2)
	2	71(5)	85(4)	27(8)	91(1)	15(2)	20(6)	35(5)	48(3)	27(3)	42(5)	18(3)	20(5)
	3	-	88(6)	43(4)	59(3)	15(3)	64(3)	43(3)	53(3)	36(4)	15(2)	14(3)	17(4)
	4	72(2)	125(7)	48(7)	66(2)	74(3)	21(6)	51(5)	53(5)	41(2)	50(7)	27(6)	19(3)
	5	68(3)	55(2)	-	47(4)	-	37(4)	45(4)	-	56(2)	50(2)	22(1)	24(2)
	6	-	-	-	-	-	-	-	-	-	-	-	-

TABLE A.6.2. Cont'd.

PART 2. Means of sub-totals of data in PART 1.

		Treatment and mean duration of diapause (days).					
		1	4	5	6	7	8
(a) origin of parents	1	98	35	16	29	54	12
	2	90	44	32	35	52	18
	3	86	52	36	39	41	21
	4	87	43	33	45	39	19
(b) interval in oviposition cycle	1	88	46	34	35	47	15
	2	95	40	26	37	41	20
	3	89	47	42	35	51	22
	4	91	50	32	50	54	20
	5	85	63	33	39	51	16
	6	64	16	56	21	47	10
	7	73	47	28	41	43	7
(c) [1]sex of pupa	male	80a	40a	27	36	38a	17
	female	97b	56b	37	40	54b	20

[1]Means followed by different letters were significantly different at the 5% level.

APPENDIX 7 LIGHT TRAP DESIGN AND TRAP CATCH DATA FROM THE SOUTH AUSTRALIAN DEPARTMENT OF AGRICULTURE

Light Trap Design

The type of light trap used in this study at both the Waite Institute and at Mortlock Experiment Station is shown in Fig. A.7.1. The light source was a 240 volt 125 watt mercury vapour globe. This was mounted over a metal funnel of 35 cm diameter, the inner surface of which was painted white and the outer surface, black. Attached to the bottom of the metal funnel was a collecting bottle with a dichlorvos-impregnated strip used as the killing agent - the strip was replaced every 3-4 weeks. The top of the metal funnel was 70 cm above ground level. The light was connected to a time clock - switching the light on at sunset and off at sunrise.

Trap catch data from the South Australian Department of Agriculture

A light trap, of similar design as that described above, was run by the South Australian Department of Agriculture for the years 1960 to 1976. The data was kindly made available by P. Birks and is reproduced here (Table A.7.1).

Peaks in catches of moths that were used to define the dates of flights of moths used in Chapter 2 for the studies on timing of diapause termination in the field and migration are indicated on the data in Table A.7.1.

FIGURE A.7.1.

The type of light trap used at Waite Institute and Mortlock Experiment Station.

TABLE A.7.1.

Nightly catches of moths of *H. punctigera* at Turretfield, S. Australia, for the period 1960 - 1976. Data collected by the South Australian Department of Agriculture. Months excluded for any year were times when the light trap was not operated. Peak catches of moths used to indicate time of arrival of assumed migrations (1972, 1973, 1974) or emergence of local moths during spring are marked with two asterisks and either one or two asterisks respectively.

1960

DATE	SEP	OCT	NOV	DEC
1	0	11	2	6
2	0	37	2	22
3	0	27	1	6
4	0	3	15	2
5	0	10	2	5
6	*21	10	12	9
7	0	19	*540	22
8	0	3	28	8
9	1	7	1	0
10	5	1	340	10
11	4	8	46	4
12	1	3	5	1
13	2	38	4	7
14	15	54	7	14
15	12	9	115	11
16	1	*254	170	25
17	1	6	21	26
18	0	5	2	112
19	0	6	40	153
20	8	44	37	150
21	7	132	13	150
22	6	*127	27	48
23	*28	150	29	240
24	0	50	18	60
25	0	*150	21	100
26	15	130	4	120
27	15	21	7	15
28	*45	*150	0	80
29	19	4	3	70
30	1	4	8	-
31		3		17
TOTAL	207	1476	1520	1493

1961

DATE	JAN	FEB	AUG	SEP	OCT	NOV	DEC
1	13	6	-	0	0	6	7
2	1	40	-	0	0	20	15
3	32	41	-	-	0	*90	0
4	26	40	-	0	4	4	7
5	28	20	-	0	1	0	0
6	73	23	-	0	11	28	1
7	19	7	-	0	*15	18	4
8	19	-	-	4	-	24	5
9	72	-	-	1	-	12	3
10	39	-	-	2	3	1	3
11	9	-	-	1	1	-	1
12	7	-	-	3	*25	-	4
13	40	-	-	2	15	-	10
14	27	-	-	1	4	-	24
15	34	-	-	0	0	-	10
16	153	-	-	0	6	-	-
17	157	-	-	2	10	-	-
18	117	-	-	1	0	-	20
19	273	-	-	1	*15	-	1
20	150	-	-	1	0	-	6
21	150	-	-	0	2	-	9
22	50	-	-	0	0	-	-
23	-	-	-	0	0	-	10
24	530	-	-	1	1	-	15
25	120	-	-	0	2	-	15
26	500	-	-	0	3	8	0
27	160	-	-	3	3	8	0
28	110	-	1	7	2	47	2
29	56		2	*10	10	32	3
30	-		4	3	0	7	7
31	37		*11		6		1
TOTAL	3002	(177)	(18)	43	139	(305)	183

1962

DATE	JAN	FEB	MAR	SEP	OCT	NOV	DEC
1	12	5	0	-	7	3	4
2	10	1	1	8	33	6	-
3	3	1	2	18	*240	6	1
4	0	3	2	*200	55	7	2
5	3	18	0	9	3	22	2
6	15	0	1	15	1	24	7
7	8	40	1	3	49	11	2
8	29	1	2	8	18	0	8
9	4	3	2	22	0	37	-
10	7	3	3	*101	6	2	-
11	4	4	385	15	54	11	5
12	4	0	58	3	30	13	23
13	6	1	108	-	8	20	4
14	0	-	292	-	4	26	6
15	50	-	704	-	11	8	-
16	14	1	-	-	32	*246	27
17	5	0	74	24	13	60	16
18	2	0	16	47	2	0	4
19	0	0	3	1	2	163	-
20	0	6	32	3	0	19	9
21	1	1	5	0	9	722	21
22	0	1	2	109	1	173	0
23	10	0	6	67	2	41	-
24	0	0	15	*41	1	2	-
25	1	1	12	53	3	131	26
26	-	1	-	212	70	9	4
27	30	9	1	2	3	130	-
28	10	4	1	0	2	42	27
29	10		4	1	6	99	-
30	3		-	65	45	0	0
31	3		-		9		1
TOTAL	244	104	(1732)	1027	719	2033	199

1963

DATE	JAN	FEB	MAR	SEP	OCT	NOV	DEC
1	4	5	1	-	45	*322	3
2	15	4	2	-	8	136	8
3	11	2	5	-	37	28	21
4	5	3	0	-	83	10	49
5	35	5	1	0	103	217	10
6	3	14	0	2	79	28	5
7	2	0	1	11	*124	10	4
8	6	4	0	3	114	6	8
9	27	42	0	*39	127	32	7
10	140	18	0	54	136	83	14
11	61	91	2	5	60	82	0
12	27	21	5	2	0	92	2
13	20	3	6	48	4	12	36
14	22	3	6	40	16	4	10
15	5	4	0	*124	33	4	0
16	11	1	0	11	51	4	2
17	6	2	0	19	97	31	-
18	14	2	2	72	*172	68	21
19	21	9	5	114	113	56	20
20	100	35	24	*238	74	20	18
21	40	1	18	100	34	81	61
22	3	1	0	66	29	45	50
23	9	3	1	17	39	48	31
24	30	21	-	10	65	15	71
25	60	-	-	156	76	22	12
26	60	22	-	*165	30	22	34
27	0	3	-	150	25	5	30
28	4	42	-	131	-	21	28
29	6		-	2	-	5	6
30	12		-	4	6	21	4
31	30		-		1		10
TOTAL	789	361	(79)	(1583)	(1781)	1530	(575)

1964

DATE	JAN	FEB	SEP	OCT	NOV	DEC
1	12	7	3	2	0	3
2	6	11	1	-	-	13
3	1	73	0	0	-	14
4	11	11	1	2	1	1
5	64	39	4	0	0	2
6	14	16	2	0	1	50
7	7	1	0	0	0	3
8	9	1	0	3	18	3
9	4	2	3	0	10	4
10	0	-	2	-	10	1
11	2	-	5	0	*51	4
12	4	-	*9	3	27	3
13	3	-	3	0	2	0
14	1	0	7	1	18	0
15	0	2	0	1	0	5
16	2	0	0	2	0	1
17	1	-	0	0	0	2
18	1	-	1	0	5	7
19	0	1	9	0	20	1
20	0	-	3	0	31	0
21	1	2	*11	0	9	2
22	3	-	0	0	4	8
23	1	-	0	0	40	0
24	2	-	0	0	60	3
25	2	-	0	0	50	0
26	0	-	0	2	6	15
27	1	-	2	4	16	1
28	20	-	0	3	13	14
29	47	-	0	0	40	1
30	83		1	0	14	1
31	7			1		1
TOTAL	309	(166)	67	(24)	(446)	163

1965

DATE	JAN	FEB	SEP	OCT	NOV	DEC
1	1	63	-	2	12	624
2	7	43	-	2	10	57
3	1	1	-	9	-	64
4	2	28	-	30	30	59
5	3	31	-	50	*113	141
6	2	94	-	*412	4	140
7	2	27	0	76	84	111
8	5	3	-	9	65	7
9	26	8	0	4	0	22
10	1	6	0	10	0	12
11	11	-	4	30	0	188
12	8	-	0	10	15	30
13	0	-	0	*160	80	23
14	88	-	6	20	105	13
15	52	-	0	4	*594	18
16	7	-	*52	0	7	3
17	7	-	7	1	8	10
18	4	-	19	1	12	163
19	14	-	41	0	8	445
20	42	-	*444	5	26	186
21	9	-	36	7	340	200
22	18	-	132	0	876	404
23	1	-	62	36	4	73
24	11	-	492	5	280	30
25	90	-	*540	*84	280	101
26	291	-	448	24	262	91
27	251	-	12	28	17	40
28	7	-	72	20	130	-
29	-		84	20	205	-
30	8		24	60	54	463
31	30			1		44
TOTAL	(999)	(304)	(2475)	1120	(3621)	(3762)

1966

DATE	JAN	FEB	MAR	SEP	OCT	NOV	DEC
1	0	23	5	-	4	0	79
2	68	9	5	-	0	0	326
3	18	1	-	-	8	1	1
4	1	1	-	-	25	16	34
5	0	0	0	1	5	0	21
6	15	14	0	0	30	28	404
7	23	7	-	0	53	-	14
8	0	6	0	1	54	3	1
9	-	4		4	114	5	94
10	-	5		2	184	-	40
11	-	3		4	*180	-	21
12	6	2		*12	200	0	534
13	172	13		16	380	8	-
14	48	1		-	88	5	540
15	0	0		0	11	0	75
16	33	2		0	56	8	13
17	14	2		0	*252	10	54
18	1	0		14	138	0	50
19	-	8		-	6	16	95
20	72	3		0	2	1	132
21	65	12		0	8	0	-
22	18	8		-	55	40	59
23	16	3		0	*161	6	44
24	22	3		0	90	200	0
25	96	3		12	24	24	30
26	4	-		8	0	24	110
27	-	0		20	0	128	0
28	65	0		*80	20	-	88
29	-			3	-	-	51
30	22			19	2	-	112
31	27				0		108
TOTAL	(806)	133		(196)	(2150)	(523)	(3130)

1967

DATE	JAN	FEB	AUG	SEP	OCT	NOV	DEC
1	56	40	-	1	-	12	0
2	134	192	-	9	-	20	0
3	55	30	-	*38	0	12	0
4	60	50	-	15	34	4	-
5	140	60	-	0	-	7	-
6	30	240	-	4	5	0	-
7	33	6	-	*54	18	0	0
8	20	5	-	0	*16	5	4
9	45	19	-	0	24	0	4
10	49	31	-	2	0	*32	2
11	56	88	-	8	4	0	18
12	25	-	-	6	12	-	-
13	23	4	-	0	0	4	1
14	52	13	-	*49	3	0	0
15	17	12	-	0	6	1	24
16	63	-	-	1	0	4	-
17	5	-	-	19	0	2	1
18	90	-	-	4	0	1	0
19	45	-	-	0	0	12	0
20	48	-	-	2	0	9	0
21	19	-	-	7	3	12	1
22	30	-	-	34	2	5	8
23	4	-	-	2	24	4	10
24	30	-	-	*68	0	2	20
25	3	-	-	17	24	3	4
26	5	-	-	4	24	1	4
27	8	-	-	5	*56	6	7
28	7	-	-	-	18	-	2
29	3		*16	5	13	4	0
30	20		4	0	4	0	2
31	20		17		6		6
TOTAL	1195	(790)	(37)	(354)	(296)	(162)	(118)

1968

DATE	JAN	FEB	SEP	OCT	NOV	DEC
1	1	0	-	-	4	8
2	3	-	-	31	4	0
3	-	2	-	14	*56	0
4	3	6	-	*232	12	0
5	2	3	-	-	5	1
6	6	1	-	4	5	2
7	2	0	-	31	11	4
8	8	3	-	*184	7	0
9	2	-	-	35	42	3
10	5	0	24	-	21	12
11	3	1	24	120	5	4
12	-	0	20	170	48	4
13	0	1	7	100	2	14
14	10	0	29	*320	4	44
15	3	1	19	-	10	47
16	3	16	48	40	22	26
17	1	-	34	186	-	2
18	-	-	*160	84	-	25
19	6	-	50	*136	-	30
20	0	-	4	48	1	17
21	0	-	24	150	-	10
22	8	-	*133	1	-	30
23	8	-	11	154	5	31
24	6	-	3	-	6	6
25	0	8	-	4	14	23
26	3	9	12	5	-	17
27	8	6	*160	*268	8	11
28	2	2	84	21	1	10
29	0	10	16	69	0	13
30	16		-	52	2	13
31	4			-		15
TOTAL	(113)	(69)	(862)	(2459)	(295)	422

1969

DATE	JAN	FEB	AUG	SEP	OCT	NOV	DEC
1	13	24	-	18	3	1	0
2	7	-	-	11	2	13	3
3	-	8	-	9	4	-	1
4	12	6	-	*23	4	-	2
5	60	20	-	7	5	2	5
6	-	22	-	0	3	0	5
7	-	-	-	0	7	1	1
8	28	8	-	3	8	-	6
9	54	27	-	1	2	0	-
10	36	80	-	11	*10	0	-
11	-	44	-	0	8	3	1
12	10	40	-	-	0	5	4
13	10	35	-	0	18	*88	2
14	29	42	-	0	1	108	0
15	10	-	-	0	4	52	1
16	12	25	-	1	0	32	0
17	0	15	-	3	1	16	1
18	60	136	-	0	5	4	0
19	8	160	-	0	4	1	2
20	-	81	-	2	3	0	0
21	-	8	-	0	1	1	1
22	-	13	-	2	5	1	0
23	17	13	-	0	1	0	1
24	46	23	-	0	-	0	0
25	55	21	-	0	4	1	0
26	20	-	-	1	2	0	0
27	16	13	-	1	8	2	6
28	16	20	4	1	2	3	3
29	-		0	0	-	18	0
30	73		*16	2	0	3	0
31	21		10		4		0
TOTAL	(613)	(884)	(30)	(96)	(119)	(355)	(45)

1970

DATE	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1	1	1	1	0	0	-	0	0	0	*11	0	1
2	2	3	0	0	0	-	0	0	0	5	5	0
3	2	0	0	0	0	-	0	0	6	2	1	-
4	3	2	0	0	0	-	1	0	0	0	5	1
5	1	9	0	0	0	-	0	0	0	0	4	5
6	0	0	0	0	0	-	0	0	0	0	10	1
7	1	0	1	0	0	-	0	0	1	1	15	6
8	4	0	0	0	0	-	0	0	3	0	*25	1
9	-	1	0	0	0	-	0	0	0	0	12	2
10	3	5	1	0	0	-	0	0	2	0	11	3
11	1	7	1	0	0	-	0	0	0	0	1	6
12	4	2	0	0	0	-	0	0	2	0	1	0
13	0	1	0	0	0	-	0	0	0	0	1	0
14	0	0	0	0	0	-	0	0	2	0	0	3
15	1	2	1	0	0	-	0	0	2	0	5	2
16	3	7	1	0	0	-	0	0	0	0	1	-
17	5	1	2	0	0	0	0	0	0	0	2	2
18	2	0	0	0	0	0	0	0	1	0	3	5
19	1	0	0	0	0	0	0	0	0	0	9	4
20	0	0	0	0	0	0	0	0	0	0	6	4
21	1	0	0	0	0	0	0	2	4	0	1	8
22	0	1	0	0	0	0	0	2	2	0	0	5
23	0	-	3	0	0	0	2	0	0	0	-	16
24	1	1	0	0	0	0	0	0	1	0	15	20
25	1	0	0	0	0	0	0	0	0	0	1	40
26	1	1	0	0	0	0	0	0	0	0	8	8
27	5	0	0	0	0	0	0	0	0	0	-	20
28	15	1	0	0	0	0	0	5	1	1	14	7
29	5		0	0	-	0	0	0	1	0	2	7
30	6		0	0	-	0	0	0	2	0	17	6
31	3		0		-		0	0		1		-
TOTAL	(72)	(45)	11	0	(0)	(0)	3	9	30	21	(175)	(183)

1971

DATE	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1	19	11	1	0	0	1	0	0	0	18	3	5
2	4	5	2	4	0	4	0	0	6	0	4	2
3	11	17	5	4	0	0	0	0	1	1	17	9
4	6	4	0	8	3	1	0	0	0	12	6	1
5	38	15	7	2	0	0	0	0	2	*40	5	7
6	78	-	11	2	0	0	0	0	4	44	0	6
7	125	3	0	0	0	0	0	0	10	1	0	16
8	38	6	3	0	0	1	0	0	*40	48	0	4
9	1	-	1	0	0	0	0	0	3	2	0	5
10	3	1	4	0	0	1	0	0	1	2	4	8
11	8	1	0	0	0	1	0	0	3	21	14	3
12	1	8	25	2	0	1	0	0	*64	*75	1	7
13	3	0	0	1	0	0	0	3	0	-	4	11
14	18	30	1	4	0	0	0	1	0	61	8	6
15	4	62	1	0	3	0	0	1	2	6	6	32
16	5	84	3	0	1	0	0	4	48	0	16	7
17	1	21	0	1	1	1	0	1	24	4	35	1
18	0	5	0	0	1	0	3	1	*91	3	15	2
19	7	10	0	1	2	0	0	3	0	16	36	7
20	-	4	0	0	0	0	0	1	10	41	26	16
21	9	11	0	0	3	0	1	3	16	*84	3	25
22	75	4	4	0	0	0	0	4	32	31	10	9
23	80	2	9	0	0	0	0	1	*48	50	12	25
24	18	0	1	0	0	0	1	0	65	14	30	11
25	-	1	1	0	0	0	0	0	0	68	7	15
26	8	0	0	0	0	0	6	0	6	3	0	21
27	9	4	1	0	0	1	1	0	15	7	2	11
28	5	13	4	1	0	1	0	1	11	4	1	15
29	4		5	0	0	0	4	0	*25	1	0	-
30	2		0	0	2	0	2	0	32	0	0	-
31	2		0		1		0	0		0		9
TOTAL	(582)	(322)	89	30	17	13	18	24	559	(657)	265	(296)

1972

DATE	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1	1	-	0	0	-	1	11	6	3	17	16	-
2	1	0	0	-	1	-	0	-	1	**140	4	1
3	1	0	0	0	-	0	13	-	2	117	-	34
4	9	3	2	0	-	0	13	-	1	76	8	51
5	8	0	0	4	-	1	12	-	0	7	20	-
6	8	6	0	5	-	1	*21	-	0	12	*31	13
7	-	14	0	0	0	0	1	-	0	48	19	3
8	0	-	-	0	0	0	9	-	3	*150	25	33
9	-	-	0	0	0	0	2	5	3	40	-	43
10	14	12	0	0	1	0	0	0	7	3	-	66
11	1	-	9	0	0	0	6	8	9	2	-	2
12	2	4	0	2	0	0	0	7	8	17	-	-
13	3	5	0	1	0	0	0	3	3	18	-	-
14	0	2	-	0	0	1	0	6	3	23	-	16
15	4	5	-	-	0	0	0	15	6	2	-	0
16	-	12	-	-	0	0	0	-	**20	5	4	4
17	2	8	-	-	0	0	0	-	0	5	6	3
18	0	4	-	0	0	1	0	1	0	6	0	5
19	2	6	-	-	0	0	0	1	0	11	4	-
20	1	1	-	-	0	0	1	0	0	1	0	-
21	2	0	-	-	0	0	3	1	6	1	1	-
22	3	3	-	-	0	0	1	1	8	0	16	21
23	0	12	-	0	0	0	3	0	0	30	-	10
24	1	-	-	0	0	0	0	0	1	7	-	1
25	-	-	-	1	0	3	0	1	5	1	-	-
26	4	12	-	1	0	0	0	1	0	0	-	-
27	10	-	-	-	0	0	4	0	8	8	-	-
28	3	17	-	0	0	0	*10	1	3	*60	4	-
29	0	1	-	0	0	0	2	0	3	21	-	-
30	5		-	0	0	27	1	0	8	7	-	-
31	3		-		0		0	3		12		-
TOTAL	(88)	(127)	(11)	(14)	(2)	(35)	113	(60)	111	847	(158)	(306)

1973

DATE	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1	-	0	-	17	-	8	0	-	5	50	7	20
2	-	0	1	14	0	0	0	-	17	20	4	18
3	1	0	1	5	0	0	0	-	8	*35	0	72
4	0	4	1	13	4	3	0	-	0	45	1	-
5	0	2	2	21	0	0	0	-	0	21	0	-
6	1	0	0	14	0	-	0	-	15	21	0	-
7	5	2	1	32	1	2	0	-	2	2	0	0
8	3	3	5	4	1	4	0	-	12	21	1	0
9	9	-	7	15	0	0	9	0	17	*50	2	0
10	1	2	7	17	0	1	0	0	*32	8	0	0
11	2	-	-	-	0	0	-	1	29	0	1	1
12	11	-	10	0	-	0	-	1	-	36	0	0
13	1	-	2	-	1	0	-	2	28	5	7	5
14	3	1	1	-	0	0	-	0	1	16	0	7
15	3	5	1	3	0	0	-	-	19	20	2	33
16	4	4	7	1	-	0	-	0	20	12	0	5
17	1	1	5	0	-	0	-	0	5	-	0	1
18	1	3	0	1	-	0	-	1	10	-	7	65
19	5	7	5	1	4	0	-	0	3	26	2	17
20	0	5	4	0	-	1	-	4	**230	25	2	7
21	1	1	3	4	-	0	-	*12	15	-	1	6
22	3	6	-	1	-	4	-	12	19	*50	0	-
23	7	6	8	3	3	2	-	3	7	10	5	-
24	2	8	4	3	0	2	-	6	21	52	29	-
25	7	7	8	0	2	0	-	8	*79	15	1	-
26	20	2	27	-	0	0	-	0	-	32	3	-
27	17	-	0	-	1	0	-	2	44	27	35	-
28	24	0	12	-	0	0	-	0	-	9	0	-
29	16		13	-	5	0	-	2	9	21	2	-
30	14		2	-	-	1	-	7	5	28	88	-
31	2		5		7		-	8		5		-
TOTAL	(164)	(69)	(142)	(169)	(29)	(28)	(9)	(69)	(652)	(662)	200	(257)

1974

DATE	JAN	FEB	MAR	APR	MAY	OCT	NOV	DEC
1	-	72	0	0	0	-	0	27
2	7	9	1	0	0	-	0	15
3	-	-	0	3	0	-	10	5
4	17	-	0	0	0	0	3	15
5	4	5	3	0	0	0	3	0
6	1	2	26	0	0	0	5	3
7	0	-	12	1	0	17	4	13
8	0	18	17	1	-	7	1	0
9	130	0	0	4	-	**51	3	0
10	8	0	26	0	-	5	*24	25
11	1	0	8	0	-	15	32	0
12	21	1	0	0	-	3	8	0
13	31	0	25	0	-	4	2	0
14	27	13	13	0	-	16	3	0
15	36	0	15	0	-	10	-	17
16	21	0	20	0	-	0	-	7
17	0	5	0	0	-	5	-	17
18	0	11	4	0	-	1	-	30
19	0	2	15	0	-	8	-	19
20	31	2	10	0	-	32	-	0
21	17	0	22	0	-	18	-	0
22	63	0	0	0	-	19	-	0
23	18	3	12	0	-	**125	-	9
24	30	0	0	0	-	5	-	19
25	42	0	0	0	-	3	0	7
26	127	0	0	0	-	5	0	5
27	96	4	0	0	-	3	0	5
28	52	0	0	0	-	7	0	5
29	126		0	0	-	17	0	7
30	-		0	0	-	5	0	15
31	-		20		-	1		19
TOTAL	(906)	(147)	249	9	(0)	(382)	(98)	284

1975

DATE	JAN	FEB	MAR	APR	SEP	OCT	NOV	DEC
1	0	5	4	0	-	1	1	0
2	0	0	0	0	-	0	1	-
3	8	24	0	0	-	1	1	4
4	30	14	0	0	0	9	6	1
5	0	125	-	0	1	*14	4	0
6	16	3	0	0	0	-	0	19
7	4	40	0	0	0	-	0	17
8	8	20	0	0	9	7	0	21
9	0	0	2	0	2	2	2	18
10	10	55	0	0	2	0	1	-
11	9	27	4	0	10	1	0	-
12	16	9	0	0	5	4	12	4
13	12	10	4	0	5	9	-	3
14	-	6	0	0	*18	0	-	8
15	1	12	0	0	2	0	-	2
16	0	1	0	0	0	0	-	-
17	1	21	0	0	1	0	0	-
18	7	0	1	0	2	0	-	-
19	0	17	1	0	2	2	0	1
20	0	10	0	0	0	0	7	4
21	2	2	0	0	4	0	5	3
22	4	0	0	0	0	1	0	1
23	30	0	0	0	0	-	4	8
24	40	5	0	0	0	0	50	-
25	2	0	0	0	0	4	0	0
26	4	1	0	0	*24	0	5	0
27	0	0	1	0	7	0	2	17
28	0	2	0	0	0	-	0	41
29	0		0	0	1	-	-	7
30	51		0	0	2	-	-	17
31	0		0			0		0
TOTAL	(255)	409	(17)	0	(97)	(55)	(101)	(196)

1976

DATE	JAN	FEB
1	2	0
2	21	4
3	0	
4	-	
5	-	
6	0	
7	0	
8	0	
9	0	
10	0	
11	0	
12	0	
13	0	
14	5	
15	0	
16	0	
17	0	
18	0	
19	1	
20	-	
21	0	
22	3	
23	0	
24	2	
25	0	
26	0	
27	2	
28	2	
29	2	
30	0	
31	0	
TOTAL	(40)	

APPENDIX 8 THE SWEEP-NET METHOD FOR SAMPLING LARVAE AND PREDATORS OF HELIOTHIS PUNCTIGERA IN LUCERNE

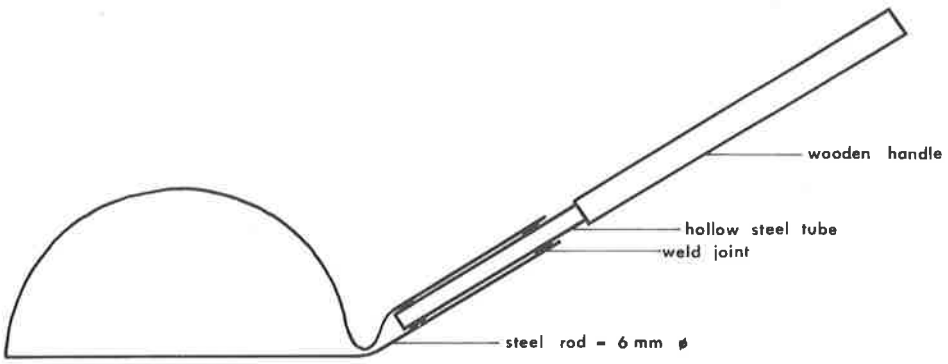
To investigate the survival of larvae of Heliothis punctigera in lucerne and to obtain an indication as to the importance of predators (see section 3.4.3), estimates of their density were required. The cost (in time) of obtaining large numbers of absolute estimates would have been prohibitive and it was decided that a relative sampling method would be more feasible. Use of a sweep-net was considered.

The efficiency and advantages of the sweep-net in collecting insects from vegetation have been reviewed by Southwood (1966). An attempt at estimating the efficiency of a sweep-net was made by Pedigo et al. (1972) on green cloverworm in soybeans. Here, sweep-net sampling resulted in greater overall precision (cost and accuracy) when compared to a cage (absolute) technique; however, larvae of this species occur mainly in the upper well-expanded leaves of the canopy, which is conducive to their efficient collection. Shephard et al. (1974) compared the efficiency of using a sweep-net, D-vac and ground-cloth sampling technique to estimate the abundance of insects in soybeans. Compared to the ground-cloth technique, the sweep-net method underestimated the abundance of Heliothis spp. larvae (especially the early instars) and Nabis spp. adults, but the trends in abundance with time were similar. Good agreement between these two methods was achieved in estimating the abundance of an adult coccinellid. Despite the fact that no detailed studies were found on the efficiency of the sweep-net method in sampling Heliothis spp. larvae in lucerne, information on this method for other crops suggested the sweep-net could be used for the present study with acceptable reservations.

A sweep-net with a D-shape aperture was constructed (see Fig. A.8.1.); nets of this design are more efficient in collecting insects in short crops (Beall 1935) and more easily handled than conventional nets with circular aperture.

FIGURE A.8.1

The sweep-net used to collect larvae and predators of *H. punctigera*.



calico net - side view

Scale : 1 cm = 10 cm

Factors affecting the efficiency of sweep-nets include sweeping technique, vegetation and target insect (Southward 1966).

The sweeping technique was standardised for the present study as a swath through 180°, starting on the right-hand side of the operator. The net was drawn through a crop at a depth such that the curved section of the frame was level with the top of the canopy. Because only one operator was involved, sweeping technique was consistent. One sampling unit involved a series of ten consecutive sweeps with each sweep at intervals of one pace. The number of sampling units constituting a sample varied between five and ten.

The approximate area of crop sampled in one pass of the net was calculated as follows :-

Surface projection of area of crop sampled, A = (surface projection of transverse of net in one sweep) x (adjustment for curvature of net aperture) x (correction factor for crop canopy type, F_c) i.e.

$$A = \left[\frac{\pi r_1^2}{2} - \frac{\pi r_2^2}{2}\right] \times \left[\frac{\pi r_3^2}{2} \div 2r_3 \cdot r_3\right] \times F_c$$

$$= \left[\pi \frac{(1.05)^2}{2} - \pi \frac{(0.60)^2}{2}\right] \times \frac{\pi}{4} \times F_c$$

$$\doteq 0.9\ m^2.F_c$$

where r_1 = distance from operator to distal point of net aperture

r_2 = distance from operator to proximal point of net aperture

r_3 = radius of net aperture.

Naturally, the height of the crop canopy and distribution of larvae down the canopy will alter the proportion of crop sampled by the net (i.e. F_c). For young, short lucerne crops, flowers and foliage were observed to be reasonably well-distributed through the canopy and the distribution of larvae probably follows this trend. Therefore, the proportion of the canopy sampled would simply be the ratio of sampling depth to crop height. In

older, taller crops, this is not the case because there is less food (leaves, flowers) towards the lower level of the canopy.

Attempts made to calibrate the sweep-net with an absolute method of sampling were inconclusive, primarily because too few samples were collected to obtain sufficiently reliable estimates of absolute abundance. For a complete calibration, different crop types would also have to be included e.g. crops of different heights, densities and growth stages.

For the purposes of the present project, the sweep-net was found to produce mean values for the abundance of larvae with acceptable standard errors (see Fig. A.8.2). For very precise work such as life table studies, more refinement would be necessary (Southwood 1966).

When the relationship between the mean and variance is examined, a contagious distribution of larvae is apparent (see Fig. A.8.3).

For the more abundant predators of H. punctigera larvae, the standard errors of mean densities expressed as percentages of the mean are plotted in Fig. A.8.4. From this, the abundance of Oechalia schellenbergii and Nabis kinbergii was estimated reasonably well and better than that for Coccinella repanda.

Despite obvious shortcomings in using the sweep-net (e.g. inability to convert the data to estimates of absolute abundance), it was considered sufficiently accurate to draw the conclusions made in Chapter 3 concerning the survival of the larval stage of H. punctigera in South Australia.

The mean number of larvae and the associated standard error for the studies undertaken at Mortlock Experiment Station and Booborowie are given in Tables A.8.1. and A.8.2. respectively.

FIGURE A.8.2

The standard error of the mean number of *H. punctigera* larvae (expressed as a percent. of the mean) collected using the sweep-net plotted against the mean number of larvae for each sample.

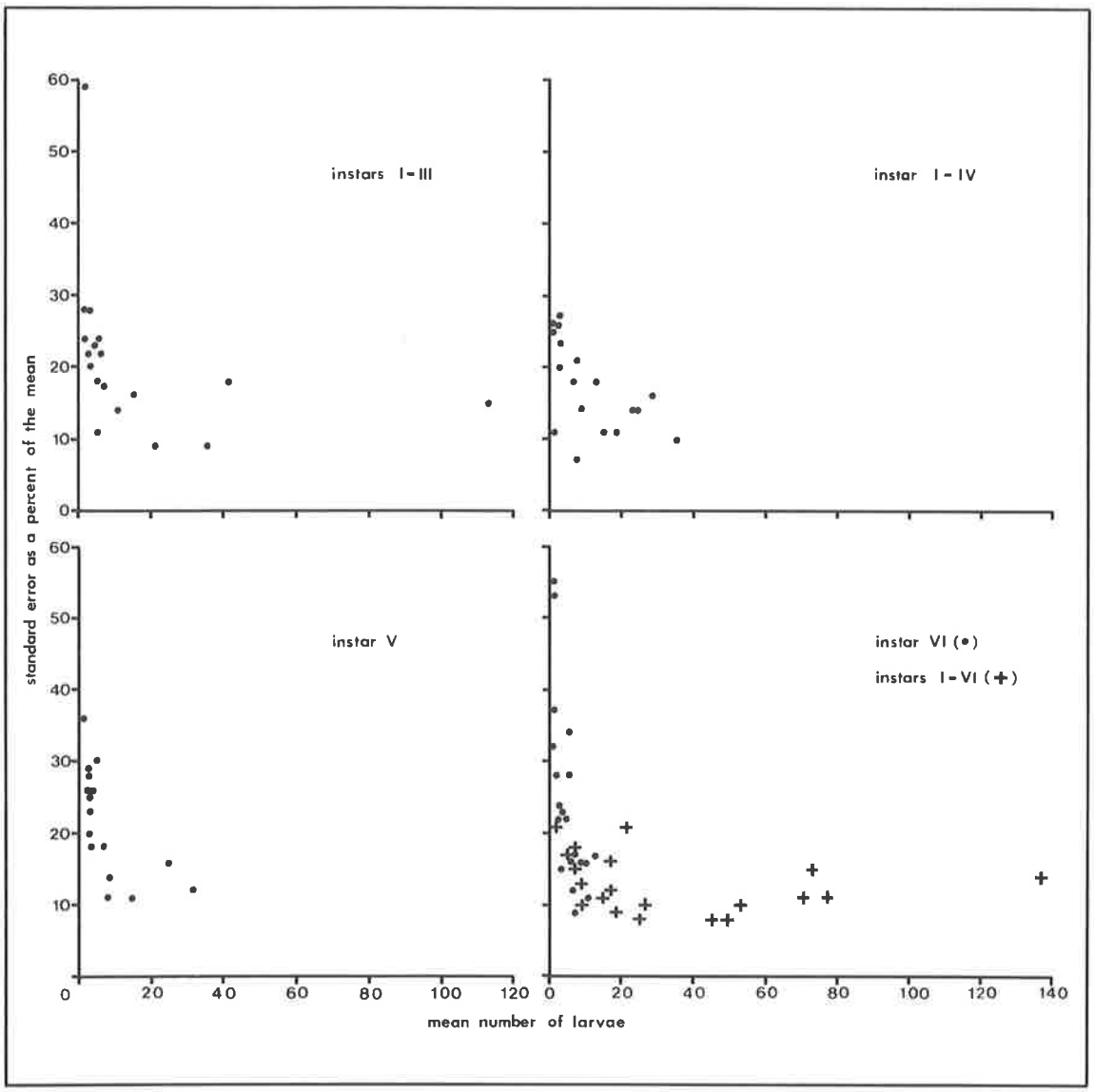

instars I-III
instar I-IV
instar V
instar VI (•)
instars I-VI (+)
standard error as a percent of the mean
mean number of larvae
0
10
20
30
40
50
60
0
20
40
60
80
100
120
140

FIGURE A.8.3

The variance of the mean number of H. punctigera larvae collected using the sweep-net plotted against the mean for each sample.

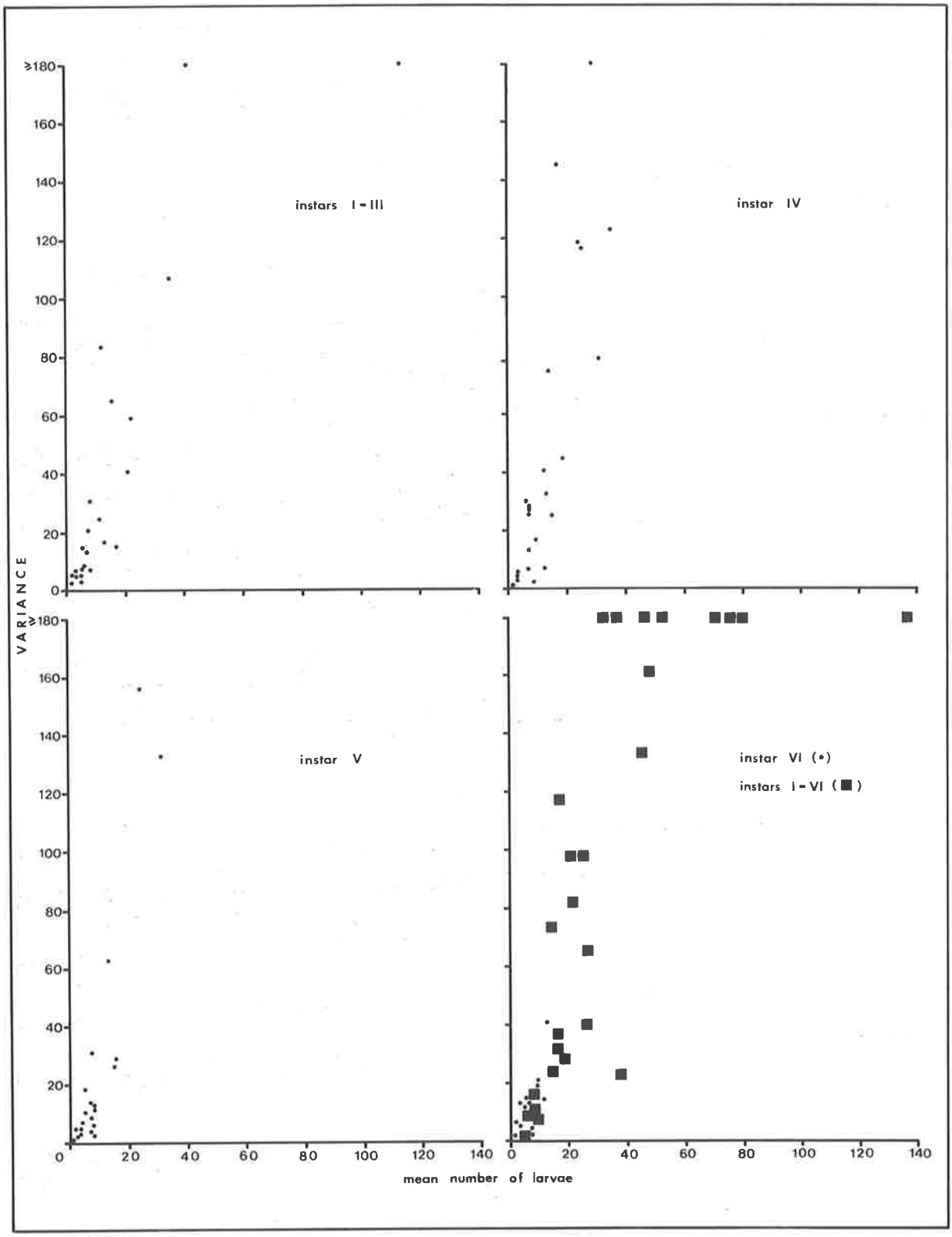

VARIANCE
≥180
160
140
120
100
80
60
40
20
0
instars I - III
instar IV
instar V
instar VI (•)
instars I - VI (■)
0
20
40
60
80
100
120
140
mean number of larvae

FIGURE A.8.4.

The standard error of the mean number of the predators *O. schellenbergii*, *N. kinbergii* and *C. repanda* (expressed as a percent. of the mean) collected using the sweep-net plotted against the mean number of predators for each sample.

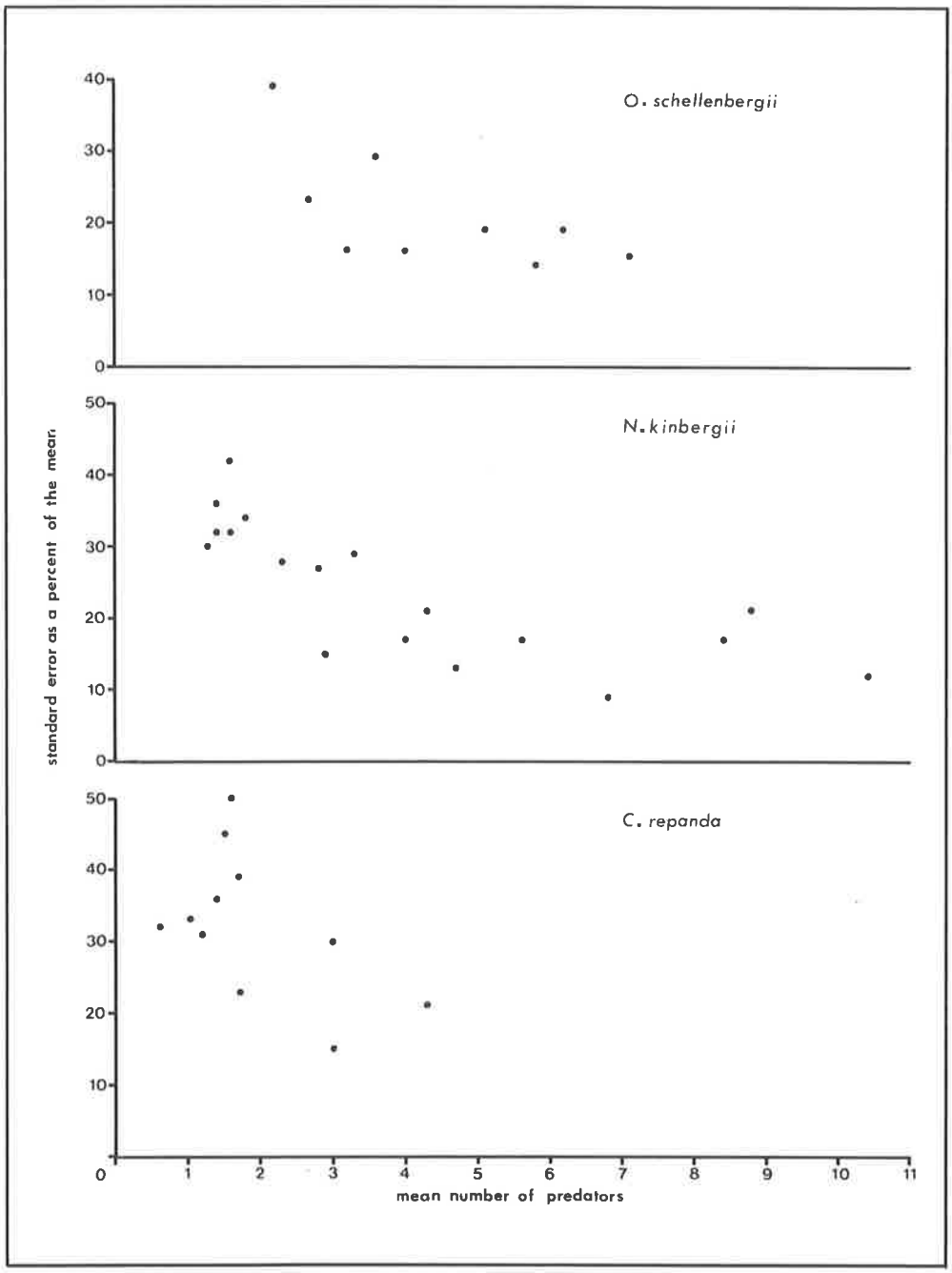

O. schellenbergii
N. kinbergii
C. repanda
standard error as a percent of the mean
mean number of predators
0
10
20
30
40
50
1
2
3
4
5
6
7
8
9
10
11

TABLE A.8.1 Details of the results of sweep-net sampling for H. punctigera larvae at Mortlock Experiment Station.

a) Dryland lucerne

Sampling date	No. sampling units	Area sampled	Instar, mean no. larvae/sampling unit and standard error (as % $\bar{x}$)									
			1-3		4		5		6		1-6	
			$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$
5.xi.1975	10	A	0.7	43	0.4	55	0	-	0	-	1.2	21
11	10	A	5.4	11	2.5	28	0.8	36	0	-	8.8	10
18	10	A	1.9	24	2.8	20	2.2	18	0	-	7.0	15
25	5	A	6.0	22	6.6	18	3.4	26	0	-	16.2	16
25	5	B	1.8	59	0.6	40	0.8	46	1.0	55	4.2	17
2.xii.975	10	B	109.0	14	24.7	14	2.4	29	0	-	136.3	14
9	10	B	15.8	16	35.3	10	24.3	16	1.2	37	76.6	11
15	10	B	5.2	18	14.6	11	14.6	11	10.6	11	45.5	8
23	10	B	1.1	28	3.2	23	7.9	10	2.1	22	14.3	11
30	6	B	0	-	0.3	70	1.3	38	0.3	70	2.0	37
30	5	C	4.4	23	12.2	23	4.6	31	0	-	21.2	21
6.i.1976	9	C	3.4	20	1.6	26	2.9	6	0.4	85	8.3	13
13	10	C	17.4	7	6.4	18	2.2	28	0.3	50	26.3	9
19	10	C	35.8	9	9.5	14	2.7	26	0.3	50	48.3	8
26	10	C	21.3	9	24.1	14	6.7	18	0.8	53	52.9	10
3.ii.1976	10	C	5.0	24	7.4	7	3.8	18	2.1	5	18.3	9
9	11	C	2.2	22	2.0	26	2.5	24	0.3	47	6.9	18
16	6	C	0	-	0	-	0	-	0	-	0	-

TABLE A.8.1. (cont'd).

(b) Irrigated lucerne

Sampling date	No. sampling units	Area sampled	Instar, mean no. larvae/sampling unit and standard error (as % $\bar{x}$)									
			1-3		4		5		6		1-6	
			$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$
5.xi.1975	10	A	0	-	0	-	0	-	0	-	0	-
11	10	A	1.6	25	0.5	44	0	-	0	-	2.2	19
18	10	A	1.1	25	1.0	30	0.7	30	0	-	2.8	20
25	5	A	1.0	55	0.6	67	0	-	0	-	1.8	27
25	5	B	4.2	29	0.8	16	0.4	60	0	-	5.8	23
2.xii	10	B	41.2	18	28.3	16	2.1	22	0.5	44	72.1	16
9	10	B	11.4	14	18.6	11	31.4	12	9.1	16	70.5	11
15	10	B	3.0	28	7.8	21	8.0	14	7.3	9	26.1	10
23	5	B	0	-	1.4	17	8.0	20	6.8	24	16.2	17
30	5	B	0	-	0	-	0	-	2.0	53	2.4	39
30	5	C	1.6	38	1.8	75	0.8	35	0	-	4.2	30
6.i.1976	10	C	2.0	25	0.6	52	0	-	0	-	2.7	27
16.ii	5	C	0.8	46	0	-	0	-	0	-	0.8	46
2.iii	5	C	4.0	27	3.0	45	1.6	51	0	-	8.6	36
9	5	C	2.2	36	1.2	61	0.6	40	0.8	46	4.8	33
16	5	C	0.8	46	2.0	28	3.0	24	1.4	17	7.2	8
23	5	C	0	-	0.4	60	1.0	45	2.2	39	3.6	37
30	5	C	0	-	0.4	60	0	-	0.6	67	1.0	32
6.iv	5	C	0	-	0	-	0	-	0.6	67	0.6	67
13	5	C	0	-	0	-	0	-	0	-	0	-

TABLE A.8.2. Details of the results of sweep net sampling for H. punctigera larvae at Booborowie.

Sampling date	No. sampling units	* Bay sampled	Instar, mean no. larvae/sampling unit and standard error (as % $\bar{x}$)									
			1-3		4		5		6		1-6	
			$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$	$\bar{x}$	$s_{\bar{x}}$
8.iii.1976	5	1	8.4	25	2.2	39	1.4	49	0	-	12.2	27
16	5	1	13.2	13	28.6	11	25.2	20	12.6	30	79.6	10
23	5	1	3.2	25	16.0	22	21.2	26	32.4	19	72.8	14
30	5	1	0	-	1.8	21	3.8	33	13.2	18	18.8	13
6.iv	5	1	0	-	0	-	0.6	67	1.4	70	2.4	50
2.iv	9	2	13.9	20	5.7	31	2.3	33	0.7	41	22.6	22
6	9	2	21.2	20	4.8	24	3.4	27	0.9	34	30.3	18
13	9	2	15.0	23	15.7	28	6.4	37	3.9	39	41.0	23
20	9	2	10.4	21	11.0	25	4.2	22	1.2	30	26.9	17
27	9	2	3.1	20	4.6	28	2.8	20	1.2	30	11.7	18
4.v.	8	2	1.0	38	1.3	43	1.0	63	0.5	38	3.8	39
11	9	2	0	-	0.6	30	0.4	73	0	-	1.2	33
27.iv	5	3	1.0	45	2.2	34	4.2	23	5.8	18	13.2	16
4.v	5	3	1.4	36	1.6	32	1.8	21	5.6	38	10.4	16
11	5	3	0	-	0.8	46	0.8	46	0.8	46	2.6	26

* bays 1, 2 on the property of Collinsville; bay 3 on the property of Nth. Bungaree.

APPENDIX 9 LARVAL INSTAR DETERMINATION IN HELIOTHIS PUNCTIGERA AND THEIR RATE OF DEVELOPMENT

For the present study it was necessary to be able to classify larvae of Heliothis punctigera into their appropriate instar and also to have quantitative knowledge of the rate of development of each instar with temperature (see Chap. 3). The methods adopted for these purposes and their derivation are described below.

a) Classification of larvae to instar

A relatively simple method of categorizing larvae to instar was essential because of the large numbers of larvae that were to be collected. The parameter on which other classification systems for Heliothis spp. larvae had been based and the one chosen here, is the width of the head capsule (e.g. Kirkpatrick 1961a, Hardwick 1965, Twine 1975).

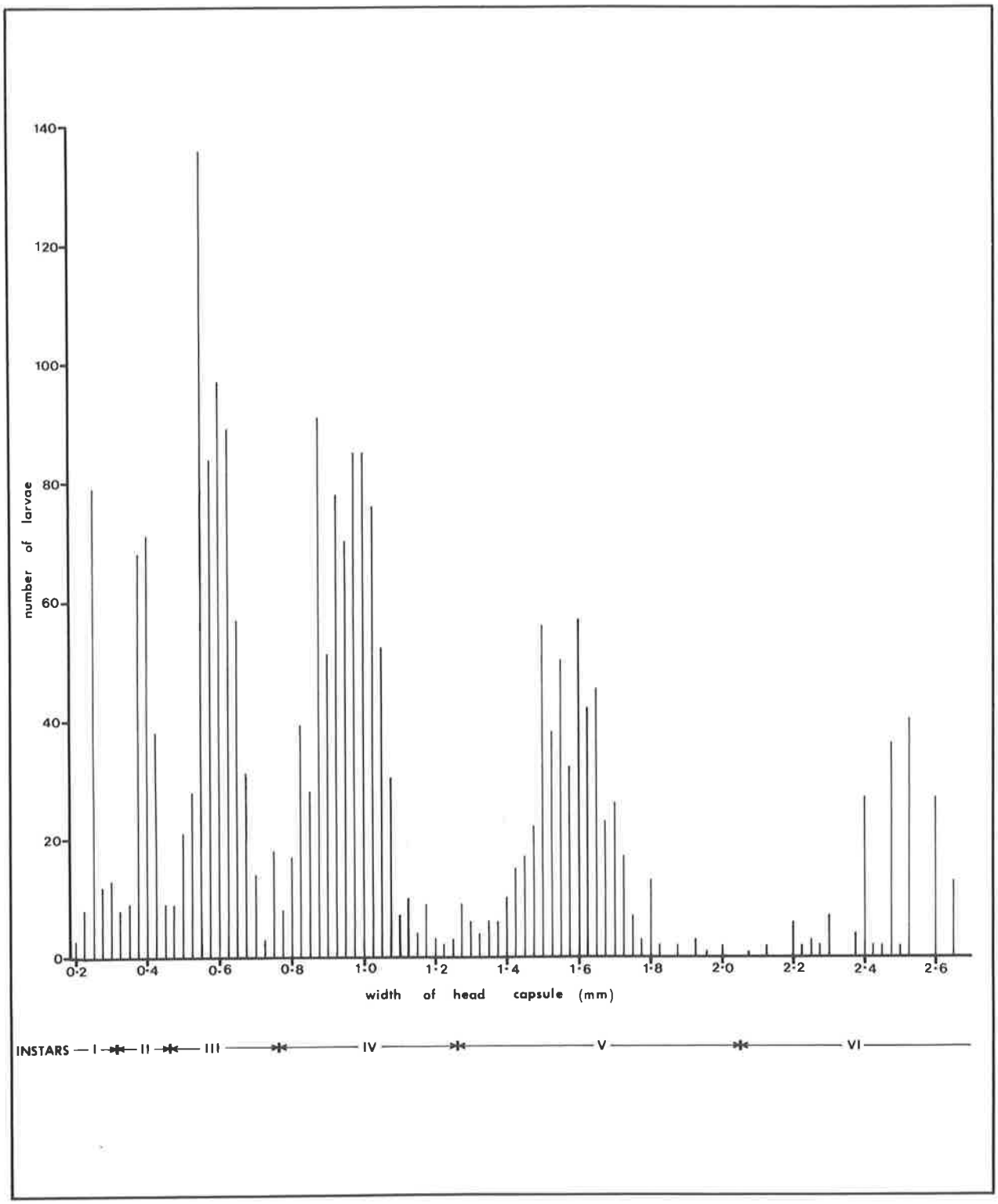
Larvae collected from lucerne at Brecon, M.E.S. and Booborowie (see Fig. 3.1) were used. The width of their head capsules was measured using a stereo dissecting microscope with a calibrated graduated eyepiece. The head capsule was measured at its maximum width (i.e. just anterior to the prothorax) and measurements were rounded to the nearest 0.025 mm.

The frequency of head capsule widths is plotted in Fig. A.9.1. From this, six instars were separated and the range in widths for each instar was determined by eye from the graph. These ranges and the average head capsule width for each instar are given in Table A.9.1. Also included here for comarison are the data obtained by Hardwick (1965). (A printing error in Hardwick's paper seems to have made for the fourth instar). While Hardwick (1965) found that H. punctigera would pass through seven larval stadia, the proportion that did so was small (6 percent). The occurrence of a seventh instar during this study was not confirmed and is neglected.

The distribution of head capsule widths was found to be continuous (Fig.

FIGURE A.9.1.

The frequency of head capsule widths and the range of widths for the six defined instars of *H. punctigera* larvae collected in the field.


number of larvae
140
120
100
80
60
40
20
0
0·2
0·4
0·6
0·8
1·0
1·2
1·4
1·6
1·8
2·0
2·2
2·4
2·6
width of head capsule (mm)
INSTARS — I — II — III — IV — V — VI

A.9.1). Therefore, the ranges of head capsule widths as well as the averages were used for the determination of larval instars in this study. Circles with diameters corresponding to these widths were drawn on stiff white cardboard and covered with clear plastic. Larvae, whose instar was to be determined, could be placed over the various circles until the circle of diameter nearest to the width of the head capsule was found. This could be done by eye and allowed for a quick and accurate method for ageing larvae.

Table A.9.1. Head capsule widths of the instars of H. punctigera larvae.

Instar	Head capsule width (mm)		
	Range (present study)	Average (present study)	Average (Hardwick 1965)
1	0.300	0.255	0.264
2	0.352 - 0.450	0.396	0.391
3	0.475 - 0.750	0.596	0.589
4	0.775 - 1.250	0.958	0.589 ?
5	1.275 - 2.000	1.574	1.64
6	2.025	2.472	2.48

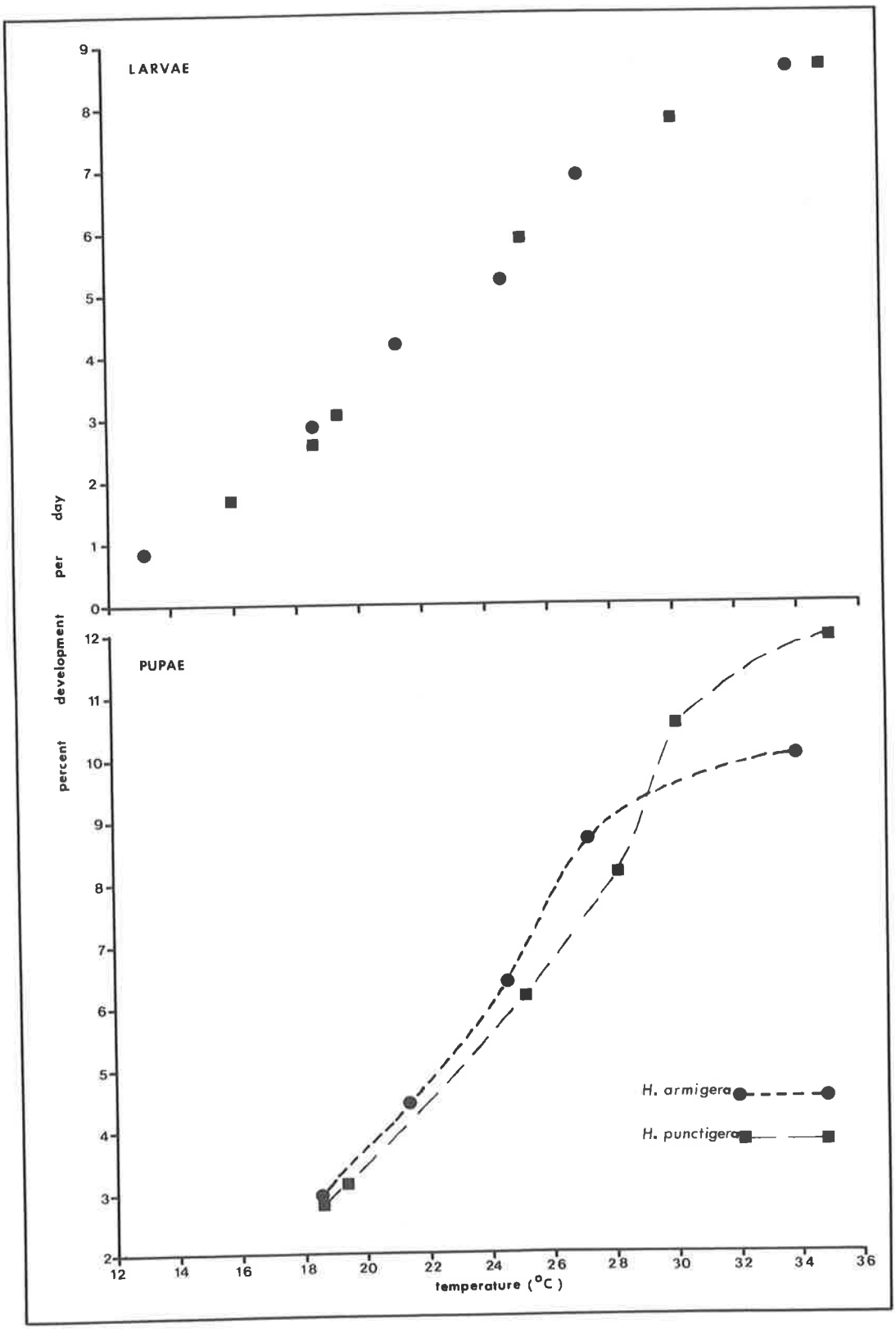
b) Rate of larval development by instar

In order to obtain reasonable estimates of the mortality of each instar (see Chap. 3), it was necessary to know their rate of development. However, there was insufficient time available during the study to enable this work to be carried out and an alternative method was devised.

The relationship between the rate of development and temperature for the larvae of H. armigera has been examined by Twine (1978) and is similar to that for H. punctigera as determined by Cullen (1969) (see Fig. A.9.2 and Room (1983)). In the same figure is the comparison for pupae and a

FIGURE A.9.2.

A comparison of the rate of development of larvae and pupae of H. punctigera and H. armigera reared at constant temperature (as reported by Cullen (1969) and Twine (1978), respectively).


LARVAE
PUPAE
percent development per day
temperature (°C)
H. armigera
H. punctigera

noticeable difference in rates of development is apparent, especially at high temperatures. While this latter deviation suggests that basic physiological differences exist between the two species at least for the pupal stage, the similarity for the larval stage indicated that it may not be unreasonable to extrapolate from Twine's data to define development rates for H. punctigera larval instars.

A closer examination of Twine's data on the rate of instar development with temperature revealed that the proportion of the larval stage occupied by each instar varied only slightly among temperatures (see Table A.9.2). One modification of Twine's date was necessary - the pre-pupal stage had to be included in these calculations because Cullen's work considered the larval stage as occurring to pupation. The pre-pupal stage was found to occupy 15.6% of larval stage for H. punctigera (Cullen 1969) and 15.4% for H. armigera (Twine 1978).

TABLE A.9.2 Proportion of larval stage occupied by each instar for H. armigera (from Twine 1978).

Instar	% of larval stage [+] (average for all rearing temperatures)[*]	S.E. of proportion average (as % of average)
1	7.78	0.39 (5.0)
2	7.74	0.45 (5.8)
3	12.16	0.96 (7.9)
4	14.15	0.44 (3.1)
5	15.86	1.74 (11.0)
6	26.93	1.30 (4.8)

[+] larval stage included pre-pupal stage which accounted for 15.4%.

[*] six temperatures used - 13.1, 18.5, 21.3, 24.6, 27.1, 33.9°C.

The assumption was therefore made that the proportion of the larval stage occupied by each instar of H. armigera was the same for H. punctigera. Using the relationships between larval development and temperature as determined by Cullen for H. punctigera and temperatures to which the larvae were exposed in the field, it was possible to determine the proportion of larval development that had been completed over one day. (This was based on two-hourly mean air temperatures as described by Andrewartha and Birch (1954), and was based on data obtained from the M.E.S. thermograph). These values were accumulated until the appropriate percentage development for a particular instar was reached. At this time, the instar concerned would have moulted to the succeeding stage and the process of percentage development accumulation repeated.

Hence, provided suitable temperature data were available, it was possible to determine the time of occurrence of each of the instars (see section 3.4.3.4 on the study at Mortlock Experiment Station).

APPENDIX 10 THE NATURAL ENEMIES OF HELIOTHIS PUNCTIGERA

A wide range of natural enemies of Heliothis punctigera was recorded during the present study. Most were mentioned in Chapter 3. A complete list of species observed and the stage of H. punctigera they attack are given in Table A.10.1., which may be compared with other similar reports (e.g. Twine 1973, Bishop and Blood 1977, Room 1979).

The roles of each of the more important predators and pathogens in the mortality of H. punctigera were discussed in sections 3.4 and 3.5, but the effect of the species of parasites was considered in total only. Initially, it was planned to collect larvae of H. punctigera from the field and rear them individually to assess the relative importance of each species of parasite. However, mortality of larvae from undefined causes when reared in the laboratory was high and necessitated adopting a system whereby external signs that larvae had been attacked by parasites were used to score parasitism. Tachinids and the Ichnemonid wasp Netelia producta were the most abundant species.

Larvae of H. punctigera that were parasitised by Tachinids either had a white oval-shaped egg attached to the cuticle or, if successful entry of the fly larvae into its host had been made, an aperture with sclerotized perimeter was present in the cuticle of the larva (see Figs. A.10.1.a, b). Tachinid eggs were shed when the host larva moulted.

Wasps of N. producta attach a black kidney-shaped egg to the cuticle of H. punctigera larvae via a pedicle (see Fig. A.10.1.c and Riek 1970). This ensures the egg is not dislodged during a moult by its host, since the eggs appeared to hatch only after the larvae entered the soil to pupate. The characteristic black silk cocoons of N. producta pupae were observed while digging for H. punctigera pupae in the field (see Section 3.5) - a further indication of the presence of this parasite.

TABLE A.10.1 The natural enemies of *H. punctigera* recorded during the study.

(a) Predators

Order	Family	Species	[1] Stage of *H. punctigera* attacked
Hemiptera	Nabidae	*Nabis kinbergii* Reuter	Le_2
	Reduviidae	*Coranus* sp.	L (n.o.)
	Pentatomidae	*Cermatulus nasalis* (Westw.)	Le-m
		Oechalia schellenbergii (Guer-Men.)	Le-m
Neuroptera	Hemerobiidae	unident. sp.	E-Le
	Chrysopidae	unident. sp.	E-Le
Coleoptera	Carabidae	*Calosoma schayeri* Erich.	Le-1
	Coccinellidae	*Coccinella repanda* Thunberg	E
		Verania frenata Erichson	E
	Elateridae	unident. sp. (larva)	P
Hymenoptera	Eumenidae	*Eumenes* sp.	Lm

(b) Parasites

Order	Family	Species	Stage of *H. punctigera* when species: Attacks	Emerges
Diptera	Tachinidae	*Chaetophthalmus* sp.	Le-1	Lm-1,Pp
		Exorista sp.	Le-1	Lm-1,Pp,P
		Goniophthalmus sp.	n.o.	P
Hymenoptera	Ichneumonidae	*Heteropelma scaposum* (Morley)	n.o.	P
		Lissopimpla excelsa (Costa)	n.o.	P
		Netelia producta (Brulle)	L1	Pp
		Pterocormus promissorius (Erichson)	n.o.	P
	Braconidae	[3] *Microplitis* sp.	Le	Lm
	Scelionidae	[4] *Telenomus* sp.	n.o.	-
	Trichogrammatidae	[4] *Trichogramma ivelae* Pang and Chen	Ee	-
(Nematoda)	Heterorhabditidae	*Heterorhabditis bacteriophora*	n.o.	P

TABLE A.10.1. cont'd.

(c) Pathogens

Type			Stage of *H. punctigera* from which pathogen recorded
Virus	-	NPV	L
		GV	L
Fungus	-	*Beauveria bassiana* (Bals.)	L
Bacterium	-	*Bacillus thuringiensis* Berliner.	P

1. stages of *H. punctigera*: E = egg; e - early stage
   L = larva; e - instars 1, 2
   m - instars 3, 4
   l - instars 5, 6
   Pp = mature larva - prepupa
   P = pupa

2. n.o. = no observations made

3. identification of this species not confirmed

4. M. Carver (pers. comm.)

FIGURE A.10.1.

Signs of parasitism of larvae of *H. punctigera*

(a) Tachinid egg externally.

(b) Aperture in larval cuticle of host associated with the presence of a Tachinid larva internally (posterior of fly larva with spiracles held next to the aperture in heavily sclerotised cone-shaped tissue apparently secreted by the parasite).

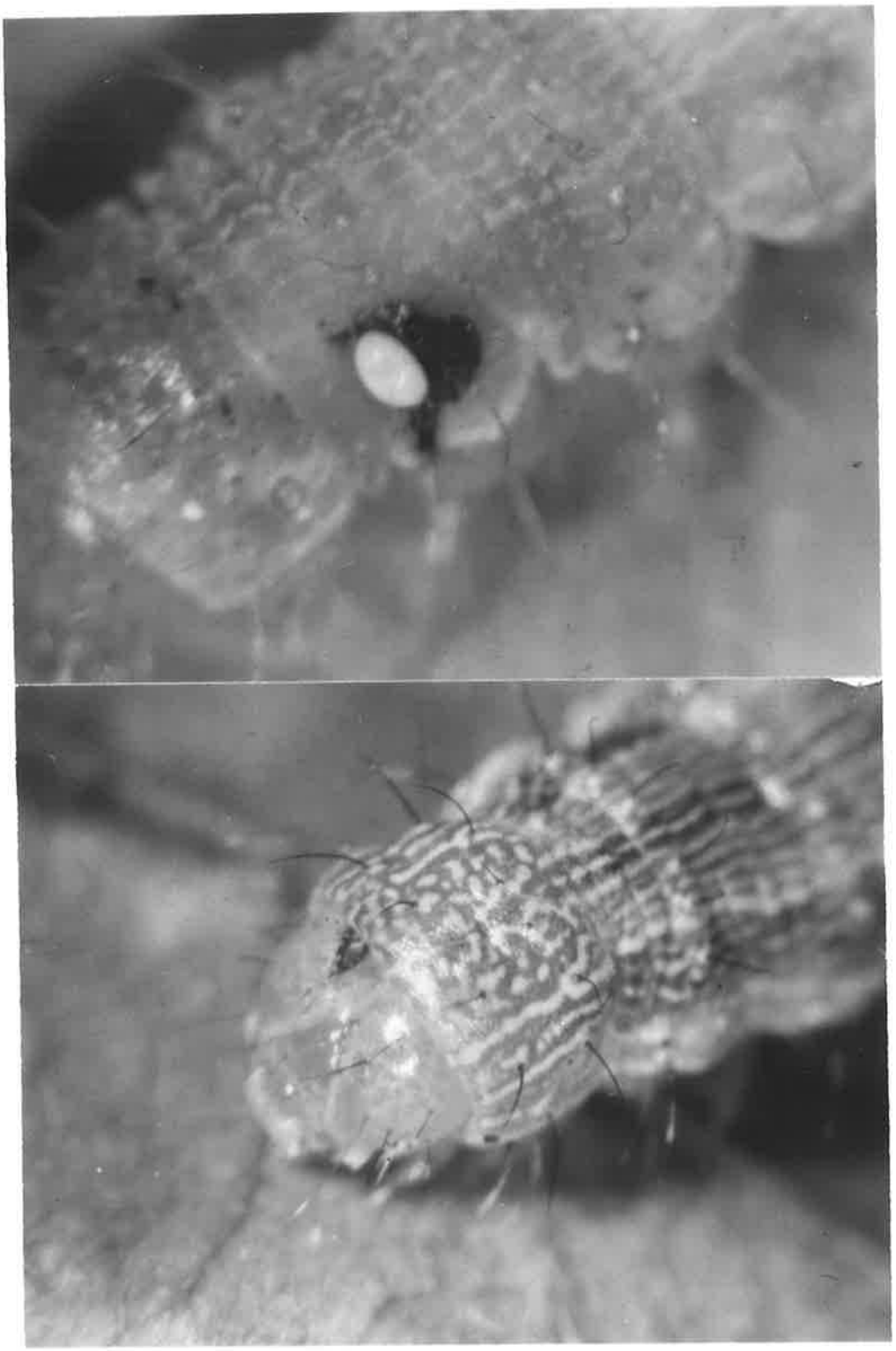


FIGURE A.10.1. (cont'd.)

(c) eggs of the wasp Netelia producta

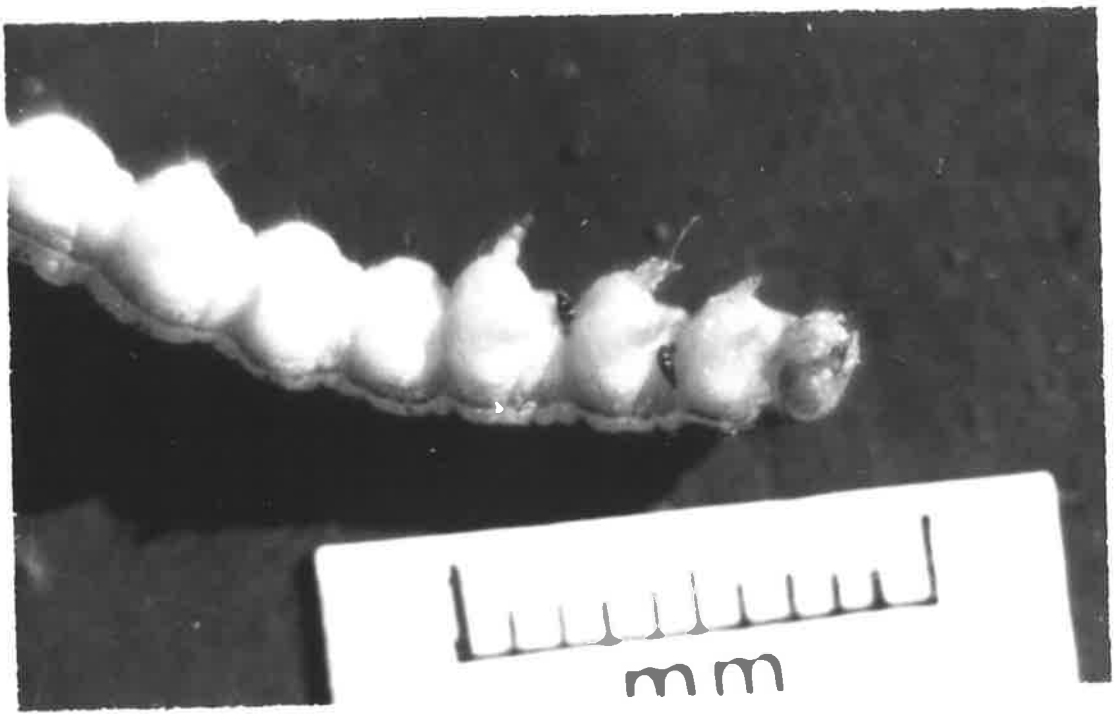
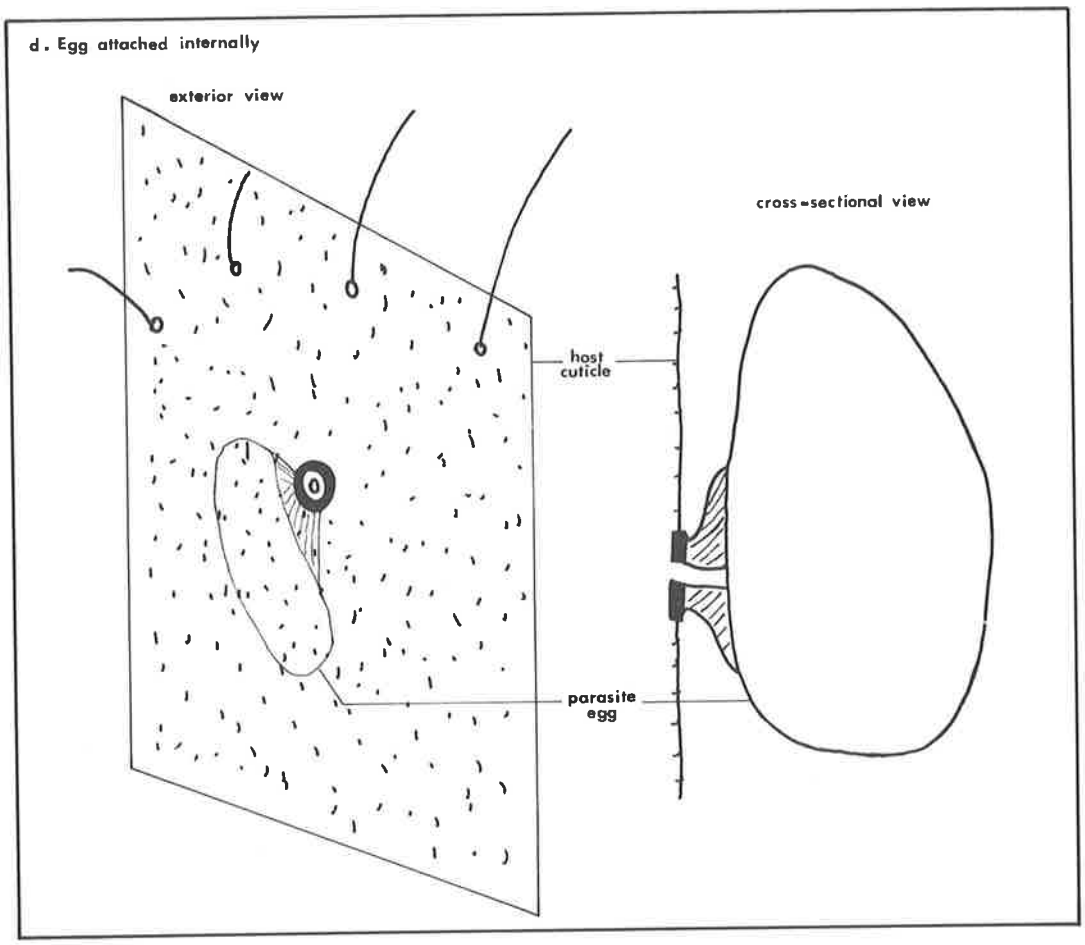

mm

FIGURE A.10.1. (cont'd.)

(d) diagrammatic representation of the attachment of an egg to the interior surface of the host cuticle by an unidentified parasite.


d. Egg attached internally
exterior view
cross-sectional view
host cuticle
parasite egg

Another sign considered to indicate parasitisation of larvae was the presence of a black ovoid-shaped egg attached internally (see Fig. A.10.1.d). The identity of the parasite(s) involved was not determined.

The inability to distinguish species using the above technique (except for N. producta), precluded any studies on their individual importance. For this reason and considering the high mortality rate of larvae reared in the laboratory, parasitism was therefore considered in total.

An indication of the relative importance of the above three groups of parasites and the stage of larvae attacked was provided by observation on the larvae collected at Booborowie (see Table A.10.2). For comparison, a recent detailed study by Broadley (1984) on parasites of Heliothis spp. in Queensland may be examined.

TABLE A.10.2. The proportion of H. punctigera larvae collected at Booborowie that were parasitised - according to the presence of certain indicators (see text).

	Parasite species and rate of parasitism (%)				
				all parasites	
H. punctigera instar	Tachinidae	N. producta	unident. (egg internally)	no. larvae collected	% parasitism
1-3	0.8	0	0.7	861	1.5
4	2.0	0.1	1.4	733	3.5
5	4.5	0.4	2.6	530	7.5
6	3.6	8.8	2.4	419	14.8
average for all instars	2.4	1.6	1.6	2543	5.6

In order to undertake detailed studies such as those required for the construction of life tables, more information concerning the parasites is required. Some of these aspects include the identification of the

particular species of Tachinidae involved (e.g. studies on egg architecture and larval mouth-hooks to determine species); the fate of larvae parasitised in the early instars (e.g. at what instar does the fly emerge i.e. when is the host killed). Other unknowns include the identification of the parasite(s) responsible for the egg laid internally and signs of parasitism relevant to wasp species other than N. producta.

The daily cumulative proportion of larvae that succumbed to NPV in the field collections described in Chapter 3 (see Figures 3.10 and 3.10) was examined to determine whether mathematical equations could be used to describe or model it.

The nature of the curves suggested that asymptotic regression (i.e. Mitscherlich's curves) might be appropriate. Parameters of such regressions for collections providing sufficient data for analysis are given in Table A.10.3. Residual mean squares of experimental error provide the best guide as to the goodness of fit but without similar analyses being found in the literature it is not possible to compare the parameters to make conclusions about the appropriateness of the fit. Certainly the larger residual mean squares for collections 1-3 and 1-3-1 indicate a poorer fit, (probably as a result of the high mortality in the first days of incubation in the laboratory). Also it is apparent that as the number of residual degrees of freedom increases, so the residual mean square for experimental error decreases.

Further data are required before the appropriateness of this or other models can be determined.

TABLE A.10.3 Parameters of Mitscherlich's curves derived for the data on cumulative daily NPV mortality described in Figures 3.10 and 3.13.

Collection details	*P1 (asymptote)	*P2	+RMS	xRDF
Fig. 3.10				
Temp. 19°C	68.91 (4.53)	0.59 (0.11)	11.81	3
Temp. 25°C	81.89 (0.77)	0.81 (0.04)	3.43	8
Fig. 3.13				
1-2	35.38 (1.16)	0.14 (0.01)	2.37	12
1-3	71.13 (3.87)	0.55 (0.13)	39.33	7
1-3-1	70.19 (3.55)	0.56 (0.20)	41.65	7
2-3	29.29 (0.89)	0.41 (0.04)	1.89	7
2-4	79.96 (1.14)	0.55 (0.04)	8.25	10

Mitscherlich's curves of the form Y = P1 (1-exp (-P2 Time)).

All curves pass through the origin.

* Numbers in brackets are standard errors

+ RMS = residual mean square of the experimental error

x RDF = residual degrees of freedom of the experimental error.

APPENDIX 11 STATISTICAL ANALYSIS OF THE INTERACTION AMONG LARVAL DIET - REARING TEMPERATURES - AND LARVAL MORTALITY CAUSED BY NUCLEAR POLYHEDROSIS VIRUS (NPV)

Analyses of data in Table 3.9; based on independence tests of the data arranged in r x 2 contingency tables using X^2 statistic; see Steele and Torrie (1960).

i) 6 x 2 contingency tables, environment (diet/temperature combination) v^s mortality.

$$X^2 = 7.34 \text{ n.s.}$$

ii) 3 x 2 contingency table, diet (at 25°C) v^s mortality.

$$X^2 = 0.15 \text{ n.s.}$$

iii) 3 x 2 contingency table, diet (at 19°C) v^s mortality.

$$X^2 = 0.61 \text{ n.s.}$$

iv) 2 x 2 contingency table, temperature (lucerne diet) v^s mortality.

$$X^2 = 3.07^*$$

v) 2 x 2 contingency table, temperature (artificial diet) v^s mortality.

$$X^2 = 1.66 \text{ n.s.}$$

vi) 2 x 2 contingency table, temperature (artificial diet without formaldehyde) v^s mortality.

$$X^2 = 1.85 \text{ n.s.}$$

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