Studies on the biology of immature stages of the buffalo fly *Haematobia irritans exigua* de Meijere (Diptera:Muscidae)

Thesis submitted by

Ian Murray Cook, M.Sc. (Qld.)

in March 1980

as partial fulfilment of the requirements for the degree of Doctor of Philosophy in the department of Zoology, James Cook University of North Queensland.

I, the undersigned, the author of this thesis, understand that the following restriction placed by me on access to this thesis will not extend beyond three years from the date on which the thesis is submitted to the University.

I wish to place restriction on access to this thesis as follows:

> Access to be restricted to staff and students of the James Cook Univeristy of North Queensland for a period of 3 years.

After this period has elapsed I understand that the James Cook University of North Queensland will make it available for use within the University Library and, by microfilm or other photographic means, allow access to users in other approved libraries. All users consulting this thesis will have to sign the following statement:

> "In consulting this thesis I agree not to copy or closely paraphrase it in whole or in part without the written acknowledgement for any assistance which I have obtained from it."

March, 1980

DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institute of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references given.

Ian Murray Cook
March 1980

ACKNOWLEDGEMENTS

My sincere thanks are due to the following people:

Dr R.E. Jones, Dr I.R. Bock and my associate supervisor, Associate Professor R.P. Kenny, for their advice on all aspects of the project and their valuable criticism of the manuscript;

my room-mates, Dr I.J. Kneipp and Dr P. Arnold for stimulating discussion and for their tolerance of my laboratory experiments;

Mr P. Archer, manager of Pioneer Stud, for permission to collect field samples from his property;

Mr R. Dixon, Mr L. Whiteman, Mr J. Hodgkinson and the staff of the C.S.I.R.O. field station at "Lansdown" for their interest in the project and their help with collections of flies;

Mr F.J. Schmidt for assistance with fly collection and management of stalled cattle;

Professor R.S.F. Campbell, of the Department of Tropical Veterinary Science, James Cook University, for generously allowing access to his department's facilities;

Mr D.F. Sinclair for his helpful advice on statistical matters; and

Dr A.V. Spain, my friend and supervisor, for his patience, vigorous participation and unwavering support.

ABSTRACT

This thesis reports the results of experiments designed to provide information on the developmental biology of the immature stages of the buffalo fly. It is divided into six chapters dealing respectively with rates of growth in relation to substrate temperature and moisture, the effects of variation of these two factors on mortality, the effects of sub-lethal temperature and dung moisture conditions on pupal size and shape, the potential significance of the pH and osmotic concentration of dung fluid, the physical nature of cattle dung and its behaviour in the field, and seasonal variation in adult fly numbers.

The durations of individual pre-imaginal stadia were measured at a number of constant temperatures. The rate of development of eggs was not linearly related to temperature, and asymptotic equations were calculated to relate temperature to elapsed time for all instars. Durations of combined pre-imaginal stadia for male and female flies were 777.7 and 745.2 hours respectively at a constant temperature of 17.5°C, and 192.7 and 184.4 hours at 35.0°C. The combined pre-pupal stadia were 584.9 hours at 15.0°C, but pupae did not survive to adulthood at this temperature. Rates of growth were reduced if dung moisture fell below 85 per cent. of wet weight.

Several aspects of the physical and physico-chemical nature of dung were examined as possible sources of mortality. These were temperature, moisture content, pH and osmolality. Eggs survived temperatures between 11.0 and 37.0°C, while larval and pupal development was restricted to temperatures between 15.0 and 35.0°C. Larval

growth ceased if dung moisture fell to 64 per cent. of wet weight. At near-lethal levels of temperature or dung moisture, the size and shape of pupae were also affected.

Eggs hatched normally within the pH range 4.5 to 9.0, and larvae survived between 5.8 and 9.0. There was no effect of osmolality on hatch within the range 62.5 - 980.0mOsm, and larvae pupated successfully between levels of 62.5 and 510.0mOsm. Mortality among eggs and pupae increased if humidity levels fell below levels close to saturation. Eggs were also killed by even short periods of immersion in water, and very few survived if the immersion period approached the duration of the total pre-hatch period.

The nature of these potential mortality factors in dung was evaluated. Naturally occurring levels of pH and osmolality did not approach lethal levels, either in fresh dung or after periods of exposure. Temperatures within dung pads frequently exceeded upper lethal limits for all instars, and must be considered a probable cause of mortality. Dung moisture was found to fall quickly below the lower limit for larval growth. Estimations on rate of moisture loss from dung and growth rate of flies were made using results of these experiments, and data on fly populations and weather records from Rockhampton. Pre-imaginal stadia appeared to increase about eight-fold in exposed pads and ten-fold in shaded pads during winter, compared to those of summer. However, the rate at which pads lost moisture did not vary much in monthly estimates made for the period from April to November. It is therefore likely that larvae die in winter because dung dries to lethal levels before they have developed to pupation.

CONTENTS

CONTENTS (Continued) Page

CONTENTS (Continued) Page

CONTENTS (Continued) Page

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF PLATES

INTRODUCTION

The buffalo fly, *Haematobia irritans exigua* (de Meijere) (Diptera : Muscidae), is an introduced parasite of cattle, buffaloes and horses in tropical Australia. Adults live on the host animal and feed by lacerating the skin and imbibing from the resulting pool of tissue exudate. The larval stages are passed only in the dung of cattle and buffaloes. Current control measures rely heavily on the use of chemicals. The future role of chemicals as a control measure may be limited by environmental considerations, for technical reasons such as resistance or by the cost of the high labour component of treatment operations.

There is little detailed knowledge of the developmental biology of this species in spite of its economic importance. This thesis attempts to remedy this deficiency in part and reports the results of a number of experiments designed with this in mind. The first chapter describes the effects of substrate temperature on the rates of development of immature stages. In the second, mortality levels for these stages are determined over a range of combinations of substrate moisture and temperature. The third chapter reports the results of a morphometric study of the puparium and its variation in shape and size as affected by temperature and moisture conditions during larval development. Chapter four describes the effects of other factors of potential importance to development of immature stages. These are immersion of eggs, and the pH and osmolality of the substrate before hatch and during larval growth. In experiments described in chapter five, dung under field conditions was monitored with respect to all of the above factors to set the laboratory studies in a field context. Measurements of moisture, internal temperature, pH and osmolality were made on fresh dung, and on dung after various periods of exposure to measured atmospheric conditions. In the final chapter a

series of counts of fly numbers, made weekly over two years, is reported. Flies were counted on a herd of British-bred cattle, and on two herds of Africander-cross cattle, to provide a measure of breed difference in attractiveness to flies, and to illustrate variability in seasonal levels of fly numbers.

Modern pest management systems require detailed knowledge of the population dynamics of the pest species. The purpose of this study was to provide some knowledge of two important aspects of buffalo fly biology *viz.* the rate at which a generation of flies will develop, and the extent to which some environmental factors cause mortality among pre-imaginal stages.

REVIEW OF LITERATURE

1. Taxonomy

1.1 Systematic position *Haematobia irritans exigua*

Family : Muscidae

Sub Family : Stomoxyinae

Genus *Haematobia* Lepeletier and Serville, 1828.

1.2 Synonymy The buffalo fly *Haematobia irritans exigua* de Meijere was so named by Snyder (1965), being designated by this author as a sub-species of the horn fly *Haematobia irritans* Linnaeus. It was first described by de Meijere (1903) and named *Haematobia exigua* de Meijere. The full synonymy is presented below:

Haematobia exigua de Meijere, 1903, in Schat: Meded. Proefst. Oost-Java, III (44) : 17.

Lyperosia flavohirta Brunetti, 1910 : Rec. Ind. Mus. IV : 89.

Lyperosia exigua (de Meijere) : Summers, 1912 : Ann. Mag. Nat. Hist. 9 (10) : 506.

Siphona exigua (de Meijere) : Norris, 1946 : J. Counc. scient. ind. Res. Aust. 19 : 65.

Haematobia irritans exigua de Meijere : Snyder, 1965 : Ins. Micronesia 13 (6) : 194.

2. Distribution

Buffalo fly has been reported as indigenous to India (Malloch, 1932; Mackerras, 1933; Snyder, 1965), Indonesia (Mackerras, 1933), the Philippine Islands (Malloch, 1932; Snyder, 1965) and northern China (Anon. 1934). Areas to which the fly has been introduced include Australia (Tillyard, 1931), the Solomon Islands and New Britain

(Mackerras, 1933). It is the only representative of the genus *Haematobia* in Australia (Pont, 1973). The species described by Malloch (1932) as *Haematobia (Haematobia) australis* sp. nov., collected near Darwin, Australia, was probably *H. irritans exigua*. Snyder (1965) stated that the taxonomic relationships of the Australian, Micronesian and Asian populations could be clarified only by rearing and cross-breeding. Considering that the southern limit of distribution in Australia is approximately 23° South, the record from Northern China indicates either that there are physiological strains of *H. irritans exigua*, or more probably that a formal taxonomic distinction exists, as yet unclarified in the literature.

The history of the introduction of the buffalo fly into Australia has been documented by Tillyard (1931) and reviewed by Roberts (1952) and Seddon (1967). Flies are thought to have entered the country on domestic buffalo, imported from Timor and landed on Melville Island in 1825. Following this introduction, little note was taken of their presence until Gilruth (1912, in Tillyard, 1931) reported large numbers on cattle in the Northern Territory.

Observation of the biology of the species was initiated in 1929 (Anon, 1929), in Darwin, N.T. and in Timor and Java. These latter locations are areas of natural occurrence of buffalo fly. Subsequent observations of the spread of flies to the eastern and western coasts of Australia were recorded by Anon (1931) and Jenkins (1945) and are reviewed by Roberts (1952) and Seddon (1967). Both of these authors consider the fly to have reached its southern limit in Queensland at latitude 23° South, a limit predicted by Handschin (1932 a) following laboratory studies of the temperature relationships of immature stages. Earlier speculation that buffalo fly could spread to Sydney (Anon, 1931) appear not to have been correct. Populations have recently been reported

in northern areas of New South Wales (Anon. 1978). Possible reasons are the evolution of cold-tolerant strains or periodic recruitment of flies on cattle travelling south.

There is little recorded information on the speed at which buffalo fly infestation can spread. Ferrar (1969) reported a natural reinfestation of Magnetic Island by flight over at least 7.2 km of open water. The colonisation of Mornington Island over 32 km of water was also recorded (Anon, 1931). This latter account also noted that an infestation had spread 135 km in a single wet season in the Northern Territory. There was no indication whether the infestation was spread by flight, or by transport on travelling cattle.

3. Biology

The life cycle and general biology of the buffalo fly were described by Hill (1917) and Handschin (1932). Adult flies rarely leave the host animal, except to oviposit on freshly-dropped dung. It was reported by Hill (1917) that females visited the dung pad for several minutes only before returning to the host and in this time deposited up to seventy eggs on the pad. On hatching, larvae move into the dung pad, and feed there until pupation. There are three larval instars. Pupation occurs in the pad, or in soil under or surrounding the pad. Buffaloes are the original host and were reported by Hill (1917) to suffer less damage to hides than do cattle. Some individuals in a herd of cattle may be more attractive to flies than others. Tillyard (1931) observed that in herds of mixed cattle, entire bulls carried more flies than cows, steers or calves. Hill (1917) further noted that particular animals, regardless of sex, appeared more attractive to flies, and consistently carried heavier fly burdens. Handschin (1932) also observed higher fly numbers

on animals in poor condition. Krijgsman and Windred (1933) found that buffalo dung was preferred over that of domestic cattle for oviposition sites. Despite the well-established relationship between fly and buffalo, populations are maintained very successfully on domestic cattle in the absence of buffalo. These authors also found that flies bred in the dung of horses, pigs, rabbits and wallabies, if the dung remained sufficiently moist while larvae were growing. There are, however, no reports of natural infestations of buffalo flies being maintained in the dung of these animals.

The face fly, *Musca autumnalis* de Geer, is similarly restricted to a few types of dung because of moisture considerations. Bay *et al.* (1968) found that the dung of sheep and deer was not attractive as an oviposition site, nor was it normally suitable for development of larvae. However, if such dung were artificially moistened it became suitable as a rearing medium.

Quantitative studies on the effects of temperature and moisture on survival and rate of development were conducted by Handschin (1933), Krijgsman and Windred (1933) and Sardey and Thakare (1977). Handschin's study concerned the effects of temperature on duration of the life cycle, and the lethal effects of low temperature. Krijgsman and Windred observed survival of eggs and pupae under varying humidity regimes, and made some measurements of the ability of larvae to survive under varying conditions of dung moisture. They also studied the lethal effects of immersion of eggs, larvae and pupae in water. Sardey and Thakare reported the effects of temperature and atmospheric humidity on the speed of development and survival of larvae.

Plate 1. Hide damage due to feeding by adult buffalo flies.

1a. Fresh lesions

1b. Healed lesions

1c. Oedematous reaction

Plate 1a

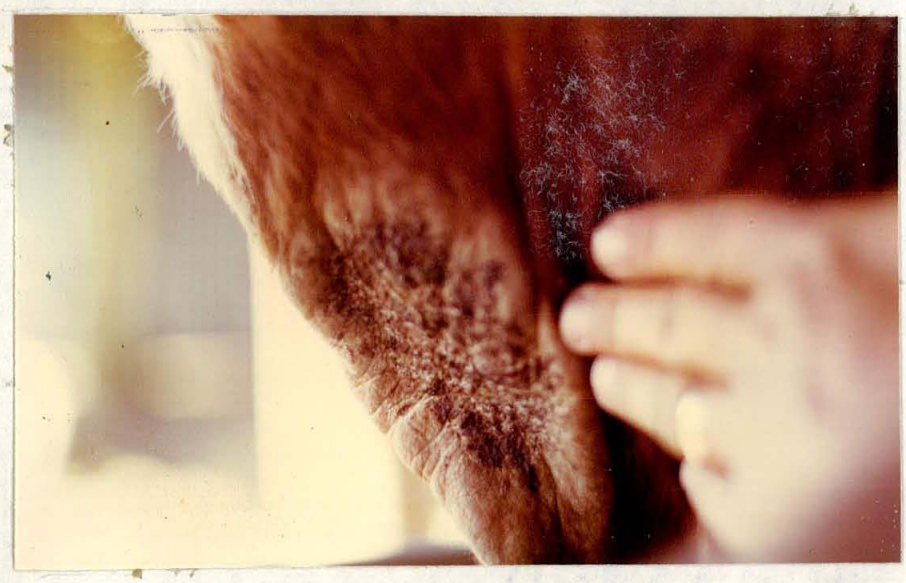


Plate 1b

Plate 1c

Some of the experiments by the above authors were repeated in this study in closer detail. Their reported results are dealt with in the appropriate chapters.

4. Effects on host

The effects of flies on host cattle were described by Handschin (1933a). The biting by flies, and the efforts of the animal to relieve the irritation by rubbing against solid objects and by striking with hind legs, produce areas from which skin and hair may be completely removed (Plate 1a). When these lesions heal, the affected area remains hairless and calloused (Plate 1b). An oedematous reaction may accompany the formation of such lesions (Plate 1c). The speed with which lesions form was demonstrated in a report by Norris (1946) that an estimated 100-200 flies (a light infestation), caused visible lesions on cows in five days. Loss of blood to buffalo flies has not been measured, but McLintock and Depner (1954) reported an estimated loss of fifteen ml per animal per day due to the feeding of "abundant" horn flies. Harris and Frazer (1970) recorded intakes of blood by horn flies in the laboratory as being 9.9 - 14.9 mg per fly per day for males, and 10.7 - 21.3 mg for females. They concluded that this rate of removal of blood would not affect animal production and that losses were probably caused by irritation, and its effects on feeding behaviour and milk production.

Loss of production due to infestations of buffalo fly has not been reported. However, loss due to effects of other biting flies has been measured, in both milk and meat production. Cutkomp and Harvey (1958) controlled populations of horn fly and stable fly, *Stomoxys calcitrans* L., on beef cattle, and measured a significant improvement in weight-gain in two out of three years. Steelman *et al.* (1972) and

Cheng (1958) were similarly able to demonstrate an increase in weight-gain by protecting cattle from mosquitoes, and from a complex of horn flies and stable flies. Further work by Steelman *et al.* (1976) and Steelman and Schilling (1977) demonstrated the cash gains resulting from protection of cattle against mosquitoes. This work was based on the use of thresholds of infestation, above which loss of production occurred. There was less injury caused by mosquitoes to cattle with some Brahman ancestry than to those of pure Hereford breeding. Protection against mosquito attack thus was only a financial advantage where animals were inherently susceptible.

Granett and Hansens (1957) reported an increase in milk yield from dairy cattle when protected from a complex of biting flies which included horn flies, tabanids and mosquitoes. However, Miller *et al.* (1973) were able to detect depression of milk yield by infestations of up to sixteen hundred stable flies per animal in only one out of six trials.

The buffalo fly does not appear to transmit diseases of domestic livestock. Johnston and Bancroft (1920 b) discussed its possible role in the transmission of the equine stomach worms *Habronema* spp. (Nematoda). These authors pointed out that buffalo flies breed in horse dung, and will feed on horses. Other muscid flies are known to be intermediate hosts of these parasites (Roberts, 1952). There is, however, no evidence of transmission of *Habronema* spp. by buffalo flies. Experiments to test whether buffalo flies could transmit bovine onchocerciasis were conducted by McEachran and Hill (1915) and reviewed by Johnston and Bancroft (1920 a). No transmission occurred.

It has been claimed that buffalo flies are theoretically capable of acting as vectors of anthrax (Handschin, 1933a) and surra (Handschin, 1933a; Gee, 1970). These claims have not been tested, and there are no records implicating the flies in the etiology of these or other infectious diseases of livestock.

5. Control

Despite the undefined economic status of the buffalo fly, much research has been conducted on control measures. It was observed (Anon, 1928, 1929) that fly numbers in Java were generally low, despite the ready availability of bovine hosts. A project was initiated to study the possible biological control of buffalo flies in Australia using the parasites and predators considered to be responsible for controlling fly populations in Java (Anon, 1930). Hill (1917) had reported several species of ants preying on eggs, and Johnston and Bancroft (1920 b) noted the existence of hymenopterous parasites of other muscoid flies in eastern Australia. Low levels of parasitism of buffalo fly pupae by unnamed species of ichneumonid wasps were found (Anon. 1928) in Java, but there is no record of attempts to introduce these into Australia. A wide range of parasites was studied by Ferriere (1933), Scheerpeltz (1934) and Handschin (1932, 1933, 1934a, 1934b), with most emphasis placed on species of the genus *Spalangia* (Pteromalidae : Hymenoptera). These parasites were *Spalangia sundaica* Graham and *Spalangia orientalis* Graham. *S. orientalis* was found in Australia, and *S. sundaica* was introduced. A hybrid of these two species was also bred, and released in Australia (Handschin, 1933). Subsequent investigations by Campbell (1938) indicated that *S. sundaica* had not persisted in the release areas. *S. orientalis* was found, but there was no apparent alleviation of the buffalo fly problem.

Attention turned to chemical control (Anon. 1944) with the establishment of a committee by C.S.I.R. and the Queensland Department of Agriculture and Stock. This committee initiated investigation of the use of D.D.T. to control buffalo fly. The method of application, and effectiveness of D.D.T. were described by Norris (1946, 1947), and the status of the chemical for ectoparasite control was reviewed by Mackerras (1947). Until withdrawn for environmental reasons, D.D.T. was the standard treatment for buffalo fly. It has been succeeded by other chemicals (Nolan and Annand, 1963, Hurwood, 1968) and the use of these, as back-sprays or in self-treatment stations, remains the currently accepted control measure.

The use of non-parasitic competitors in dung to reduce survival of buffalo fly larvae was suggested by Tillyard (1931). The principle was demonstrated by Blume *et al.* (1970) who found that the presence of other arthropods in dung caused mortality among the larvae of the horn fly *H. irritans irritans*. The predatory beetle *Pachylister chinensis* was introduced into Australia as a possible control for buffalo fly. Four years after introduction it had made little progress (Bornemissza, 1968). A programme to introduce coprophagous beetles was initiated by C.S.I.R.O. (Anon, 1968) and is still in operation. The aim of the introduction was to reduce buffalo fly numbers by destruction of dung pads during larval development. The actual mechanism was described by Ferrar (1973, 1975) and Macqueen (1975). The dung burying beetle *Onthophagus gazella* Fabricius is capable of preventing the development of the bush fly, *Musca vetustissima* Walker, under insectary conditions (Bornemissza, 1970). This beetle, indigenous to Africa, is now successfully established in northern Australia, but buffalo flies still infest cattle in this region, in large numbers.

It has been shown that at times of high activity of *O. gazella*, survival of immature buffalo flies in dung is reduced (Anon, 1976). However, when beetles were numerous and active, numbers of adult flies on cattle remained high (Anon., 1977). This apparently was due to recruitment from neighboring herds of cattle in areas where beetles were less active.

TECHNIQUES

1. Laboratory propagation

There is no record of the successful laboratory-rearing of *H. irritans exigua* but a technique for continuous propagation of the horn fly *H. irritans irritans* was described by Depner (1962). The method involved rearing flies on a live steer, enclosed in a screen cage. Use of this system was not practicable in the present study. Further work on the horn fly resulted in development of a rearing procedure which did not involve the use of live cattle as a source of blood meals for adult flies. Schmidt *et al.* (1967) presented adult flies with bovine blood, A.C.D. anticoagulant, nystatin and chloromycetin. The flies fed, and subsequently produced viable eggs. From these eggs developed adults which in turn produced viable eggs when fed in the same manner. Schmidt *et al.* (1968) later modified the diet slightly by replacing the sodium citrate component of the anticoagulant with potassium oxalate. This change produced more consistent egg production. Other changes included the addition of penicillin to retard decomposition of blood. Horn flies were also successfully fed on bovine blood by Harris and Frazar (1970), in a quantitative study of intake.

In the course of the present study, an attempt was made to feed field-collected adult buffalo flies on bovine blood, using the method of Schmidt *et al.* (1967). The flies did not feed, and survived approximately 24 hours after collection, as was normal for unfed flies. Handschin (1933a) found that females which died in captivity were exhausted of mature eggs, and stated that further feeding, and possibly further copulation, might be necessary for further egg production. Tillyard (1931) also suggested that females probably needed to feed continuously to

ensure survival and continued production of eggs. Eggs laid by field-caught flies were therefore used in all experiments.

2. Field collection of flies

Throughout the study, eggs were provided by flies swept from the backs of several herds of Droughtmaster steers. The flies were anaesthetised in the net with carbon dioxide, and transferred to humidified, lightly-ventilated plastic food containers. They were then transported to the laboratory and held at 24°C for egg-collection.

Flies were held in the laboratory in two-litre clear plastic containers, each of which held approximately 300 flies. It was found that longevity of flies was reduced at higher densities. Overcrowding was avoided in case of possible reduction in fecundity, although Schmidt *et al.* (1973) found that there was no effect of crowding on egg-production by horn flies.

3. Collection of eggs

Eggs were deposited by flies on the walls of the containers. When eggs were due for collection, the flies were again anaesthetised with carbon dioxide, and transferred to a new container. Eggs were washed from the container-walls with a wash-bottle, and the suspension of eggs in water was transferred to a paper towelling filter. When the water had passed through the filter, the sections of towelling on which the eggs had been deposited were placed in sealed humidified plastic boxes. They were held in these boxes until transferred to the breeding medium prior to hatch.

4. Rearing of larvae

Larvae were usually reared to pupation in dung-filled containers. Sections of paper-towelling carrying the appropriate numbers of eggs were excised from the main sheet, and placed on the surface of the dung, with eggs uppermost. The containers were fitted with tight lids, each having four ventilation holes, 2 mm in diameter.

After hatch, the towelling was removed from the dung surface with forceps, and the numbers of hatched and unhatched eggs counted under a low-power dissecting microscope. Hatching was indicated by partial detachment of the hatching panel, and the absence of the embryo. The numbers of eggs per culture were arranged to allow at least 2.0 gm dry weight of dung per larva. Bay *et al.* (1970) found that the provision of 1.8 gm per larva of the face fly *M. autumnalis* resulted in maximum pupal weight.

The quality of dung has been found to influence survival and vigour of several species of flies. Greenham (1972a) found a wide seasonal variation in the moisture and nitrogen-content of dung, from a number of pasture types and from several localities. These variations were reflected in the survival of the bush fly, *M. vetustissima*, when reared in dung of variable quality. Bay *et al.* (1969) found that moisture variation affected the size of pupae of the face fly *M. autumnalis*, and Morgan and Graham (1966) recorded that dung from cattle on a high plane of nutrition (alfalfa) produced larger pupae of the horn fly *H. irritans irritans* than did dung from cattle on a low-nutrition diet (praire hay).

Considering the possible effects of diet on size, survival and vigour of fly larvae, it was decided to use a standard larval diet. This diet was provided by a single Shorthorn bull, available for the course of the study. The animal was fed lucerne chaff when dung was required, and collection of dung was commenced at least five days after the change to the chaff diet. Dung was freeze-dried and stored at -10°C. When required for experimental purposes, it was reconstituted to the desired moisture level with distilled water. Unless otherwise specified, larvae were normally reared in dung with a moisture content of 85 per cent. of the wet weight.

In some experiments rearing of larvae in whole-dung cultures was not suitable. For these purposes, a modification of the method described by Morgan and Schmidt (1966) was used. A mixture of 500 g of dung and 500 ml of water was homogenised in a high-speed blender. The liquid fraction of the homogenate was then separated from the fibre under pressure through coarse-mesh cotton gauze. Pads of "Cestra" surgical cotton dressing were saturated with this liquid and placed in petri dishes. Larvae were reared in the pads, after hatching from eggs on paper towelling placed on the pad surface.

Many of the experiments required that temperatures in the rearing medium be accurately known. Direct and constant measurement of temperatures in dung cultures was not feasible, but intermittent checks using a recording thermometer were made to monitor air temperatures. A preliminary experiment, described in Appendix 1, showed that internal temperatures of dung, infested with concentrations of larvae similar to those used in later experiments were the same as those of the atmospheres of the controlled temperature cabinets.

5. Collection and storage of pupae

Flies were normally separated from the dung cultures as pupae. Those which had pupated on the walls of the container, away from the dung, were removed with a wash-bottle. The dung was then broken up by hand in water, to retrieve those pupae which had formed in the dung. Pupae were collected after they had floated to the surface. This was normally carried out shortly prior to the emergence of adults, at the stage when pupae turned from brown to a dull black colour. It was found that pupae would not float if immersed soon after pupation; this made recovery from debris difficult, and introduced error in quantitative studies. Depner (1961) noted similar properties in pupae of the horn fly, *H. irritans irritans*.

On retrieval, the pupae were washed, allowed to dry briefly on paper towelling, then placed in glass tubes stoppered with nylon gauze. These were placed in humidified plastic boxes to await emergence of adults.

6. Presentation of equations

Where equations are included with graphic presentation, constants are of the form $KE \pm n$

where K = constant, and

$$E^{\pm n} = 10^{\pm n}$$

where large numbers of zeroes would otherwise appear.

CHAPTER 1: RATE OF DEVELOPMENT OF IMMATURE STAGES

INTRODUCTION

An accurate and detailed knowledge of the duration of the life cycle of a pest insect in relation to climatic factors is of critical importance to any system aimed at control by management. Of the climatic factors, temperature is undoubtedly the most important in deciding the rate of insect development through the immature stadia. Moisture is, perhaps, more important in its influence on mortality but at extreme levels has an undoubted influence on rate of development.

Handschin (1933) studied the life cycle of the buffalo fly in northern Australia in relation to temperature but did not examine the rate of development through individual larval stadia. A more recent study by Sardey and Thakare (1977) suffers from the same deficiency in addition to insufficiently intensive observations. Most reports of holometabolic species treat the total duration of the larval stadia as a single interval. Tsitsipis and Mittler (1976) and Iheagwum (1978) measured durations of individual pre-imaginal stages of *Aphis fabae* Scop. (Hemiptera: Aphididae) and *Aleyrodes prolettella* (L.) (Hemiptera: Aleyrodidae) respectively, reared at a number of constant temperatures. In the work reported in this chapter, development rates of individual instars were studied; there is no apparent basis for assuming that such characteristics are uniform for all immature stages.

REVIEW OF LITERATURE

1. The hyperbolic function

The relationship between temperature and the speed of development of insects was recognised initially in the Development - Temperature rule quoted by Bodenheimer (1925) i.e.:

Temperature x Time = Constant.

This author pointed out the error in this rule, which postulated a constant value for the product of temperature and development time. Bodenheimer adopted Blunck's correction, which allowed for a development threshold, or "critical cold point". The development constant then became the product of time and temperature above threshold.

Handschin (1933a) used this technique to fit a rectangular hyperbola to his data on the buffalo fly *H. irritans exigua*. His calculated lower threshold(the asymptote of the hyperbola) was $11^{o}C$, and the development constant was $249.6D^{o}_{11}$ (degree-days above a threshold of $11^{o}C$). However, his observations on the geographical distribution of *H. irritans exigua* indicated that maintenance of populations was not possible where temperatures fall below $20^{o}C$ "for some time".

2. The linear function

The Blunck-Bodenheimer method has been further modified to facilitate estimation of threshold temperatures and thermal constants. The curve, if hyperbolic, may be rectified by plotting temperature and the proportion of pre-imaginal development per unit time, rather than the absolute development period. The threshold temperature, below which no development occurs, is the X-intercept, and the

thermal constant the reciprocal of the slope of the fitted straight line.

Butler (1966) observed the speed of development of eggs and nymphs of six species of predaceous Hemiptera. Linear regressions were fitted using this technique, and the associated R^2 values indicated close fit of data to the linear model. No lower thresholds were calculated, although this could easily have been done from the regression equations. Chiang and Sisson (1968) used a similar rectification technique in a study of eggs of *Diabrotica longicornis* (Say) (Coleoptera: Chrysomelidae). A lower threshold of 52°F (11°C) was estimated by extrapolation, but as no regression equation was reported, linearity was apparently assumed, and a line fitted by eye.

Dunbar and Bacon (1972) studied the development of eggs and nymphs of *Geocoris atricolor* Montandon, *G. pallens* Stål and *G. punctipes* (Say) (Hemiptera: Lygaeidae). These authors fitted linear equations to rectified data, but recognised the possibility of sigmoidal relationships when extreme values are involved, and did not extrapolate. The lower thresholds of development were estimated experimentally. Butler and Dickerson (1972), in a study of the life cycle of *Hippodamia convergens* Guerin Meneville (Coleoptera: Coccinellidae) found that the relationship between egg development and temperature was apparently curvilinear at high temperatures. However, this was ignored and an overall linear relationship was described. Departure from linearity was not noted in observations of larval stages. Butler and Ritchie (1970) modified the units of the linear equation when applying it to observations of the life cycle of the lacewing *Chrysopa carnea* Stephens (Neuroptera: Chrysopidae).

The equation took the form:

$$\log y = a + b \log x$$

for each stage of the life cycle, where x and y represented temperature and time respectively. This relationship was assumed to be linear, and the calculations of threshold temperatures, and thermal constants, were conducted accordingly.

Eckenrode and Chapman (1971), studying the development rate of *Hylemya brassicae* (Bouche) (Diptera: Anthomyiidae) used the x-intercept method for determining threshold temperatures for eggs, larvae and pupae, where a range of temperatures for each stage was available. In one series of experiments, however, only two temperatures were used for observations on pupal development. Development threshold in this case was calculated using the Uvarov expression

$$Th = \frac{dt - DT}{d-D}$$

where d = development period at temperature t

D = development period at temperature T

and Th = threshold temperature.

There was close agreement between this calculation, and that based on the x - intercept method, described elsewhere in the same study.

Many other examples could be cited, but those above illustrate the applications of the linear function. Because of its simplicity and because it is most easily applied to the prediction of development times in the field it is preferred when field temperatures do not exceed those producing maximum development rates. Unfortunately, as shown in Chapter 6, temperatures experienced by buffalo flies frequently do exceed this limit.

3. Other non-linear relationships

Several authors have reported non-linear responses of speed of development to temperature. Bachelor and Bradley (1975) measured the pre-hatch period for eggs of *Anthonomus grandis* Boheman (Coleoptera: Curculionidae) over a range 18-36°C. The shortest period (55.9 hours), occurred at 30°C. Hatch occurred at 36°C, but pre-hatch time was 70.0 hours. The longest pre-hatch period (174.4 hours), was recorded at the lowest temperature, 18°C.

Greenham (1970) measured the effects of temperature on the speed of development of the bushfly *M. vetustissima* Walker. The most rapid embryonic development occurred at 103°F (38°C). Above this temperature, the pre-hatch period was prolonged and mortality was abnormally high. This author's later study (1972b) on the speed of larval development includes only a linear treatment of temperature and rate of post-embryonic growth.

Philipp and Watson (1971) found that the minimum pre-hatch, larval and pupal periods for *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) occurred at 83°F (28°C). Above and below this temperature, intervals were longer. Pfadt *et al.* (1975) described a similar optimum temperature effect, at 26°C, for the minimum duration of pupal interval for *Hypoderma lineatum* (de Villers) (Diptera: Oestridae). Davidson (1942) discussed a similar pattern of developmental rate in the eggs of *Drosophila melanogaster* (Diptera: Drosophilidae). Maximum rate of development occurred at 30°C. At higher sub-lethal temperatures, development was slower. Further, the curve appeared non-linear over the entire range of temperature, 15-32°C. The author fitted a logistic

curve to the data and derived the following equation:

$$Y = \frac{7.0953}{1 + e^{4.45142152 - 0.20718787\ X}}$$

where Y = average percentage of development per hour

and X = temperature in degrees Celsius.

Although the fit was good up to 30°C, points above this temperature increasingly fell below the upper asymptote, until the upper temperature treatment (33.1°C) point at which development no longer occurred was reached.

In the same study, a similar pattern was shown for the eggs of *Habrobracon juglandis* Ashm (Hymenoptera: Braconidae) when hatched at various constant temperatures. In a later work, Davidson (1944) fitted the same logistic curve to data on the rate of development of pupae of *D. melanogaster* and eggs of *Musca domestica* L. (Diptera: Muscidae), *Cochliomyia hominivorax* (Coq.) (Diptera: Calliphoridae), *Phormia regina* Meig. (Diptera: Calliphoridae), *Lucilia sericata* (Meig.) (Diptera: Calliphoridae) and *Ephestia kuhniella* Zell. (Lepidoptera: Pyralidae).

This sigmoidal relationship between rate of development and temperature has been recorded for several other species. Stinner *et al.* (1974) reported this for both larvae and pupae of *Trichoplusia ni* Hubner (Lepidoptera: Noctuidae), Sping Lin *et al.* (1954) for eggs of *Tribolium confusum* Duval (Coleoptera: Tenebrionidae), and of *Oncopeltus fasciatus* (Dalls) (Hemiptera: Lygaeidae), and Huffaker (1944) for immature stages of *Anopheles quadrimaculatus* Say (Diptera: Culicidae). In addition, this decline of the relationship at high temperatures, previously discussed for several species in the work of Davidson (1942, 1944), was noted for *T. ni*, *T. confusum*, and *O. fasciatus*.

Huffaker (1944) also fitted curves which were the reciprocals of the catenary equation:

$$t = (\frac{m}{2})\ (a^{T} + a^{-T})$$

where t = time

m = optimum temperature

$a^{\pm T}$ = temperature above or below optimum

The curves used thus accommodated the departure from the upper asymptote when observed values fall below this level. Tests for goodness of fit were not presented, but the graphic data showed little departure of observed from calculated values.

4. Fluctuating temperatures

Temperatures fluctuating about a given mean have been noted by several authors to alter the rate of development in comparison with that pertaining at the corresponding constant mean temperature. An accelerating effect of fluctuating temperatures was recorded by Messenger (1964), on *Therioaphis maculata* (Buckton) (Hemiptera: Aphididae), by Toba *et al.* (1973), on larvae of *T. ni* and by Pfadt *et al.* (1975), on the pupal development of *Hypoderma bovis* (Linnaeus) (Diptera: Oestridae). Siddiqui and Barlow (1972) investigated the reaction of *D. melanogaster* to fluctuating temperature regimes. For all temperatures tested (15-27.5^{o}C) development was faster under alternating, rather than constant, temperature conditions. This effect was greatest at an amplitude of 5^{o}C, and diminished as the amplitude decreased. Pradhan (1945) proposed a more complicated system based on acceptance of a sigmoidal response in rate of development to a range of constant temperatures. The argument proposed that, at temperatures below the point of inflection, fluctuating temperatures accelerated development in comparison with constant temperatures.

Above the inflection point, the reverse would occur. Messenger and Flitters (1959) conducted a study which supported the postulate of Pradhan. Three species of fruit fly (Diptera: Tephritidae) were investigated. They were *Dacus dorsalis* Hendel, *D. cucurbitae* Coq. and *Ceratitis capitata* (Wied). At medium temperature levels, speed of development depended only on mean temperature, the degree of fluctuation having no effect. Below these levels, fluctuating accelerated development; above them, the reverse occurred.

5. Thermal constants

The use of thermal constants depends on a linear response by rate of development to change in temperature. Their application to practical situations was demonstrated by Strong and Apple (1958). In a study of the development of *Hylemya cilicrura* (Rondam) (Diptera: Anthomyidae), these workers estimated the threshold temperature for development to be $50^{\circ}F$ ($10^{\circ}C$). The thermal constants, in day-degrees Fahrenheit above threshold, for development of eggs, larvae and pupae were then estimated, using the formula

$$K = y(t - a)$$

where K = Thermal constant in day-degrees

y = Time for development in days

t = Actual temperature

a = Threshold temperature.

Under insectary conditions the thermal constant for total development was calculated to be 572.5 day-degrees F. This was compared with 600.7 day-degrees observed in the field. Using meteorological records to monitor accumulated temperature input, it was then possible to estimate the number of generations of *H. cilicrura* which would occur in any given time.

Thermal constants can be calculated from experimental data, as the products of elapsed time and temperature above threshold; they can also be estimated as the reciprocal of the slope of the straight line fitted to the regression of rate of development ($\frac{1}{\text{Time elapsed}}$) on temperature as the independent variable. Both methods depend on the value of temperature coefficient in the equation being constant, i.e. that the data are in fact rectified by using an inverse transformation of the time variable. Estimations of thermal constants based on this procedure are thus inappropriate if the relationship of reciprocal-transformed time and temperature is not linear.

MATERIALS AND METHODS

1. Egg hatch

Eggs were collected at 15 minute intervals and placed on moist 10 cm filter papers in Petri dishes which were covered and placed in constant-temperature cabinets. During the hatching period, the percentage hatched was determined by regular inspection using a binocular microscope. Newly-hatched larvae were counted and removed from the dishes with forceps. Larvae were prevented from escaping prior to counting by a moat of free water, placed around the inner edge of the floor of the dish with a fine pipette, during preparation. Table 1 shows the temperatures at which hatch was monitored, and the frequency of inspection.

2. Larval moulting

The three larval instars were distinguished by the form of the buccal skeleton and the posterior spiracle together with the presence or apparent absence of an anterior spiracle. The latter was not observed in first instar larvae, a characteristic often considered usual in the Cyclorrhapha. Kitching (1976) reported the presence of anterior spiracle in the first instar larvae of several species of acalyptrate Cyclorrhapha but commented that, because of difficulty in seeing them under the light microscope, the diagnostic significance of this presence or apparent absence remained valid.

Eggs were collected at 15 minute intervals and placed on the cotton homogenate cultures previously described. At regular intervals during moulting, four or five replicates were sacrificed by flooding with 10 per cent. formalin. This caused larvae in the cotton pads to move out

into the liquid surrounding the pads where they quickly died. They were collected and the numbers of each instar present were counted. Initial tests with non-experimental cultures showed that very few larvae remained in the cotton pads after flooding with formalin and these may have died some time prior to flooding. An advantage of the method was that only larvae alive at the time of flooding could move out of the pad and be counted. Larvae which died in the pad prior to moulting thus escaped counting and a possible bias in favour of the pre-moult instar was avoided. This procedure was followed for monitoring both larval moults. The temperatures at which moulting was observed, and the intervals at which samples were sacrificed, are shown in Table 1.

3. Pupation

In the homogenate pads previously described, mortality during pupal formation was frequently high despite successful growth to maturity of the final larval instar. Whole dung was therefore used to monitor pupation. Eggs were collected at 15 minute intervals and placed on whole-dung cultures of 85 per cent. moisture content. Prior to egg-collection, these cultures were held for 24 hours in the constant temperature cabinets which would ultimately house them during the experiment, to allow the internal temperature to stabilise. Shortly prior to pupation, larvae migrated from the dung to the sides and inner lid-surfaces of the containers. It was thus possible to make regular counts of pupae as they appeared without disturbing the culture. Pupae were only included in the count if the puparium had tanned.

4. Eclosion of adults

Flies were reared to pupal stage in whole dung cultures. Pupae were collected from dung and containers by washing and placed in

gauze-stoppered glass tubes in a plastic container humidified by moist paper towelling. The plastic container was almost completely sealed to prevent evaporative cooling from the towelling having any effect on the temperature regime.

As adults emerged, they were periodically removed from the glass tubes, counted and their sex determined. Pilot experiments showed that carbon dioxide anaesthesia before removal caused high mortality before eclosion among remaining pupae, which were subjected to regular and frequent exposures to the gas. In the recorded experiments, adults were removed by placing an empty tube mouth-to-mouth with the opened tube containing adults and pupae. The joined tubes were then oriented to light, so that adults moved towards the light, and into the empty tube. They were then anaesthetized, counted and their sex determined. The frequency of observation and temperatures used are recorded in Table 1.

ANALYSIS OF RESULTS

Initial analysis of pre-hatch data (i.e. from oviposition to hatch) was conducted using the reciprocal of elapsed time as a measure of rate of development. This technique failed to rectify the data; therefore the use of linear regression to estimate lower threshold temperatures and thermal constants was not practicable.

The regressions of the inverse of development time on temperature for the larval instars did not depart significantly ($P>0.05$) from linearity. However, in comparison with equivalent data for eggs, there were fewer treatments at the extremes of the temperature range and it was at the extremes for eggs that departures from linearity were most apparent. Because of this and so that all life stages could be treated the same way, the relationships between temperature and speed of development were treated analytically using untransformed data.

Inspection of plotted raw data for each instar or combination of instars suggested a relationship between X (temperature) and Y (duration of stadia) such that Y tended to a horizontal asymptote as X increased, and a positive finite intercept at X = 0. Such a relationship is described by the equation:

$$Y = A + BR^X$$

where Y is the duration of a stadium and X the constant temperature. At X = 0, Y has the value (A + B). As $B<1$, Y declines as X increases and is equal to the asymptote A when $X = \infty$. The coefficient B denotes the range of the value of Y from X = 0 to $X = \infty$ and R^X is the rate of change of slope.

Fitting was performed by least squares methods. Data points were the means of replicates at each temperature weighted inversely as the variance of the means of the observations at each temperature, since coefficients of variation were not uniform over the range of temperatures tested.

RESULTS

The effects of temperature on speed of development were measured directly for the following instars, or combinations of instars:

(a) Egg

(b) First larval instar

(c) First plus second larval instars

(d) Egg, plus first, second and third larval instars

(e) Pupae (Male and female separately)

In order to describe the times at which sample egg hatch, moulting, pupation or eclosion occurred, a number of measures of central tendency were considered. These were mode, median, mean and T50, the latter being the mid-point of the interval prior to the first observation when 50 per cent. or more individuals had undergone change. There were no significant differences between any of these measures, ($P>0.05$), and T50 was used in all calculations.

The dispersion in time of egg hatch at each temperature was investigated. Initial plots of non-cumulative hatching indicated a slight right-skewness in many samples. Examples appear in Fig. 1. However, the distributions were tested and found not to depart significantly from normality. Cultures used to observe larval moultings were sacrificed on inspection. Sample sizes were too small to allow rigorous testing of normality for the distribution of larval moulting with time. There was no obvious skewness and the distribution was assumed to be normal.

A summary of results for each stadium appears in Table 2. Data for the second and third larval instars were derived by subtraction.

The estimates of the regression coefficients for each stadium appear in Table 3, accompanied by estimates of the proportions of variance (Fisher's A^2) explained by the regressions for each stadium. The regressions for eggs, larvae, pupae and total pre-imaginal stages are presented graphically in Figures 2, 3, 4 and 5 respectively.

In the case of derived means for second and third larval stadia, variances were obtained by summation of the appropriate treatment variances. Thus, data for the second larval stadium were derived from the differences between estimates of the first and first plus second stadia at the temperatures selected. The large proportions of variance accounted for by the regressions for all stadia show that most of variation in developmental time is accounted for by temperature. Table 4 contains estimated durations of the total pre-imaginal developmental period and also includes estimates made by Handschin (1933) and by Sardey and Thakare (1977).

In most cultures females emerged before males (Table 2). The distribution of pupation was always unimodal, and samples of pupae collected during early, middle and late stages of pupation yielded equal numbers of male and female flies. This indicated that males and females had similar prepupal intervals, and that the difference between the sexes in development rate was restricted to the pupal stage. Tests for displacement and lack of parallelism of the two regression lines were not significant. ($P>0.25$ in each case). However, the differences between mean pupal stadia for males and females were found to be significant, at varying probability levels (Table 5). Separate regressions for each sex are therefore presented (Table 3). Neither Handschin (1933) nor Sardey and Thakare (1977) reported this difference,

but Hoelscher and Combs (1971) recorded faster development by females of the horn fly *H. irritans irritans*.

The distribution of emergence of adults was occasionally bimodal. An example is shown in Figure 6. Peaks of emergence were always approximately twenty-four hours apart and the normal sex ratios applied to samples from both peaks. In the more usual unimodal emergences, the pupal interval corresponded to the shorter of the two intervals when bimodal eclosion occurred. Formation of pupae was always unimodal, bimodal maturation of pupae being effected only between pupation and eclosion.

DISCUSSION

1. Temperature effects

Egg hatch occurred through the temperature range from 11.5°C to 37.5°C, but at both 10°C and 40°C eggs failed to hatch. The pre-hatch intervals recorded in this study do not generally agree very closely with those of Handschin (1933a) and Sardey and Thakare (1977). Handschin's figure for a pre-hatch period of 20.9 hours at 25°C is similar to that of 21.3 hours presented here. However, the figures for 35°C differ substantially. The observations of Sardey and Thakare differ considerably from those of both Handschin and the present report. There are also discrepancies in estimates of pupal stadia and of total pre-imaginal duration. Handschin reported difficulty in temperature control during his experiments, which may account for some of the differences noted. Sardey and Thakare described observations of hatching and moulting at 24 hour intervals. Since stadia are short, it is unlikely that such infrequent observation would provide accurate estimates of development times and their variability. In the present study, eggs collected over a half-hour period hatched over a 1.5-hour interval at 35°C, and a 5.3 -hour interval at 11.5°C, and frequent observations were made within these intervals. In addition, temperatures in the present study were held to within 0.5°C of the stated value.

Results from the present study suggest that there are small differences in developmental times between male and female pupae. Neither of the other reports described this difference for *H. irritans exigua* but Hoelscher and Combs (1971) recorded faster development by females of *H. irritans irritans*, the closely-related horn fly.

Melvin and Beck (1931) and Depner (1961) reported pre-hatch periods at 25°C and 30°C for *H. irritans irritans* which are very similar to those reported here for *H. irritans exigua*. Depner (loc. cit.) recorded a total prepupal duration for *H. irritans irritans* of 112.8 hours at 30°C. This again approaches closely the 110.7 hours recorded here for *H. irritans exigua*. Melvin and Beck also estimated the total pre-imaginal interval to be a mean 238.5 hours at 30°C, without reference to any difference between sexes. Again, this figure is very close to those observed for male and female buffalo flies in the present study.

None of the above authors reported the bimodal emergence of adults described above for *H. irritans exigua*. Similar experiments were conducted by Kunz *et al.* (1977) on *S. calcitrans* and again there was no reference to bimodal eclosion, nor was there a record of differential rates of development of pupae depending on sex.

2. Temperature thresholds and thermal constants

The lower temperature thresholds for development were found experimentally to be between 10 and 11.5°C for eggs, and between 15 and 17.5°C for larvae and pupae. The details of the relevant experiments appear in Chapter 2.

Thermal constants are normally calculated as the product of time units and degrees above the lower temperature threshold. In the present study there are two difficulties; firstly eggs have a lower development threshold than that measured for the combined larval instars and for pupae and secondly the relationship between egg developmental rate and temperature is not linear. Had the study been restricted to immature stages in combination only, a single threshold

of between 15 and 17.5°C would have been determined. Because eggs can develop at lower temperatures than larvae or pupae, a single development constant for all stages is not realistic. Development times for eggs and for the rest of juvenile development need to be calculated separately and summed in order to predict generation times accurately. The fact that egg development is not linearly related to temperature means that the use of a thermal constant will also be inaccurate if applied to eggs alone.

Handschin (1933) estimated a lower threshold for the combined immature stages of 11°C from the Blunck-Bodenheimer hyperbolic equation. Assuming reliability of the experiments, and the suitability of the hyperbolic function to the data, this implies that all stages can develop about 11°C, since temperature tolerances of individual instars were not investigated. Had the experiments described in this chapter been conducted similarly on combined stages only, a threshold of 15°C would have been expected, as larval and pupal growth cease below this level.

If the higher limit of 15°C (as for larvae and pupae) were applied also to eggs in a predictive exercise, error would be incurred only between 10 and 15°C, and then only in the egg stages. From Table 2, it appears that egg development occupies about six per cent. of the total immature phase, so that error induced by over estimation of the threshold would be restricted to this section of the life cycle. Secondly, in coastal areas where buffalo flies abound in Australia (north from 23°S) minimum air temperatures are generally lower than 15°C only during the winter months and then for relatively short periods around the daily minimum. Both of these factors would

mitigate error in a predictive exercise. However, accurate meteorological records and a knowledge of the lower thresholds for eggs allow such possible errors to be avoided.

The measurement of accumulated heat input in the larval environment is dependent upon knowledge of the micro-environmental temperature relationships of dung pads. A measure of atmospheric temperature would only be of use for such measurement where this relationship has been calibrated against microenvironmental conditions. This aspect of the development of pre-adult stages is discussed in a later section concerning the physical nature of cattle dung.

3. Moisture and further temperature effects

During studies of the effects of dung moisture on larval survival it was observed that larval development was slower in dry dung. Data to illustrate this appear in Table 6. More thorough investigation, using the rearing techniques described, was not possible. In dry dung, larvae tend to pupate without leaving the dung and pupation can thus not be monitored so readily.

The effects of fluctuating temperatures on other species have been reviewed earlier in this chapter. It is possible that buffalo flies would similarly respond to temperatures which are not constant.

These two factors may modify the nature of the quantitative relationships between development rate and temperature described in this chapter, either by changing thresholds, or by affecting temperatures within dung pads. Although outside the scope of this study, investigation of these factors would enhance our understanding of the effects of temperature on growth.

CONCLUSIONS

The duration of all stadia are closely defined by the constant temperature pertaining during each stadium. The asymptotic model describes the data better than does the linear model. Rectification of the relationship by inverting stadium duration would simplify calculation of thresholds and development constants, but was not used in this study since pre-hatch data were non-linear when so treated. However, life cycle duration could still be predicted under measured temperature conditions, using the equations developed for single stadia, or combinations of these. The calculation of proportions of growth can be conducted using monitored temperature and the above equations. Predictive techniques would be further refined if the effects of fluctuating temperatures, in contrast to constant temperatures, were elucidated.

	TEMPERATURE (°C)									
	11.5	13.5	15.0	17.5	20.0	25.0	30.0	35.0	36.0	38.0
Hatch	5.3/0.3	3.0/0.3	3.0/0.2	**	2.3/0.2	2.0/0.2	1.5/0.2	1.5/0.2	1.5/0.2	1.1/0.2
First larval	*	*	9.0/1.0	**	6.0/1.0	4.0/1.0	4.0/1.0	4.0/1.0	*	*
Second larval	*	*	12.0/1.0	**	10.0/1.0	5.0/1.0	4.0/1.0	5.0/1.0	*	*
Pupation	*	*	152.0/10.0	120.0/4.0	55.0/2.0	41.0/2.0	30.0/1.0	10.0/1.0	*	*
Eclosion (male)	*	*	*	75.0/5.0	50.0/5.0	18.0/2.0	12.0/1.0	8.0/1.0	*	*
Eclosion (female)	*	*	*	65.0/5.0	37.0/5.0	10.0/1.0	9.0/1.0	9.0/1.0	*	*

TABLE 1. Duration of moult/frequency of inspection, in hours. Hatch, larval moults and pupation data are from eggs collected during 15-minute intervals. Eclosion data are from pupae collected during two-hour intervals.

* Did not survive to reach this moult.
** Moults not observed.

	TEMPERATURE (°C)									
	11.5	13.5	15.0	17.5	20.0	25.0	30.0	35.0	36.0	38.0
Egg	115.2	81.5	70.3	**	29.3	21.3	15.1	12.9	12.8	12.9
L1	*	*	53.5	**	22.5	15.8	8.8	8.8	*	*
L2	*	*	87.8	**	41.5	25.7	16.7	10.9	*	*
L3	*	*	373.3	**	166.8	80.7	70.1	61.3	*	*
Total pre-pupal	*	*	584.9	352.7	260.1	143.5	110.7	93.9	*	*
Pupa (male)	*	*	*	425.0	279.2	187.3	121.5	98.8	*	*
Pupa (female)	*	*	*	392.5	246.0	172.8	108.8	90.5	*	*

TABLE 2. Duration in hours of pre-imaginal stadia at a number of selected constant temperatures. First, second and third larval stadia are designated L1, L2 and L3 respectively. L2 and L3 were estimated by subtraction

* No survival of this instar at this temperature.
** Individual stadia not measured.

	COEFFICIENTS			
	A	B	R	Fisher's A^2
Egg	11.7247 (0.3840)	838 (79.7)	0.8335 (0.0050)	98.39
L1	8.1660 (1.9006)	1046 (598.4)	0.8107 (0.0308)	98.76
L1 + L2	16.7925 (2.8941)	1780 (523.5)	0.8370 (0.0161)	99.21
L2	9.6790 (2.3682)	988 (236.5)	0.8442 (0.0143)	99.84
L3	55.0303 (7.5698)	8565 (257.6)	0.8031 (0.0166)	99.94
Total pre-pupal	89.6992 (13.9982)	11540 (4552.0)	0.8076 (0.0202)	99.99
Pupa (male)	76.1261 (10.8831)	5058 (217.2)	0.8561 (0.0194)	99.92
Pupa (female)	61.3275 (21.7989)	3286 (197.3)	0.8727 (0.0279)	99.80
Total pre-imaginal (male)	165.0671 (7.7276)	9099 (1255)	0.8558 (0.0065)	99.99
Total pre-imaginal (female)	154.6582 (14.3389)	7687 (1943)	0.8605 (0.0121)	99.98

TABLE 3. Asymptotic regression coefficients for equations relating duration of stadia to constant temperature, having the form $Y = A + B * R ** X$. A^2 estimates the percentage of variance in treatment means explained by the regression. First, second and third larval instars are designated L1, L2 and L3 respectively. The L2 and L3 regressions are based on data obtained by subtraction. Total pre-imaginal data were obtained by summation. Standard errors appear in brackets.

Temperature (°C)	Total Duration (hours)			
	Male	Female	Handschin (sex unspecified)	Sardey and Thakare (sex unspecified)
17.5	777.7	745.2		
20	539.3	506.1		
25	330.8	316.3	324	342
30	219.5	232.2		192
35	192.7	184.4	249.6	228

TABLE 4. Duration of combined pre-imaginal stadia at a number of constant temperatures, estimated by summation of the raw data of Table 2. Other estimates by Handschin (1933a) and Sardey and Thakare (1978) are included.

Temperature (°C)	d.f.	t value	Significance level
35	3	33.9	<0.001
30	2	19.0	<0.005
25	2	58.5	<0.001
20	2	12.8	<0.01
17.5	2	33.4	<0.001

TABLE 5. Values of *t* for the differences between durations of male and female pupal stadia at a number of constant temperatures.

Dung Moisture (%)	Interval to pupation (hours)
86.5	108.1 (103.2 - 113.0)
85.0	110.1 (105.9 - 114.3)
81.0	128.1 (119.3 - 136.9)

TABLE 6. Interval from oviposition to pupation at 30°C, in dung of various constant moisture content. Mean values are of four replicates and are accompanied by the 95 per cent. confidence interval (50 individuals per replicate).

Figure 1. The distribution in time of the hatch of eggs collected at fifteen minute intervals and held at various selected constant temperatures.

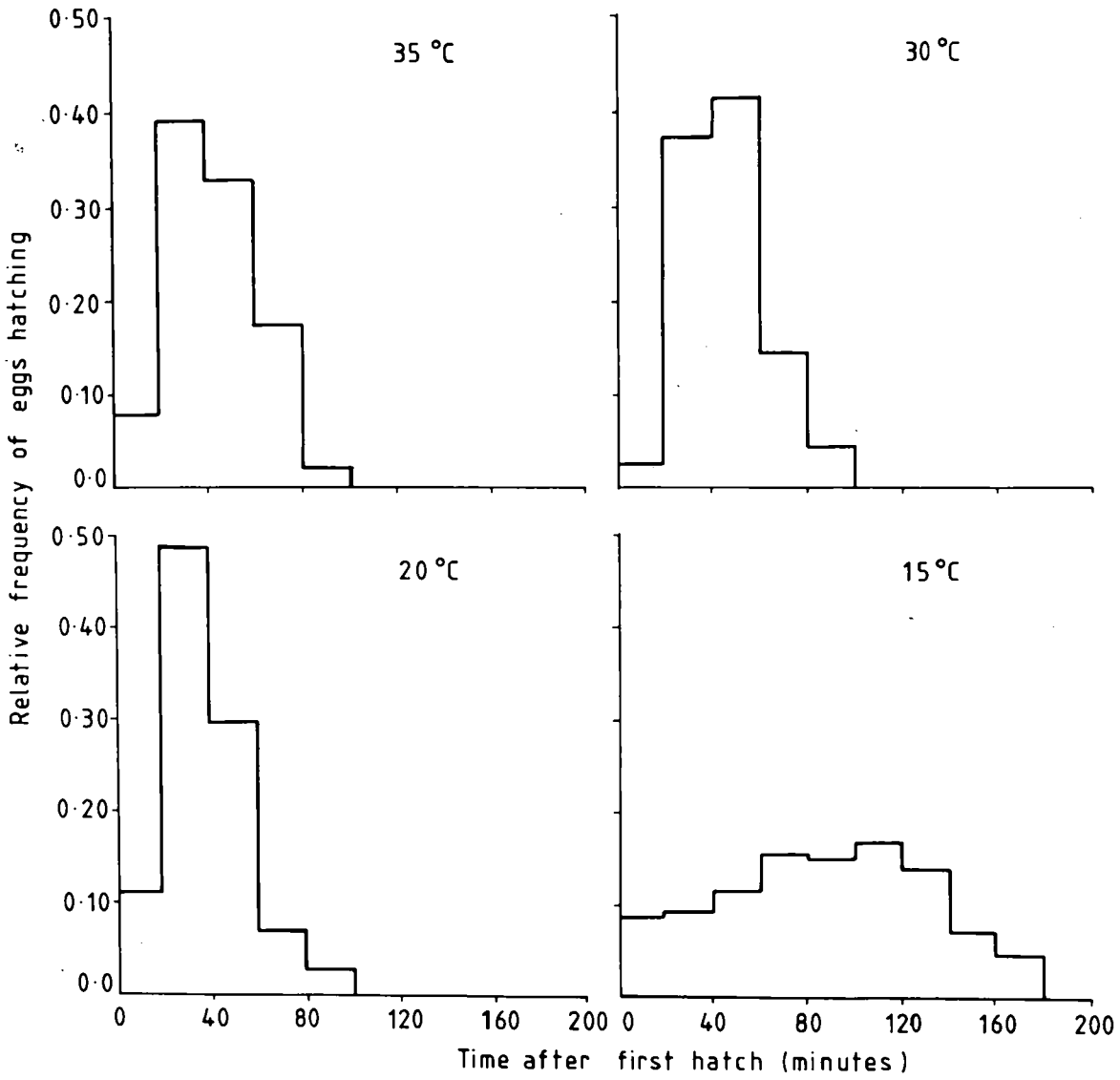

35 °C
30 °C
20 °C
15 °C
0·50
0·40
0·30
0·20
0·10
0·0
Relative frequency of eggs hatching
0
40
80
120
160
200
Time after first hatch (minutes)

Figure 2. Duration of the egg stadium at a number of selected constant temperatures.

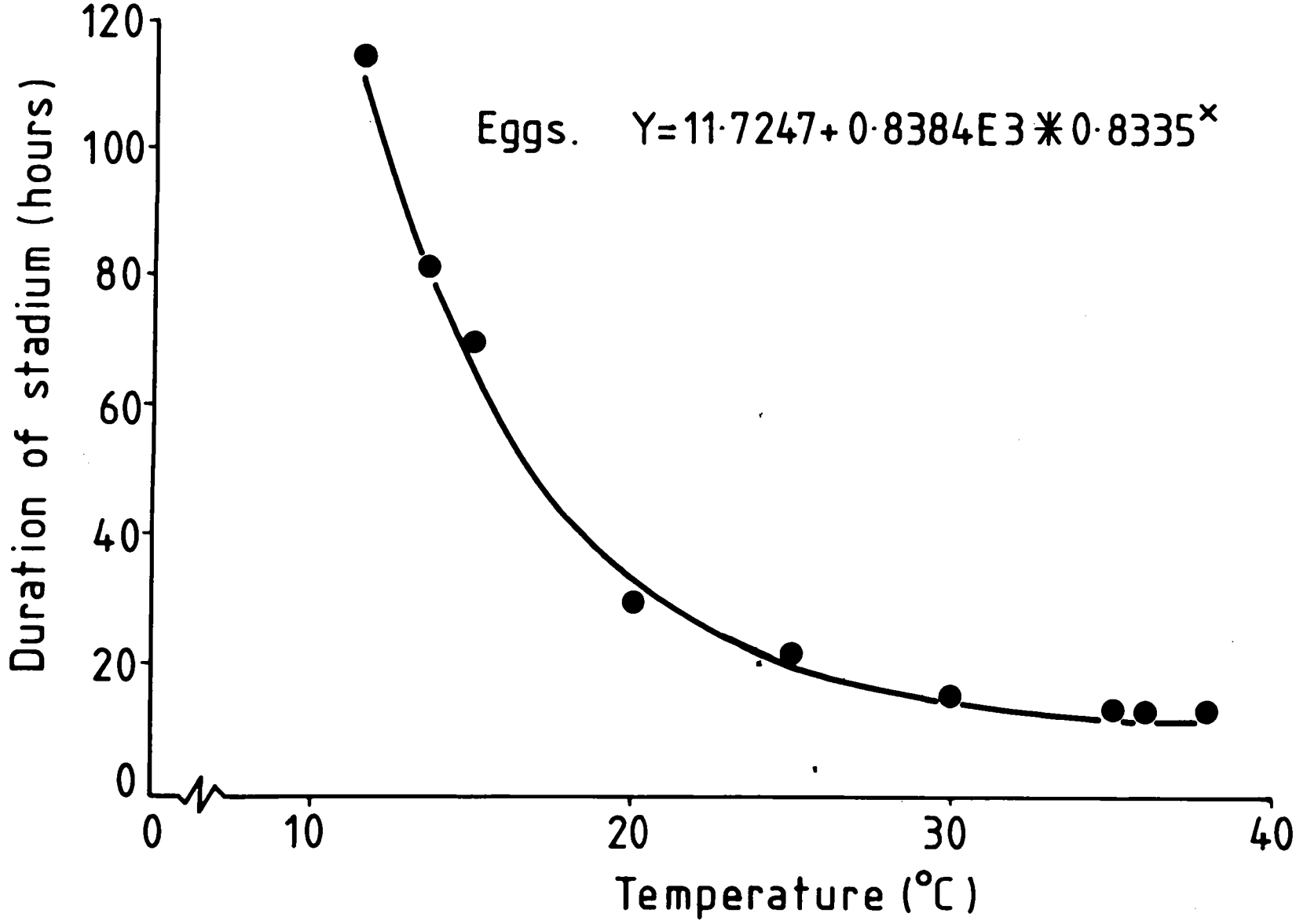

Eggs. Y=11·7247+0·8384E3*0·8335^x
Duration of stadium (hours)
120
100
80
60
40
20
0
0
10
20
30
40
Temperature (°C)

Figure 3. Durations of first, second and third larval stadia (LI, LII and LIII respectively) at a number of selected constant temperatures.

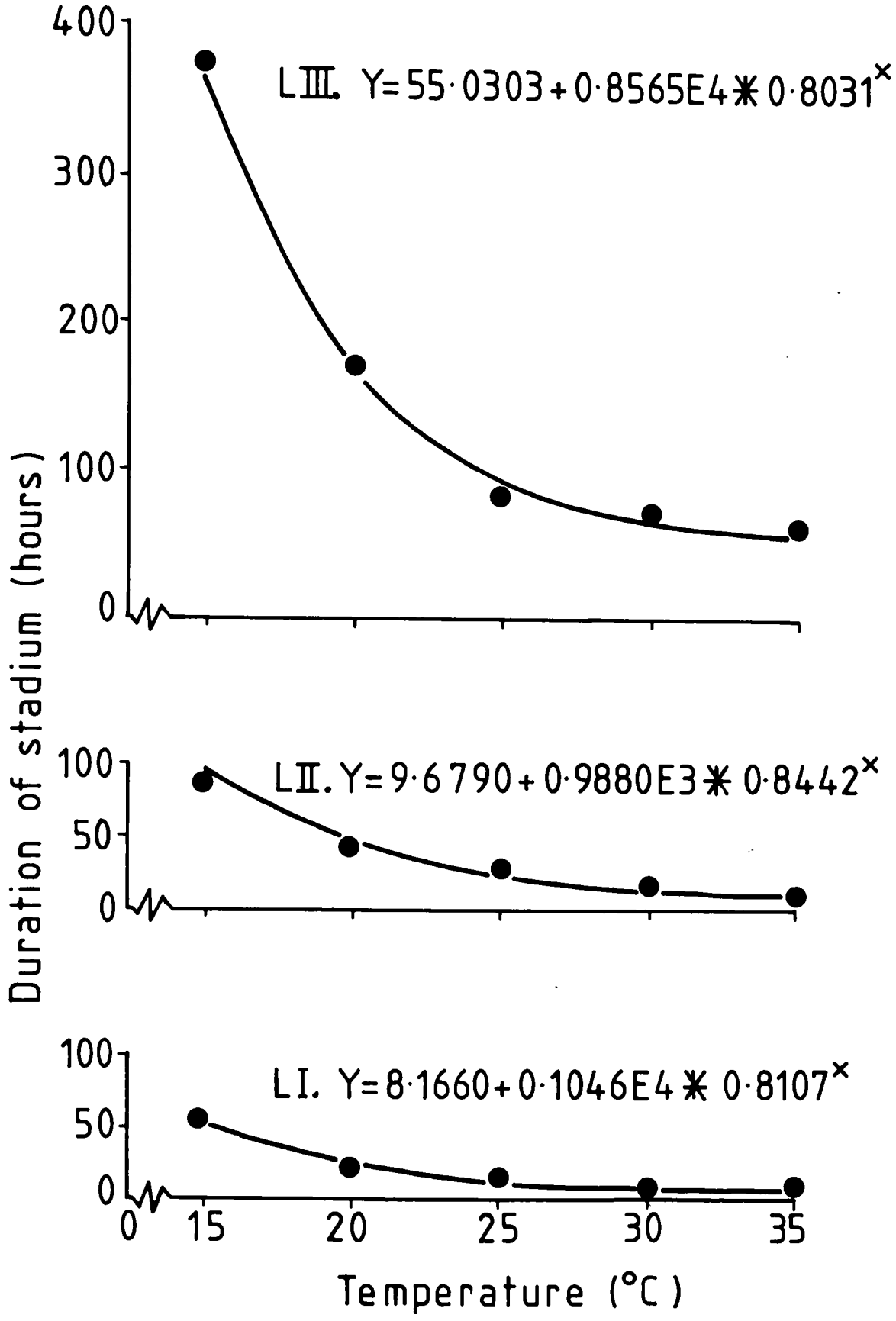

LIII. Y=55·0303+0·8565E4✱0·8031^x
LII. Y=9·6790+0·9880E3✱0·8442^x
LI. Y=8·1660+0·1046E4✱0·8107^x
Duration of stadium (hours)
400
300
200
100
0
100
50
0
100
50
0
0
15
20
25
30
35
Temperature (°C)

Figure 4. Durations of male and female pupal stadia at a number of selected constant temperatures.

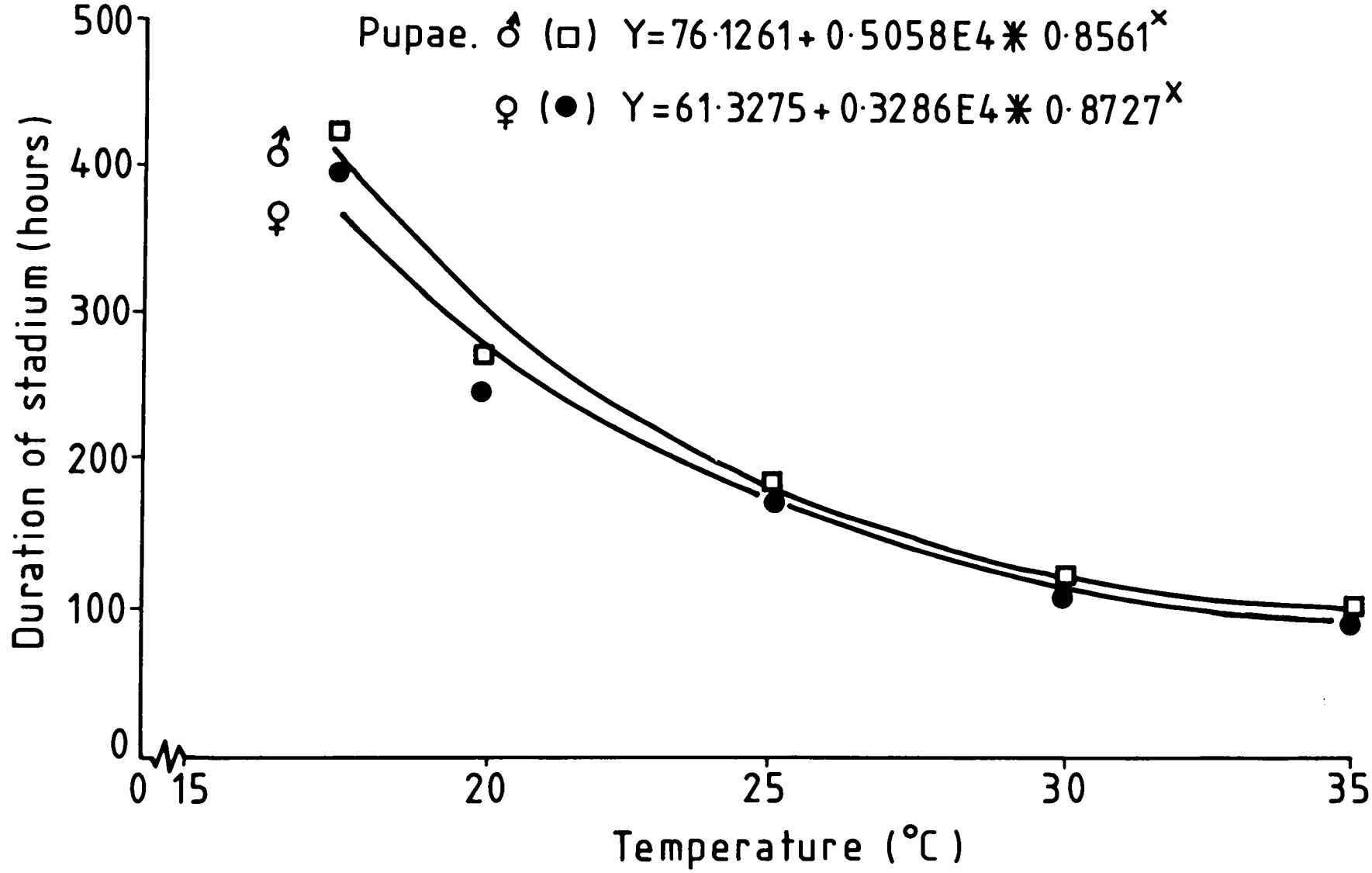

Pupae. ♂ (□) Y=76·1261+0·5058E4✱0·8561^X
♀ (●) Y=61·3275+0·3286E4✱0·8727^X
♂
♀
Duration of stadium (hours)
500
400
300
200
100
0
0
15
20
25
30
35
Temperature (°C)

Figure 5. Durations of the combined pre-imaginal stadia at a number of selected constant temperatures.

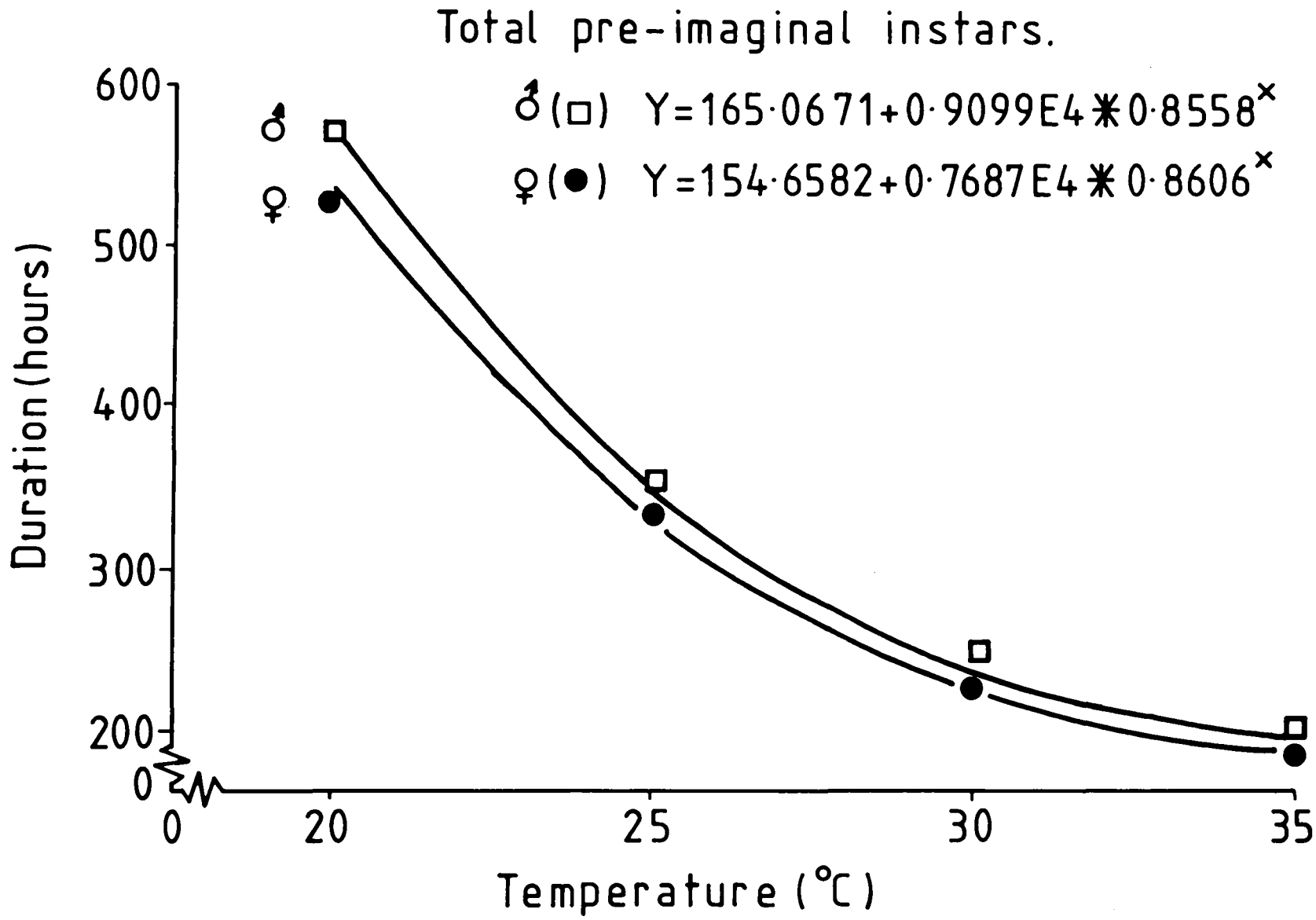
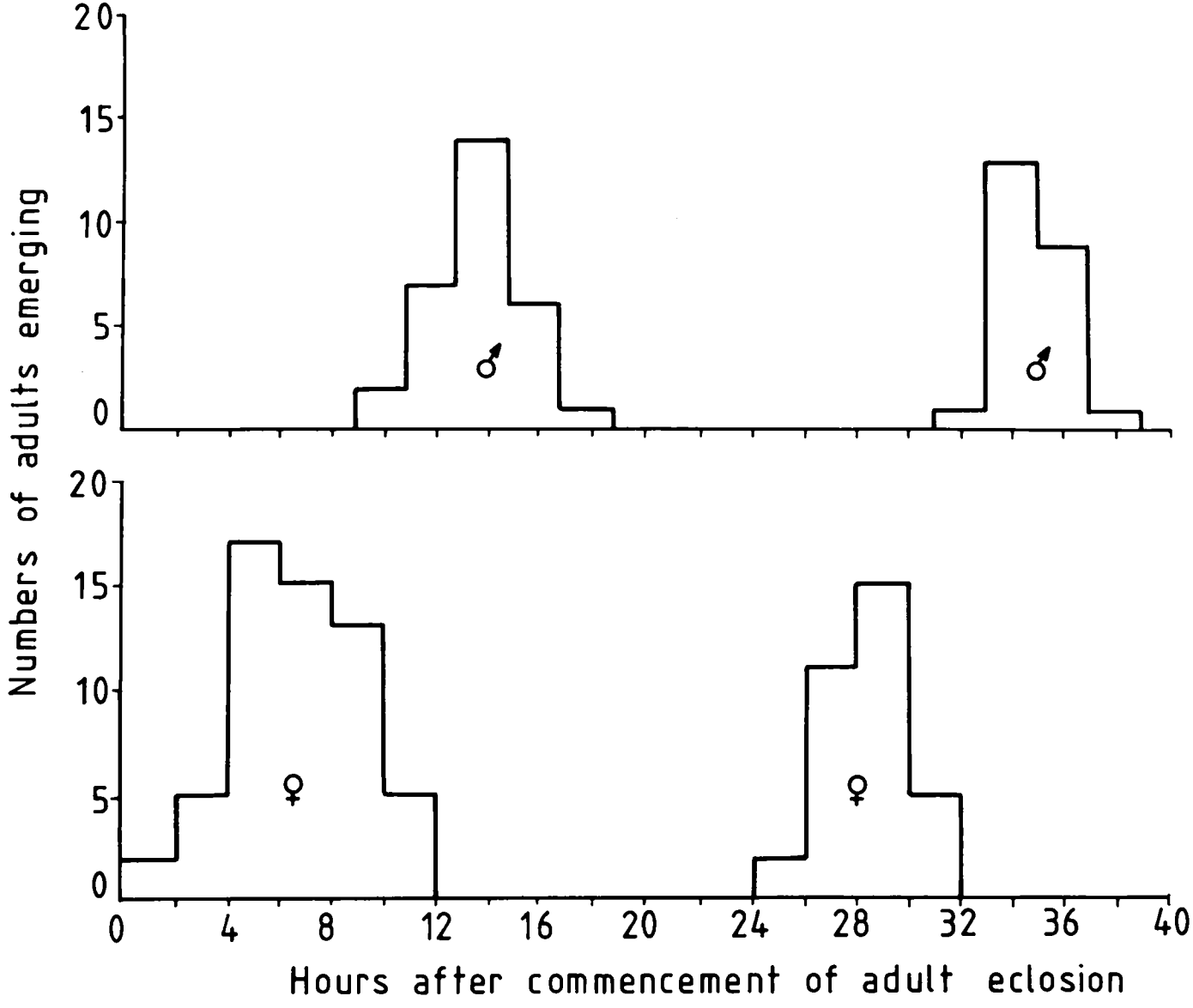

Total pre-imaginal instars.
♂(□) Y=165·0671+0·9099E4*0·8558^x
♀(●) Y=154·6582+0·7687E4*0·8606^x
Duration (hours)
600
500
400
300
200
0
0
20
25
30
35
Temperature (°C)

Figure 6. An example of bimodal emergence of male and female adult flies.


20
15
10
5
0
♂
♂
20
15
10
5
0
♀
♀
Numbers of adults emerging
0
4
8
12
16
20
24
28
32
36
40
Hours after commencement of adult eclosion

CHAPTER 2 : EFFECTS OF TEMPERATURE AND MOISTURE ON SURVIVAL OF IMMATURE STAGES

INTRODUCTION

Variation in substrate temperature and moisture play major roles in the environmental control of growth. Their effects include mortality when extreme levels are reached. Insects living in dung pads are in an isolated environment, from which there is little chance of escape if conditions become unfavorable. Dung pads are subjected to variation in atmospheric temperature, and may lose moisture by drying, or absorb it during rain or dew fall. Since the levels of these two attributes of dung are prone to daily and seasonal variation and constrain the growth and survival of insects, their effects on mortality among the immature stages of the buffalo fly were investigated.

REVIEW OF LITERATURE

1. Temperature

The effects of temperature on survival of the buffalo fly have been little studied. Handschin (1932, 1933a) estimated a minimum temperature of 11°C for development through immature stadia from oviposition to eclosion of the adult. Studies on the duration of individual stadia were not conducted and upper limits were not considered. Tillyard (1931) postulated that, short of frost conditions, low temperatures would not limit buffalo fly numbers, and that infestations might thus occur as far south as the Hunter River in New South Wales. However, there is generally little in the literature which relates thermal limits to the population dynamics of dung-breeding flies.

2. Moisture

2.1 Humidity studies - eggs Larsen (1943) tabulated the hatching rate of eggs of a number of species of Diptera under stress from lowered humidity. Among those species listed, several were taxonomically close to *H. irritans exigua*. *Haematobia stimulans* Meigen and *Lyperosia irritans* Bezzi showed marked decrease in hatch below 83 per cent. and 93 per cent. relative humidity respectively, at 77°F (25°C). Observations by Krijgsman and Windred (1933) revealed complete failure of buffalo fly eggs to hatch at 70 and 75 per cent. relative humidity.

2.2 Dung moisture studies - larvae Krijgsman and Windred (1933) also investigated the effects of dung moisture on larval development. It was found that larvae survived best in dung in which the moisture content was 68 per cent. of the moisture content at which the dung would be saturated. The optimum moisture content, expressed in this way,

would thus vary in terms of actual percentage of moisture, since dungs of different physical properties would have different water retention capacities, and thus different saturation levels. This figure of 68 per cent. has been misinterpreted to represent actual moisture content, by Roberts (1952) and Pont (1973).

Bay *et al.* (1969) found that optimum development of larvae of the face fly *M. autumnalis* took place in dung which was 85 per cent. of wet weight moisture. In the present study, dung from a lucerne-fed bull was found to be saturated when water content was artificially increased to approximately 90 per cent. wet weight. Assuming that Bay *et al.* were using approximately similar material, 85 per cent. of wet weight dung moisture would represent a level of approximately 63 per cent. saturation. This species was also found by Teskey (1969) to avoid dung of extremely high or low moisture as an oviposition site. Very wet or dry dung attracted few females and these laid reduced numbers of eggs. Valiela (1969) dismissed dung moisture as a potential mortality factor for *M. autumnalis*, considering that clustering of larvae, and the subsequent localised churning of dung, would produce an increase of liquidity, and allow feeding.

Dung moisture has been observed to influence development of the horn fly *H. irritans irritans*. Sanders and Dobson (1969) used a visual rating of viscosity from 1 (very thin) to 7 (very thick). The greatest number of flies were produced from dung of viscosity ratings 4 and 5, while none was produced from dung at either end of the range. Actual moisture contents were not indicated. Morgan and Graham (1966) also observed emergence of *H. irritans irritans* from dung of varying quality and constitution, produced by feeding cattle on different diets.

The dung least favorable for larval survival was from a "praire hay" diet. This dung had the lowest moisture content of all types. However, the authors did not discriminate between moisture and general nutritional suitability when discussing larval mortality. Further experiments using liquid extracts from these dung types showed that pupae from the praire hay dung were lighter than others. As moisture in such a diet would not be limiting, the overall plane of nutrition appears to be instrumental in the control of larval development.

The influence of dung quality, including moisture, on larval development of the bush fly *M. vetustissima* has been studied by Greenham (1972a). Dung used was from different localities and pasture types. This provided a natural variation in nitrogen and moisture content, the two parameters under observation. Multiple regression analysis showed that these factors affected only weight of adults at emergence. It was nevertheless established for all types of dung observed, that there was a marked seasonal effect on the reproductive potential of the bush fly. However, this seasonal effect could not be assigned directly to measured variation in moisture and nitrogen content. This study did not include observations on the effects of changes in dung quality during larval development.

MATERIALS AND METHODS

1. Eggs

1.1 Temperature studies Eggs were collected at 24°C, less than 30 minutes after oviposition. They were placed on several layers of moist filter paper in Petri dishes. These were placed in incubators set to operate at the required constant temperatures. To avoid prolonged exposure to the open atmosphere and accidental damage by handling, eggs were not counted until after incubation. However, all replicates comprised at least 50 eggs. After a period equal to one and a half times the longest pre-hatch interval observed during life cycle studies in Chapter 1, the numbers of hatched and unhatched eggs were counted.

1.2 Humidity studies Atmospheres of controlled constant relative humidity were provided by saturated solutions of various salts, with excess of the salt added, in sealed "Vacola" bottles. The experiment was conducted at 25°C. The salts used, and the humidity levels they provided at this temperature, appear in Table 7. Humidities produced by these methods vary with temperature. As accurate hygrometric instruments were not available to measure unknown humidities observations over a range of temperatures were not conducted. Eggs, on collection, were air-dried on fresh dry paper towelling for ten minutes. They were then removed with a camel-hair brush to further dry pieces of paper towelling, and placed on the floor of 4 cm x 4 cm plastic tubes. These tubes were suspended by cotton threads over the surface of the salt solutions inside the sealed bottles. After an extended time of twice the observed pre-hatch period for this temperature, eggs were counted to estimate the proportion which had hatched.

2. Larvae-Temperature and moisture studies Freeze-dried dung was used for the rearing of larvae. In this experiment, desired moisture levels in dung were provided by the addition of calculated amounts of distilled water. The moisture content of this reconstituted dung was checked gravimetrically. Moisture levels are expressed as a percentage of the wet weight.

Petri dishes lined with paper towelling were filled to a depth of approximately 3 mm with dung of the same moisture content as that at which larvae were to be reared. These are referred to as hatching pads. Eggs were collected in the usual manner, and placed on moist paper towelling on the surface of the hatching pads. These were covered to prevent moisture loss and incubated at 25°C. On expiry of the pre-hatch period, the hatching pads were placed on the surface of dung in the plastic rearing containers. These containers, with 500g of dung, were placed in appropriate incubators 24 hours previously to stabilise at the required temperature. The egg papers were removed, and the numbers of hatched eggs counted as a measure of the initial inoculation of first instar larvae. Exhaustive inspection after hatch confirmed that larvae were remaining in the initial hatching pads, and not migrating. Larval survival was estimated by counting the numbers of pupae, compared with the initial numbers of hatched eggs.

Pupae were collected and stored in humidified containers at 25°C. During the "black" phase, which occurs shortly prior to eclosion, a sample of pupae from each moisture/temperature treatment was weighed and measured individually. Measurements recorded were of the longest axis, and of the longest axis at right angles to it i.e. the length, and the maximum diameter. Measurements were made with a graduated eye piece in a Nikon stereoscopic microscope, at X20 magnification.

3. Pupae-Temperature and humidity studies Pupae for these experiments were produced by larvae reared at 25°C. They were collected at two-hourly intervals, only those with tanned puparia being chosen. In the temperature experiments, pupae in gauze-stoppered glass tubes in humidified sealed plastic boxes were held until emergence of adults in incubators at various constant temperatures. The humidity experiments were conducted at 25°C, pupae being exposed in the manner already described for eggs. Humidity levels were achieved over the saturated solutions of salts presented in Table 7.

ANALYSIS OF RESULTS

The effects of mortality factors were assessed in terms of the percentage of total individuals which survived any particular treatment. Confidence limits were calculated on these percentages transformed to the arcsine of square root values (in radians). Tabulated limits have been re-transformed to percentage values, and are thus not symmetrical about means.

Regression equations were calculated using the same angular transformation, and are presented in terms of the radian transform. The regressions were calculated using the "step-up" method and contain the maximum sub-set of variables in which all coefficients are significant ($P<0.05$). The regression coefficients were tested for difference from zero as each new term was calculated, the term being discarded if the difference was not significant.

RESULTS

1. Eggs Effects of temperature and humidity on survival.

1.1 Temperature The effects of temperature on the hatching success of eggs appear in Table 8. The raw data are represented in Fig. 7. At temperatures from 20°C to 35°C inclusive, the proportion hatching was uniformly high. One-way analysis of variance revealed no significant differences between hatching rates within this range. Mean hatch for all temperatures within this range was 85.52 per cent. At 15°C hatch was reduced significantly ($P<0.01$), and at 11.5°C was reduced further. No hatching occurred at 10°C. Hatching occurred at 38°C, but survival of embryos was reduced, and at 40°C hatch failed completely. Considering the range over which hatching occurred, the report by Sardey and Thakare (1977) that hatch failed at 20°C and at 37.5°C is surprising.

1.2 Humidity Variation in humidity had a profound effect on the survival of eggs (Table 9). The 93 per cent. treatment reduced hatch to 49.3 per cent., compared with an 81.5 per cent. hatch in a saturated environment. Humidities below these levels reduced hatch further. The relationship between humidity and survival is described over the range of 79 per cent. to 100 per cent. by the equation

$$Y = -4.9076 + 0.0606\ X \qquad (A^2 = 98.21\%)$$

where Y is the proportion of eggs surviving, transformed to the inverse sine of the square root in radians, and X is relative humidity expressed as a percentage. The regression is presented in Fig. 8.

It was not possible to study the possible interactive effects of temperature and humidity on hatch, due to a lack of suitable monitoring facilities. The possible effects of these factors in a field situation are evaluated in subsequent discussion.

2. Larvae Effects of temperature and dung moisture on survival.

The survival of larvae to pupation under various conditions of constant temperature and dung moisture is recorded in Table 10. At temperatures between 20°C and 30°C, where dung moisture was between 75 and 85 per cent., larval survival was uniformly high. One-way analysis of variance showed no significant differences ($P>0.50$). At 71 per cent. dung moisture survival decreased uniformly over the entire temperature range. A further uniform fall in survival occurred at 67 per cent. moisture in the 20, 25 and 30°C treatments, and at this moisture level survival was much further reduced at 35°C. No larva survived at any temperature when dung moisture was a constant 63 per cent. Above 85 per cent. moisture there was some reduction in survival at 25, 30 and 35°C, but not at 20°C. A possible artificial waterlogging effect may have operated at this moisture level (87 per cent.). Dung was held in plastic containers without drainage. Attempts were made to rear larvae in dung whose moisture level had been adjusted to 89 and 91 per cent. A layer of free water formed on the surface of these cultures, and larvae failed to survive. To a lesser extent, this mechanism may have reduced survival in the 87 per cent. treatments.

Survival at temperatures below 20°C was investigated only in dung where the moisture content was 85 per cent., a level considered

close to optimum. A reduction to 61.25 per cent. survival was observed at 17.5°C, and at 15°C only 5.14 per cent. of larvae pupated. At all moisture levels except 85 per cent. slight falls in survival were noted at 35°C, compared to that of the 20, 25 and 30°C treatments. Survival at 35°C in the 85 per cent. treatments remained anomalously high, in relation to neighboring treatments. At 37.5°C no pupation occurred.

A step-up multiple regression was fitted to the data using the least squares method and retaining only those coefficients significantly different from zero, at $P<0.05$. Data were analysed as the inverse sines of the square root of the proportion surviving and expressed as radians. Dung moisture percentage (X_1) and temperature (X_2) were not transformed. The data are described by the equation:

$$Y = -18.1541 + 0.2888X_1 - 0.0021X_1^2 + 0.8721X_2 - -0.0343X_2^2 + 0.0004X_2^3 - 0.0012X_1X_2. \qquad (A^2 = 53.78\%)$$

This equation is presented graphically in Figure 9. The main feature of this plot is that the predicted favorable temperature/moisture combinations are generally similar to those evident on inspection of the data i.e. 20-35°C and 75-85 per cent. respectively. However, the plot also indicates a second favorable area above a slight trough at 35°C in the same moisture range. As this was considered to be biologically improbable, the data were analysed in the same manner, with the omission of results from the 35°C/85 per cent. treatments, where survival was previously described as being anomalously high. The equation of best fit was then found to be:

$$Y = -22.3150 + 0.3488X_1 - 0.0021X_1^2 + 1.0413X_2 - 0.0365X_2^2 + 0.0004X_2^3. \qquad (A^2 = 53.39\%)$$

This equation is presented graphically in Figure 10. The most favorable combinations of treatments and the maximum survival values are similar to those predicted by the previous equation, and the initial improbable second favorable area commencing above 35°C, does not appear in the present plot within the range of treatments tested.

3. Pupae Effects of temperature and humidity on survival.

3.1 Temperature Pupae survived uniformly well under constant temperatures between 20°C and 35°C, (Table 11). One-way analysis of variance showed that there were no significant differences within this range ($P>0.50$). The pooled mean survival rate for all replicates at all of these temperatures was 87.2 per cent. Mortality was total at 38°C. Rearing at 17.5°C significantly reduced survival and no pupae survived at 15°C.

3.2 Humidity The effects of relative humidity on survival of pupae to eclosion are shown in Table 12. The relationship is described by the equation

where X is untransformed relative humidity (per cent.) and Y is transformed survival (Figure 11). At levels of 71.2 per cent. and above, survival was high. However, the 66.0 per cent. treatment caused an increase in mortality, and only 3.6 per cent. of pupae survived the next lowest humidity level of 31.0 per cent.

During these experiments, adult flies were frequently able only to eclose partially, and died with only the head and thorax free of the puparium. Others eclosed fully but were unable to expand the

wings before dying. These were counted as pupal deaths in the assessment of survival. Larsen (1943) noted a similar occurrence when rearing pupae of the house-fly *M. domestica* under dry conditions.

In none of the temperature or humidity treatments was there a discernible differential response due to the sex of pupae. Chi-square tests revealed no significant departure from a 1 : 1 sex ratio among resulting adults ($P>0.05$).

DISCUSSION

1. Eggs

1.1 Temperature Studies Buffalo fly eggs survived experimental temperatures which are commonly experienced as daily atmospheric maxima in tropical Australia. The experiments reported here showed that most eggs hatched normally if incubated at a constant temperature of 35.0°C. In a field situation, exposure to temperatures above this level would not last for the complete pre-hatch period. Thus, although the lethal upper constant temperature was found to lie between 38.0°C and 40.0°C, eggs might survive brief exposure to temperatures beyond this level. In addition, eggs are protected by behavioural devices. Oviposition normally occurs on the under-side of pads, away from direct sunlight. As eggs are normally laid only on fresh, wet dung, evaporative cooling may further protect against high temperatures.

Eggs are able to develop slowly at 11.5°C, but fail to do so at a constant temperature of 10°C. If this failure is due not to immediate death, but to cessation of development, it is likely that eggs could survive short periods of lower temperatures. If low temperature affects survival of eggs, it is more likely to be by indirect means, such as delay of hatching, allowing factors such as drying of dung or desiccation of eggs to occur. Severe frost may injure eggs directly by causing freezing and although not frequent in fly-infested areas, may cause occasional mortality.

1.2 Humidity studies The effects of lowered humidity on egg survival (Table 9) indicate that this could be an important mortality factor under field conditions. Larsen (1943) conducted studies of

the effects of humidity on the hatch of eggs of *M. domestica*, *S. calcitrans*, *H. stimulans*, *L. irritans*, and *Scatophaga stercoraria* (L.) (Diptera : Muscidae) all dipterans taxonomically close to *H. irritans exigua*. The experiments were conducted at 25°C, as were those described above. The results of Larsen's work are reproduced in Figure 12, and include raw data from experiments on *H. irritans exigua* described above. All species show a generally similar dependence on high levels of relative humidity for survival of embryos although the hatch under saturated conditions for the species studied by Larsen are higher than was found above for *H. irritans exigua*. Larsen's data gave humidity levels where no eggs hatched, but the exact lower lethal levels for these species were not defined.

As discussed in Chapter 1, the effects of atmospheric conditions on hatch may be modified by the microclimate immediately surrounding the egg. The effects of placement on a wet dung surface and of being surrounded by air which lies immediately over this surface may protect the egg from any desiccation, except perhaps under extremely severe conditions.

2. Larvae

2.1 Temperature studies The response of larval survival to temperature variation follows a pattern similar to that shown by Valiela (1969) for *M. autumnalis* in that at both ends of the favorable range, decrease in survival is abrupt, rather than gradual. Strangely, *H. irritans exigua*, a tropical species, proved to have lower thresholds, both upper and lower, than those demonstrated for *M. autumnalis* by Valiela.

Larvae failed to survive constant temperatures below 15°C. As previously pointed out, buffalo flies in Australia occur in areas where daily minimum temperatures only fall below this level for brief periods during the night, if at all. It is thus unlikely that mortality among larvae in habitually infested areas is directly due to low temperatures. The sharp decrease in fly numbers which occurs during winter months in such areas is probably not due to larval mortality resulting from low temperatures. Mortality among larvae is more likely to arise indirectly. As low temperatures delay development other environmental elements may become unfavorable. Prolonged pre-imaginal development may allow dung to become dry to the point where larvae are unable to feed, even though humidity may be high enough to prevent desiccation of larvae.

The upper thermal limit for larval survival between 35 and 37.5°C, is a temperature often exceeded in infested areas. Upper thermal limits for development in insects are generally thought to be due to direct lethal affects, not merely cessation of activity, in contrast to low temperature thresholds. Thus high temperature, unlike low temperature, is probably a direct cause of mortality among buffalo fly larvae. The temperatures actually reached in dung pads in a temperate area have been observed by Greenham (1970), and are further dealt with in Chapter 5. The lethal effects of high temperature may be produced after fairly short periods of exposure. A study of the interaction of time and temperature above the constant lethal limit and of the effect of previous temperature experience on tolerance of high temperatures would further clarify the contribution of this source of mortality to the variation in fly numbers during summer months.

2.2 Moisture studies The dung used in these experiments was derived from a diet consisting solely of lucerne chaff. The moisture level at which larvae could not survive was found to lie between 63 and 67 per cent. of wet weight. Humidity within the pad at these low levels was likely to be near 100 per cent. and it is improbable that larvae died of evaporative desiccation. As larvae rely on a fluid medium for feeding, availability of food depends on free moisture and would be limited by low dung moisture. It is thus more likely that mortality in dry dung is caused by starvation or by net loss of body water due to reduced intake falling short of respiratory loss. The availability of moisture for feeding may depend not only on moisture content, but on texture. Conceivably, dung of high fibre content might contain water which would contribute to moisture content, yet nevertheless not be available to constitute the free liquid medium necessary for larval feeding.

The apparent reduction in larval survival at 87 per cent. dung moisture may be artificial, due to free surface water as previously discussed. Such free water in naturally deposited dung would drain away, effectively lowering the dung moisture content. However, high moisture content may yet be an indirect cause of mortality. Cattle in tropical Australia frequently produce dung of extremely high moisture in the early summer wet season when pasture is lush and growing vigorously. Such dung is highly fluid, and tends to spread in very thin layers on falling. Even though they may be initially adequate for fly breeding, these pads tend to dry very quickly, and are also easily dissipated by heavy rain, thus being rapidly rendered unsuitable for survival of larvae.

3. Pupae Temperature and humidity studies.

The results demonstrate that pupae require high relative humidity for successful development.
This requirement has been demonstrated for *H. stimulans, L. irritans* and *S. calcitrans* by Larsen (1943). Survival of pupae appears to be threatened by naturally occurring low levels of relative humidity, especially during periods of high temperature.

The effects of high temperature alone under optimum conditions of humidity, also appear able to influence survival, as the maximum constant temperature which can be tolerated is often exceeded under summer conditions in northern Australia.

Atmospheric minima often fall below the lowest constant temperature survived by pupae. However, if this measured constant minimum implies cessation of development, rather than death, the effect of lower temperatures for short periods would appear in the rate of development, rather than in levels of survival.

Pupation by mature larvae of the buffalo fly usually occurs in the pad or in soil. When occurring in soil particularly, such behaviour would maximise any insulating effect by the pad, or by pad plus overlying soil, against extremes of temperature. Similarly pupae are not subjected to atmospheric levels of humidity. As the relative humidity in soil remains high, even at very low levels of soil moisture, pupae in soil may not normally be stressed during periods of low atmospheric humidity. Handschin (1932) stated that humidity had no effect on the biology of buffalo flies following pupation, but did not substantiate this claim. On the other hand Krijgsman and Windred (1933)

recorded no unusual mortality among pupae above 60 per cent. relative humidity, at an unstated temperature. At 35°C, a humidity of 75 per cent. accompanied 94 per cent. mortality of pupae. These authors thus appear to infer an interaction between temperature and humidity.

CONCLUSIONS

1. Temperature

Eggs are the most temperature-tolerant of all immature stages, at both low and high levels. Considering that some eggs and larvae survived constant temperatures of 11.5 and 15°C respectively, (Table 8) it is unlikely that mortality in infested areas in Australia is directly due to brief nightly exposures to temperatures below these levels during winter. The drop in fly numbers during these months is more likely to be an indirect effect of low temperatures, and is further discussed in chapter 6.

High mortality levels resulting from elevated temperatures may be of a direct nature. The placement of eggs on the shaded underside of pads and the effects of evaporative cooling of fresh dung probably protects them from all but extreme conditions of high temperature. The relatively short pre-hatch period for this species would also minimise the harmful effects of high daily temperatures.

Larvae, on the other hand, appear more prone to high temperature mortality (Table 10). Results in Chapter 5 show that temperatures in a dung pad clearly exceed atmospheric levels, which in turn often exceed the constant level shown to cause larval death (i.e. between 35 and 37°C). Pupae may be similarly susceptible, particularly if located within the pad. Where pupation has occurred in soil below the pad, the effects would be less severe.

2. Humidity

It was pointed out in discussion above that atmospheric humidity probably did not affect hatching of eggs under field conditions.

Larval mortality may be influenced indirectly by the contribution of humidity to the rate at which pads dry out. Pupae are protected by the puparium and the dung pad, and perhaps further by a layer of soil if so placed. This would buffer the effects of unfavorably low atmospheric humidity levels.

3. Moisture

The effects of dung moisture on mortality are probably restricted to larvae. Eggs are generally only deposited on fresh, very wet dung and hatch quickly, and pupae are physically protected from desiccation and do not move or feed.

Salt	Relative Humidity (%)
$NH_4H_2PO_4$	93.0
ZnSO4	90.0
Na2CO3·10H2O	87.0
NH4Cl	79.3
NH4Cl/KNO3	71.2
NaNO2	66.0
CaCl2	31.0

TABLE 7. Relative humidities of atmospheres over saturated solutions of various salts at 25°C. (Weast, 1971).

Temperature (°C)	Percentage hatch	95 per cent. confidence interval
10.0	0.0	-
11.5	5.8	3.8 - 8.2
15.0	27.9	16.9 - 40.5
20.0	85.6	80.0 - 90.5
25.0	85.6	78.1 - 91.7
30.0	85.4	76.8 - 92.3
35.0	85.5	78.3 - 91.5
38.0	62.8	41.0 - 82.1
40.0	0.0	-

TABLE 8. Percentages and 95 per cent. confidence intervals of eggs hatching at various constant temperatures, under conditions of saturated atmospheric humidity (n = 5 replicates).

Relative humidity (%)	Percentage Hatch	95 per cent. confidence interval
100	81.5	73.9 - 88.5
93	49.3	42.4 - 55.6
90	27.6	24.9 - 29.8
87	10.7	8.7 - 12.5
79	1.3	-0.62- 5.61

TABLE 9. Percentage, and 95 per cent. confidence intervals, of eggs hatching under selected constant conditions of relative humidity, at 25°C (n=5).

Temp. (°C)	Percentage dung moisture (of wet weight)						
	63	67	71	75	77	81	85	87
15.0	0.00	*	*	*	*	*	5.14 (3.45- 7.14)	*
17.5	0.00	*	*	*	*	*	61.25 (38.24-81.87)	*
20.0	0.00	55.57 (46.16-64.95)	68.89 (45.82-87.85)	78.96 (64.45-90.45)	83.84 (70.50-93.65)	84.48 (80.65-87.96)	80.92 (72.54-87.96)	83.33 (47.73-99.88)
25.0	0.00	50.17 (46.54-59.85)	68.89 (26.37-97.71)	91.66 (80.15-98.30)	81.19 (59.71-95.75)	84.98 (52.62-99.85)	78.39 (67.10-88.19)	76.79 (57.48-90.76)
30.0	0.00	50.00 (33.89-66.28)	64.62 (50.52-77.52)	82.67 (46.34-99.87)	75.75 (38.58-97.87)	83.07 (56.44-98.39)	89.18 (63.78-99.95)	77.38 (51.05-95.46)
35.0	0.00	14.15 (-3.58 - 67.26)	66.11 (17.73-98.87)	56.61 (38.92-73.47)	70.34 (51.74-85.85)	71.45 (36.05-95.96)	91.26 (76.50-99.15)	73.32 (51.40-90.55)
37.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

TABLE 10. The percentages of larvae surviving to pupation at various combinations of constant temperature and dung moisture. The 95 per cent. confidence intervals of each mean appear in brackets (n=4).

* No record available.

Temperature (°C)	Percentage Survival	95 per cent. Confidence interval
15.0	0.0	-
17.5	26.5	0.2 - 73.8
20.0	87.0	59.5 - 99.7
25.0	89.0	81.3 - 94.8
30.0	87.0	66.3 - 98.6
35.0	84.7	76.2 - 91.7
38.0	0.0	-

TABLE 11. Percentages, and 95 per cent. confidence intervals, of pupae surviving to eclosion of adults at various constant temperatures, under constant conditions of saturated atmospheric humidity (n=4).

Relative humidity (%)	Percentage of adults eclosing	95 per cent. confidence interval
100.0	89.4	80.9 - 96.4
79.3	92.6	87.8 - 97.3
71.2	74.3	63.6 - 84.3
66.0	57.1	13.7 - 95.6
31.0	3.6	0.1 - 10.8

TABLE 12. Percentages, with 95 per cent. confidence intervals, of adults eclosing from pupae under various conditions of constant relative humidity, at 25°C (n=5).

Figure 7. Percentages and 95 per cent. confidence intervals of eggs hatching at various constant temperatures.

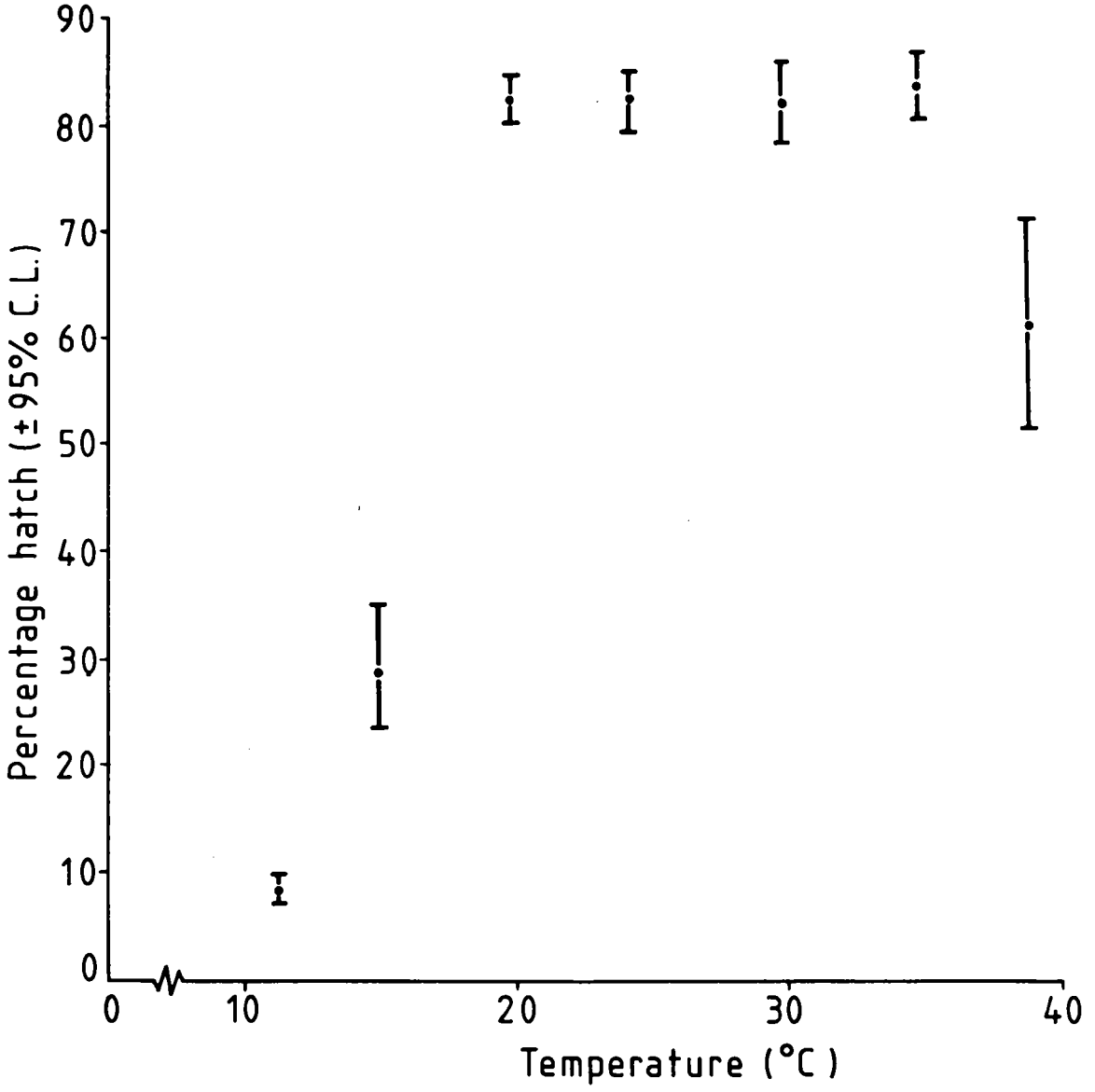

Percentage hatch (±95% C.L.)
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
Temperature (°C)

Figure 8. Proportions of egg hatching at various constant levels of relative humidity, at 25°C.

0.2 radians = 3.9 per cent.

1.2 radians = 86.8 per cent.

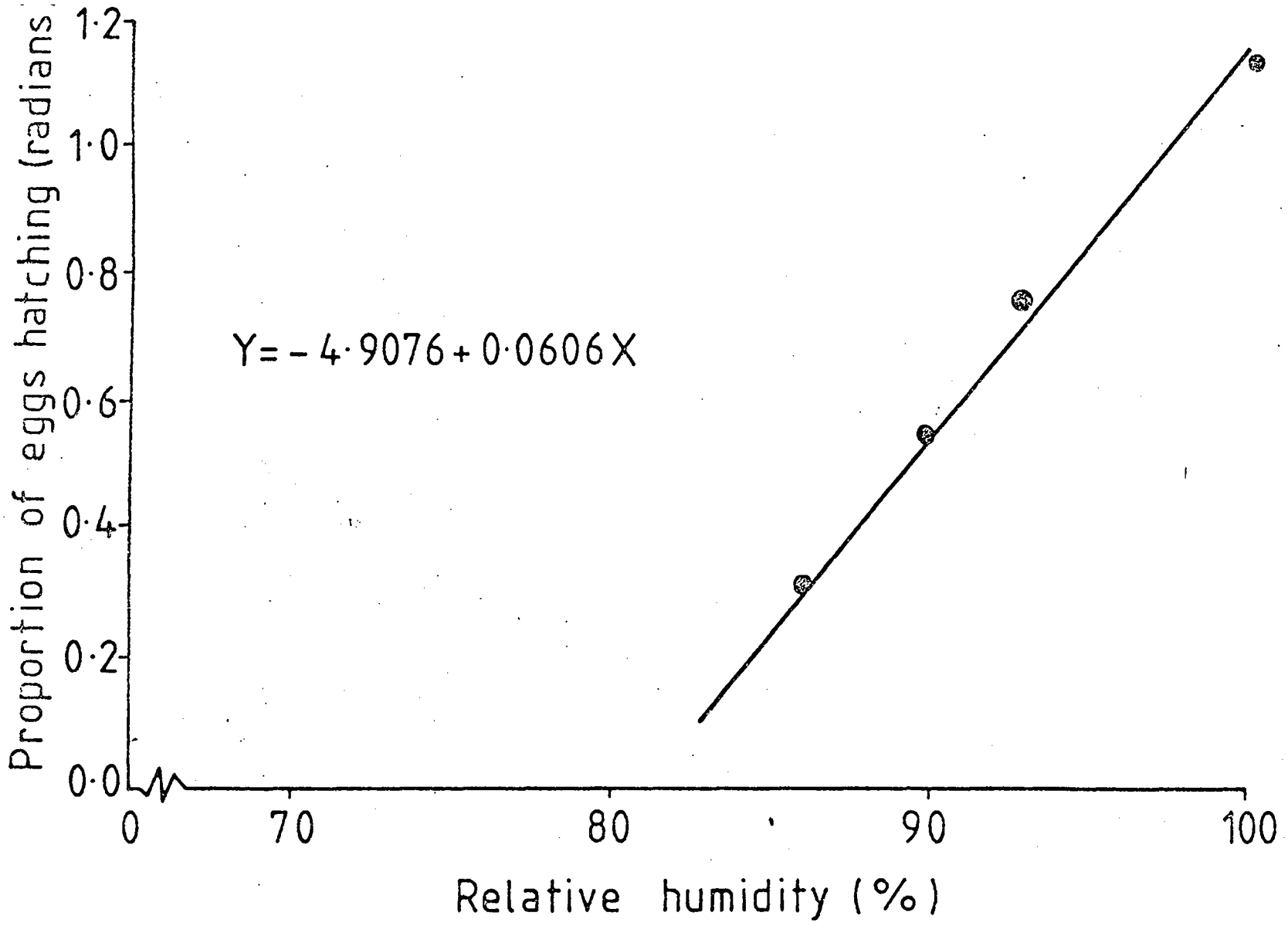

Proportion of eggs hatching (radians
1·2
1·0
0·8
0·6
0·4
0·2
0·0
Y= - 4·9076 + 0·0606X
0
70
80
90
100
Relative humidity (%)

Figure 9. Proportions in radians of larvae surviving under various combinations of constant temperatures and dung moisture levels, with the 35°/85% combination included in the analysis.

0.4 radians = 15.1 per cent.

1.3 radians = 92.9 per cent.

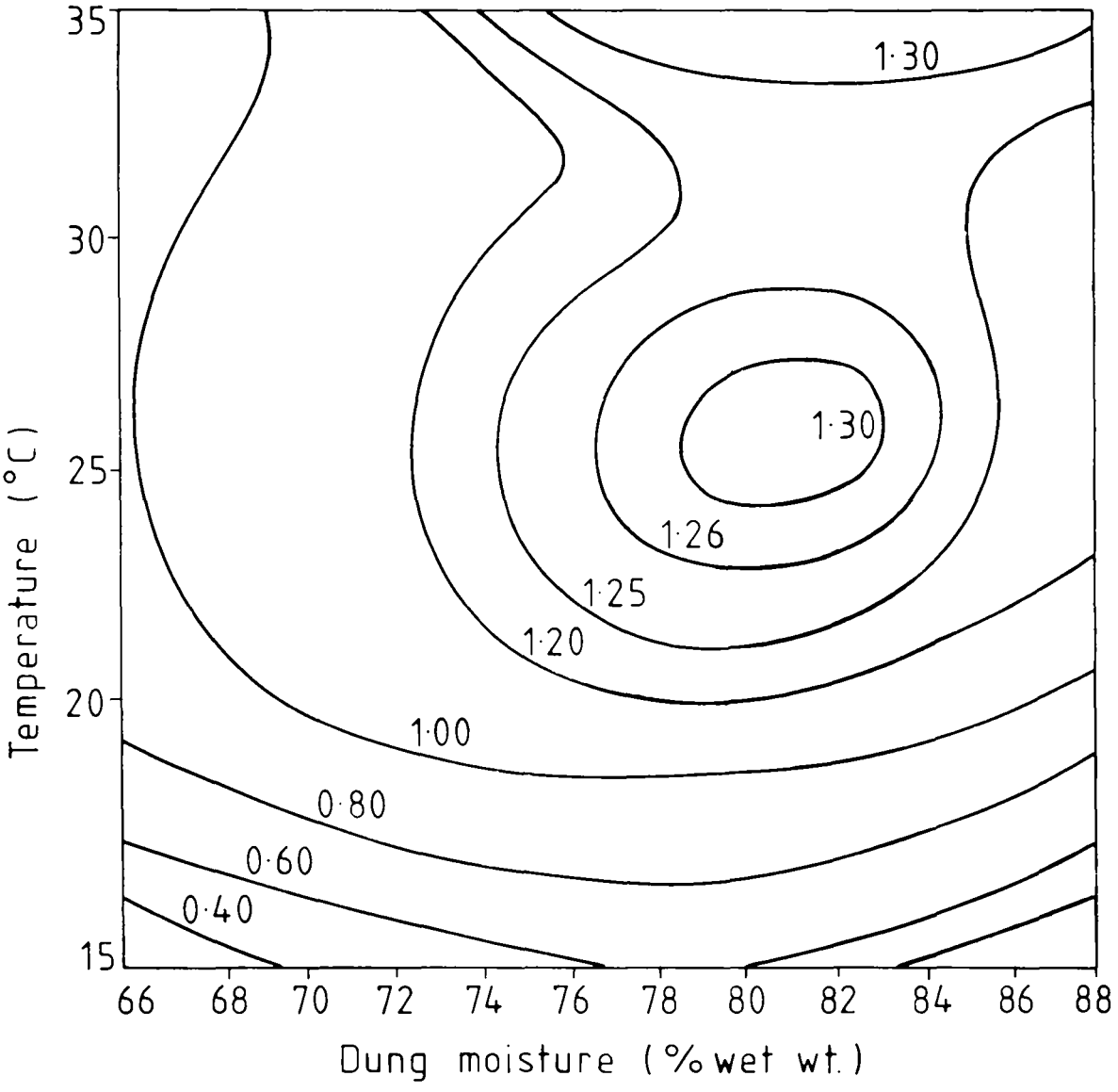

35
30
25
20
15
Temperature (°C)
1·30
1·30
1·26
1·25
1·20
1·00
0·80
0·60
0·40
66
68
70
72
74
76
78
80
82
84
86
88
Dung moisture (% wet wt.)

Figure 10. Proportions in radians of larvae surviving under various combinations of constant temperatures and dung moisture levels, with the 35/85 combination omitted from the analysis.

0.4 radians = 15.1 per cent.

1.3 radians = 92.9 per cent.

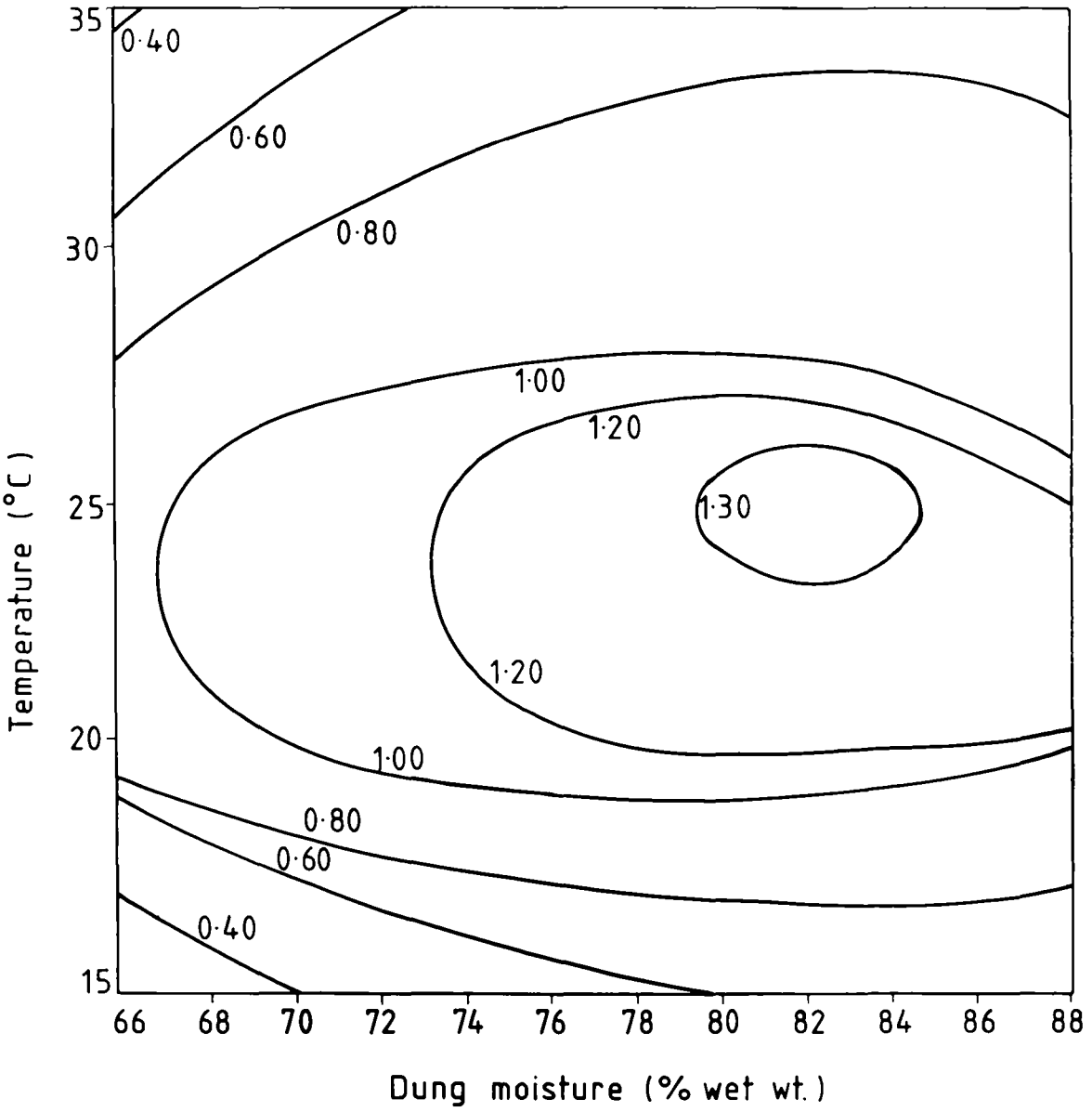

35
30
25
20
15
Temperature (°C)
0·40
0·60
0·80
1·00
1·20
1·30
1·20
1·00
0·80
0·60
0·40
66
68
70
72
74
76
78
80
82
84
86
88
Dung moisture (% wet wt.)

Figure 11. Proportion in radians of adults from pupae exposed to various constant levels of relative humidity at 25°C.

0.2 radians = 3.9 per cent.

1.3 radians = 92.9 per cent.

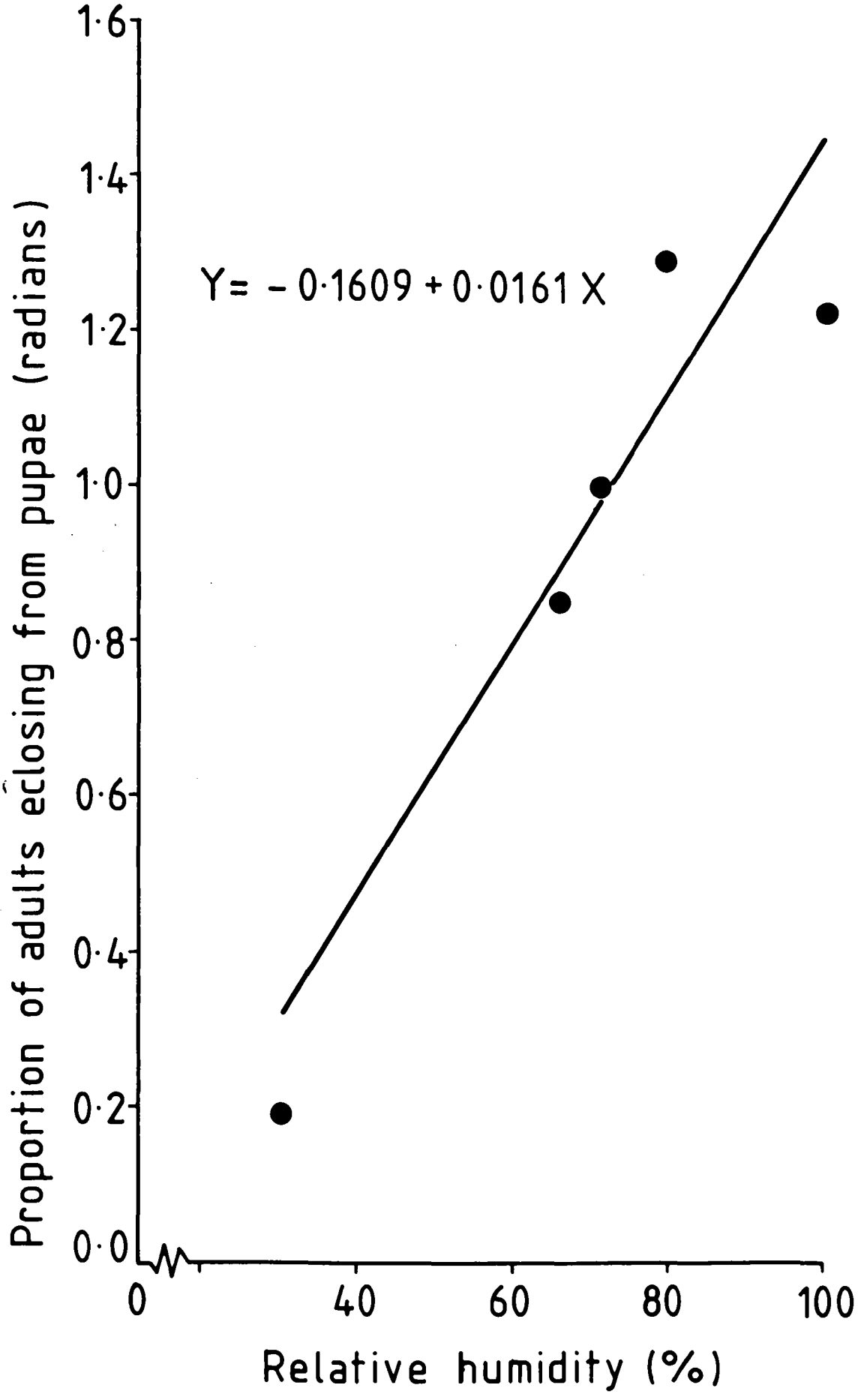
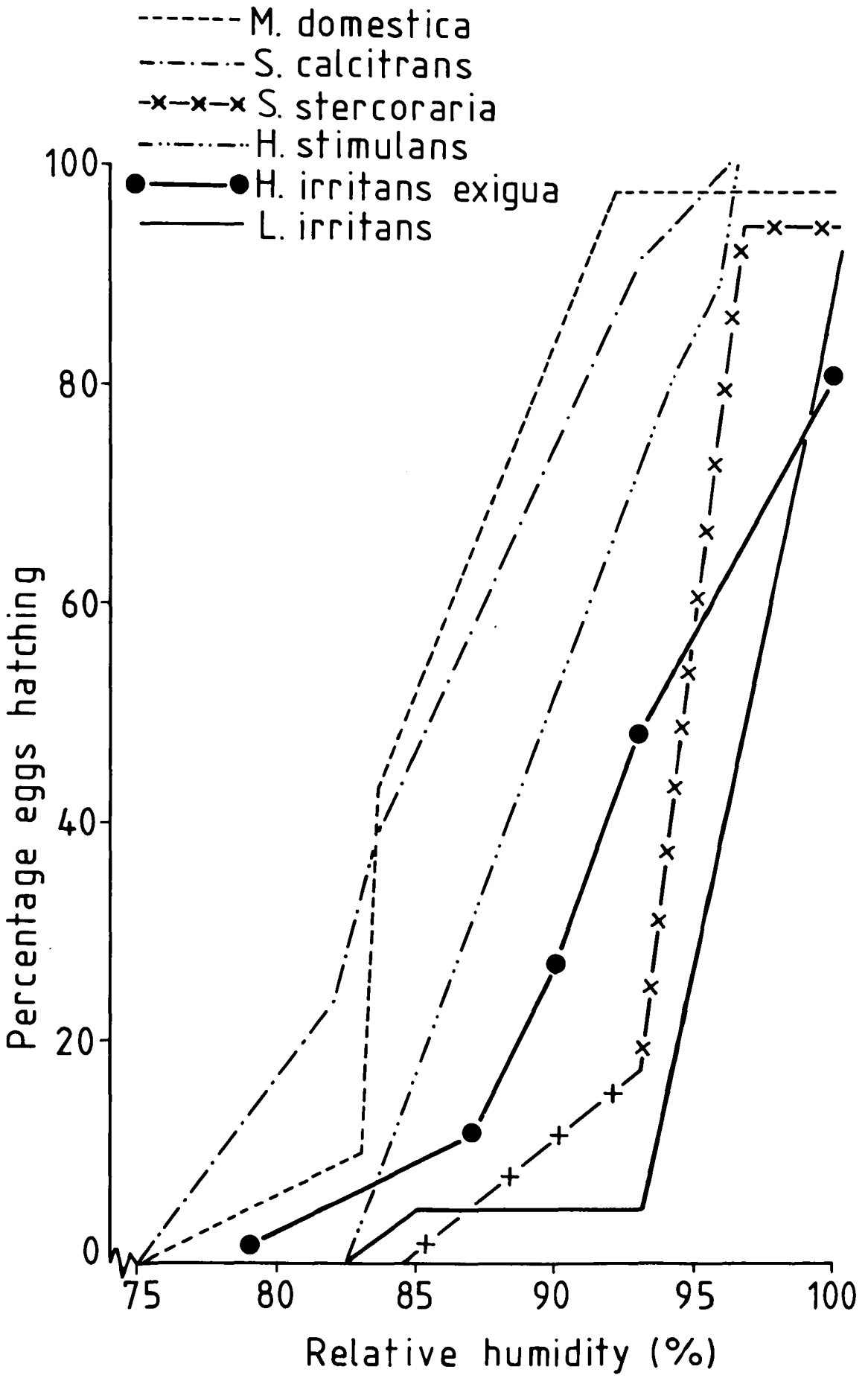

Y= - 0·1609 + 0·0161 X
Proportion of adults eclosing from pupae (radians)
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
0·0
0
40
60
80
100
Relative humidity (%)

Figure 12. Percentages of eggs of a number of species of Diptera hatching under various constant levels of relative humidity. All data except those for *H. irritans exigua* are from Larsen (1943).


M. domestica
S. calcitrans
S. stercoraria
H. stimulans
H. irritans exigua
L. irritans
Percentage eggs hatching
0
20
40
60
80
100
75
80
85
90
95
100
Relative humidity (%)

CHAPTER 3 : THE EFFECTS ON PUPAL FORM OF TEMPERATURE AND DUNG MOISTURE DURING LARVAL DEVELOPMENT

INTRODUCTION

Variability in reproductive capacity is an important consideration in a population study, and the possible effects of extreme temperature and moisture levels on this factor require clarification. Apart from direct action, such extremes could influence egg production by females if their pre-imaginal growth were impeded. The size of the adult female bush fly *M. vetustissima* has been shown to affect fecundity (Tyndale-Biscoe and Hughes, 1969).

On the assumption that pupal size is related to adult size and thus to fecundity, a study of the effects on pupae of various levels of temperature and moisture during larval development was conducted.

REVIEW OF LITERATURE

Puparial weight has been used as an index of the suitability of the breeding medium for immature forms of several species of muscid flies (Bay *et al.* 1969, 1970; Morgan and Schmidt, 1966). Valiela (1969) used the product of weight and length to represent the size of pupae of the face fly, *M. autumnalis*, when describing the growth of the immature stages under varying population densities in dung. In other studies of this species, Bay *et al.* (1968) found that puparial weight depended on dung moisture levels and that no differences existed between the dung of different species in this regard. Other factors also control the size of pupae and emerging adults. Greenham (1972) found that moisture content and nitrogen levels in the larval breeding medium partially accounted for variation in the adult size of *M. vetustissima*. Also, a high larval density in the breeding medium may limit pupal size (Bay *et al.*, 1970).

MATERIALS AND METHODS

In this chapter the term puparium is used to describe the puparium and its contents. Puparia observed were those involved in experiments to measure larval mortality as affected by temperature and dung moisture, described in Chapter 2. They were weighed and measured during the "black" phase shortly prior to emergence.

The combinations of dung moisture (6) and incubation temperature (4) used as experimental treatments, may be obtained from Fig. 13. In the following text, treatments are indicated by figures denoting a combination of moisture level and temperature. For example, the combination 67/20 refers to larvae reared at a dung moisture level of 67 per cent. of wet weight and held at a temperature of 20°C.

RESULTS

Figures 13, 14 and 15 present plots of the treatment means for length, diameter and weight, respectively, together with the fitted curves for moisture alone. The nature of the curves is discussed below. The sample size was 35 for all treatments except 67/35 and 71/30 for which it was, respectively, 20 and 34 puparia. In the case of treatment 67/35, the number of puparia available was restricted by mortality resulting from the hot, dry conditions within the breeding medium. For treatment 71/30 the data for one puparium were misplaced.

A detailed summary of treatment means and standard deviations is presented in Appendix 2.

Two way analysis of variance was performed on each variable. Puparial weights were analysed as their square roots to equalise the error variances. The temperature and moisture main effects and the temperature x moisture interaction were split into single degree of freedom orthogonal polynomial contrasts.

An interesting feature of the analyses of the three variables is that they all behaved in a very similar fashion. Moisture was by far the dominant effect for each variable, accounting for at least 74% of the total sum of squares (TSS) in each case. The temperature main effect was much smaller in comparison and accounted for approximately 2% TSS. There was a significant temperature x moisture interaction in each case, but again, at around 4% TSS, this was small relative to the moisture effect.

The analyses indicated that the puparia were significantly larger when larvae were reared at 25^{o}C than at the other three temperatures

for moisture levels of 84.5% and 87.5%. There were no significant differences in size for any of the variables between the treatments 84.5/25 and 87.5/25. This suggested that a temperature of approximately 25°C, combined with a moisture regime of approximately 85% would produce optimal growth conditions. To investigate this hypothesis a response surface approach was adopted.

Multiple regression equations were fitted to each of the puparial variables using all the significant ($P<0.05$) single degree of freedom submodel terms from the analysis of variance. While these regressions accounted for a large proportion of the variance, it was found that almost as much of the variance could be explained by simply using the moisture terms. The polynomial regressions thus obtained are presented below. A^2_1 designates the percentage variance accounted for by the polynomial regressions presented, A^2_2 denotes the percentage variance accounted for by fitting all the significant single degree of freedom submodel terms.

$$\text{Length} = 101.76 - 4.0996\ \text{moisture} + 0.557\ \text{moisture}^2 - 0.002\ \text{moisture}^3 \qquad (A^2_1 = 83.77\%,\ A^2_2 = 87.90\%)$$

$$\text{Diameter} = 21.88 - 0.9422\ \text{moisture} + 0.0137\ \text{moisture}^2 - 0.00006\ \text{moisture}^3 \qquad (A^2_1 = 79.56\%,\ A^2_2 = 82.61\%)$$

$$\sqrt{\text{Weight}} = 1.5949 - 0.0663\ \text{moisture} + 0.0009\ \text{moisture}^2 - 0.000004\ \text{moisture}^3 \qquad (A^2_1 = 72.66\%,\ A^2_2 = 77.57\%)$$

These fitted curves are shown in Figs. 13, 14 and 15 with the mean observed values for each treatment combination. The interaction least significant differences are also included. The maxima of the fitted curves for length, diameter and the square root of weight occurred at moisture levels of 84.8%, 84.3% and 85.3% respectively confirming that 85% is the optimal moisture level. Furthermore, at this moisture level

the 25°C treatment clearly produces notably larger puparia, particularly in terms of length and weight.

A canonical variate analysis of the data derived from this experiment showed that a contrast between length and diameter was an important characteristic of the resulting puparia. Thus, a further two way analysis of variance was carried out on the ratio of these two variables for all treatments. The moisture, temperature and interaction terms were all highly significant ($P<0.001$) although moisture was again the most important factor. The treatment means and accompanying least significant differences are shown in Fig. 16. Certainly, at the driest moisture level (67%) and at treatment 71/35 puparia were proportionally more elongated. The heaviest puparia were produced at a length to diameter ratio of approximately 2.5 at an estimated moisture level of 85% and a temperature of 25°C.

DISCUSSION AND CONCLUSIONS

In the present study, most of the observed variation in puparial size is directly related to the moisture level of the dung. Within the range of the treatments, puparia become longer, thicker and heavier with increasing moisture, at least to the optimum level of 85% moisture. This effect changes with temperature in a consistent manner for each of the variables (Figs. 13, 14 and 15). The shape variation shown by the canonical variate analysis and the subsequent analysis of variance (Fig. 16) does not appear to have been recorded before. However, its magnitude is small in relation to the size effects and it is most apparent at low moisture and high temperature levels.

The analyses have indicated that a dung moisture level of 85% and a temperature of approximately 25°C are optimal in terms of producing the largest puparia. A similar moisture level was found to produce the heaviest puparia in six other species of muscid flies and that above and below this level weight was reduced (Bay *et al.*, 1968). Below this moisture level range, stress is evidenced by a reduction in size and an alteration of body shape to a relatively more elongate form. The temperature level is less critical however.

In the regression analyses of puparial length, diameter and weight, the low levels of residual or unexplained variation do not leave much room for the possible contributing effects of variation in dung nitrogen concentrations or other factors. However, in this experiment, the dung was uniform and derived from an animal fed on a legume known to have a high nitrogen concentration. It would be of considerable interest to examine variation in puparia derived from cattle fed on a nutritionally inferior diet.

Morphometric variation in muscid flies resulting from changes in the physical environment has been previously reported by Greenham and Hughes (1971). In their study, it was shown that morphometric variation in the adults of the bushfly *M. vetustissima* resulted from different temperature regimes pertaining during the pupal stage. In the present study, temperature and moisture effects were not allocated to any particular larval instar.

It would be desirable to directly relate pupal characteristics to the fecundity of the emerging adults. Unfortunately, techniques for rearing adult flies of this species under conditions of controlled diet have not yet been worked out. When this is achieved, it will open the way for more intensive studies on the role of the quality of the larval substrate in determining important biological attributes of this species.

Figure 13. Treatment means, least significant differences (P=0.05; P=0.01) and fitted cubic regression for puparial length (mm).

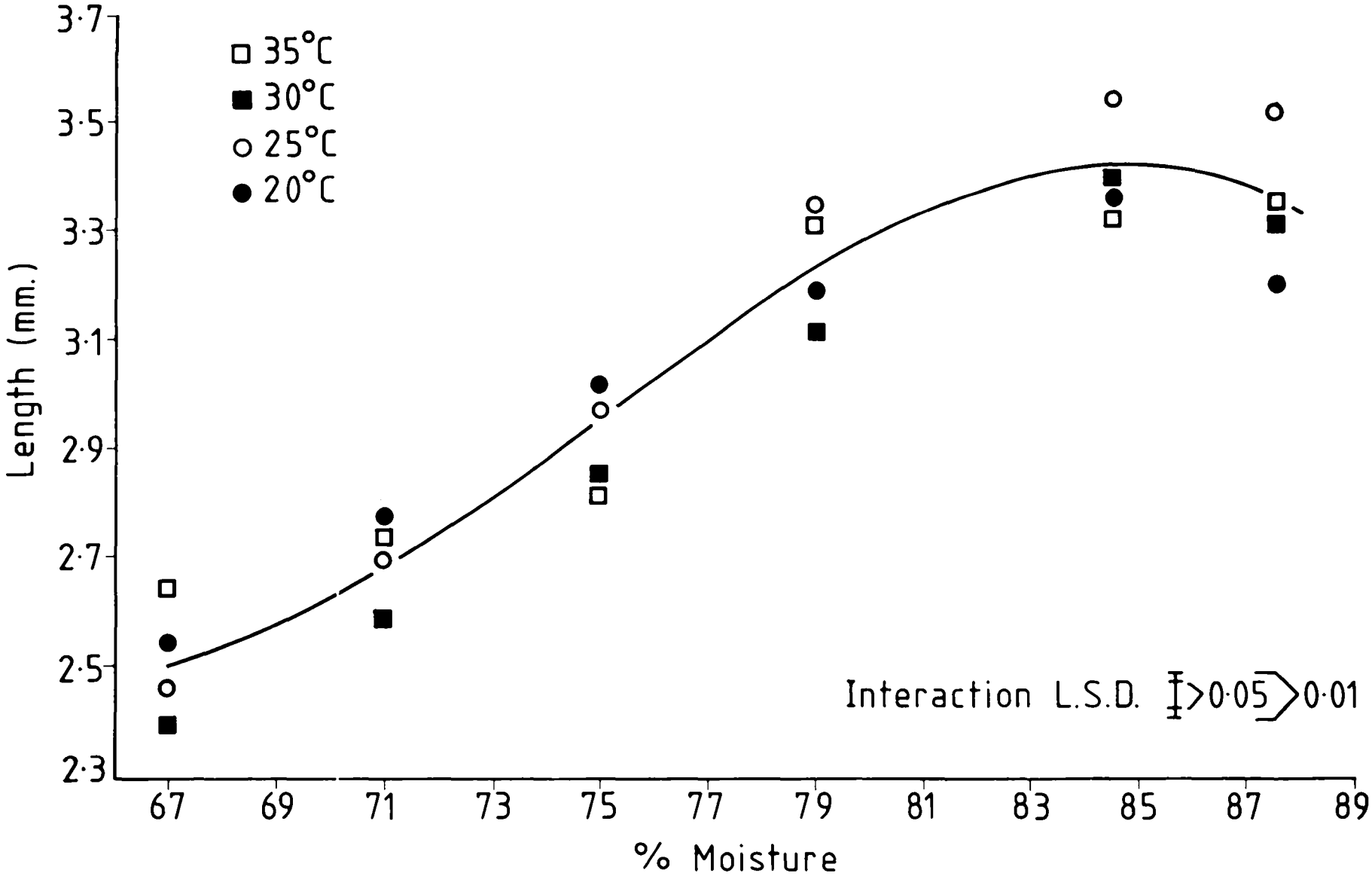

3·7
3·5
3·3
3·1
2·9
2·7
2·5
2·3
Length (mm.)
35°C
30°C
25°C
20°C
Interaction L.S.D. I>0·05>0·01
67
69
71
73
75
77
79
81
83
85
87
89
% Moisture

Figure 14. Treatment means, least significant differences (P=0.05; P=0.01) and fitted cubic regressions for puparial diameter (mm).

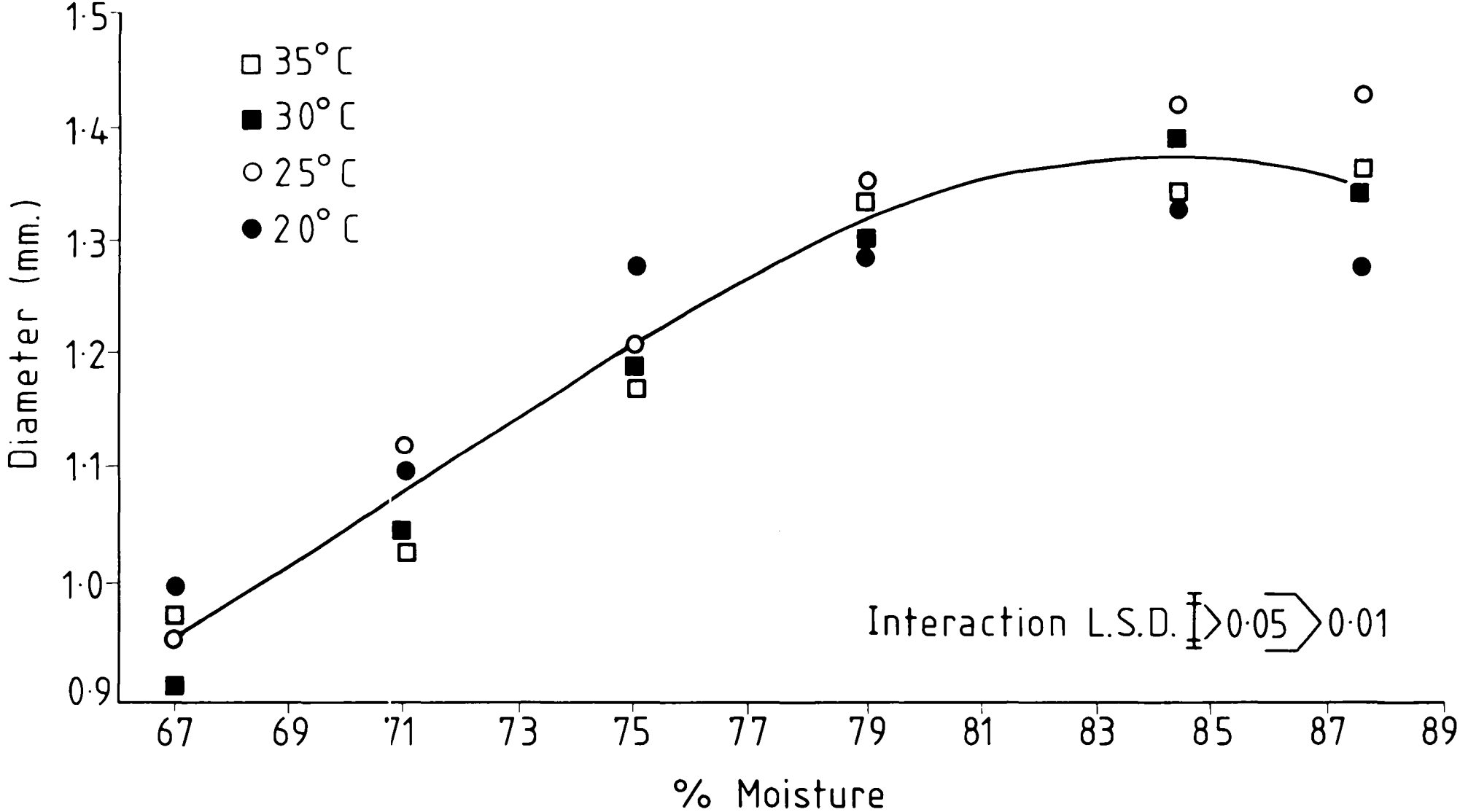

1·5
1·4
1·3
1·2
1·1
1·0
0·9
Diameter (mm.)
35° C
30° C
25° C
20° C
Interaction L.S.D. 0·05 0·01
67
69
71
73
75
77
79
81
83
85
87
89
% Moisture

Figure 15. Treatment means, least significant differences (P=0.05; P=0.01) and fitted cubic regressions for puparial weight (square root) (mg).

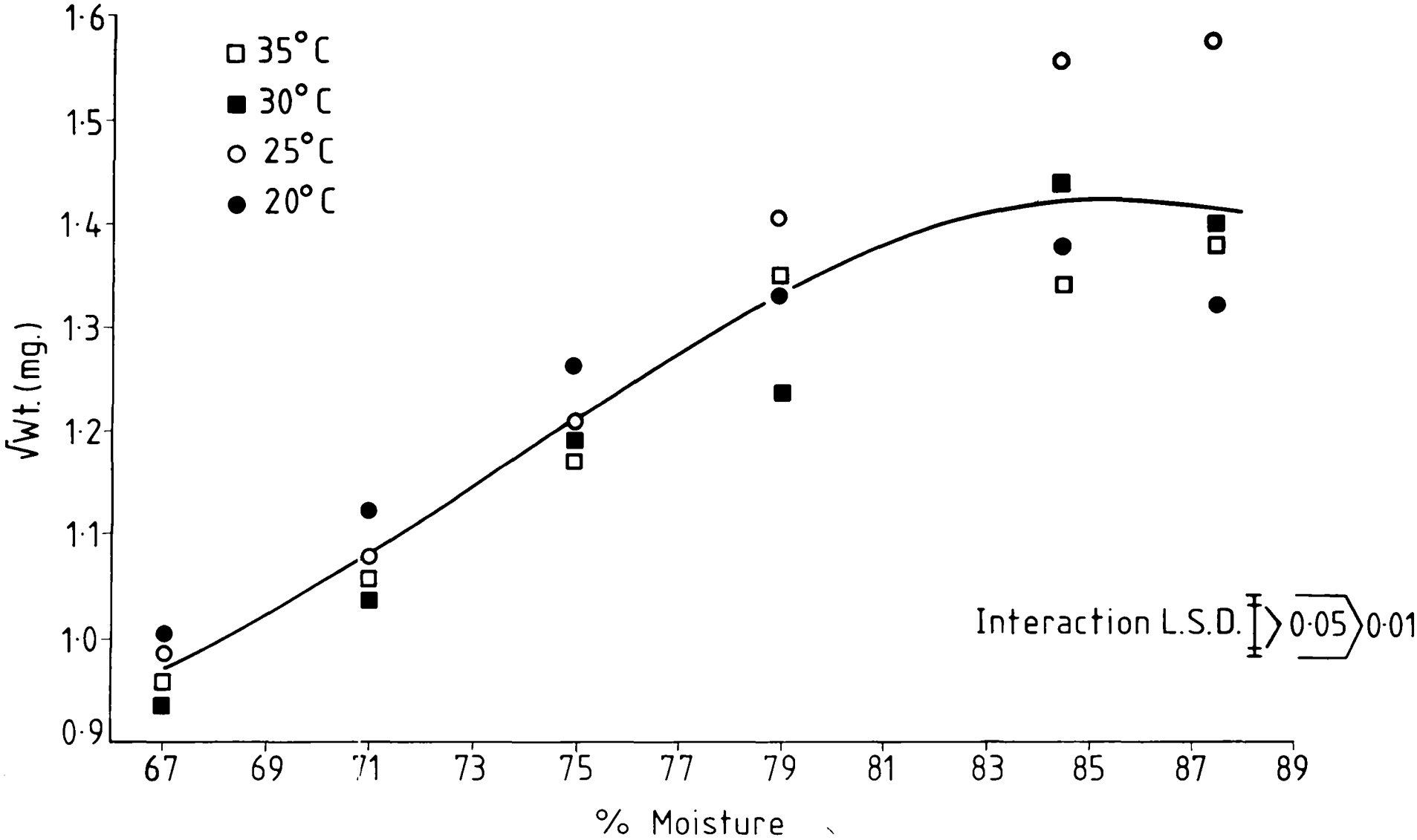
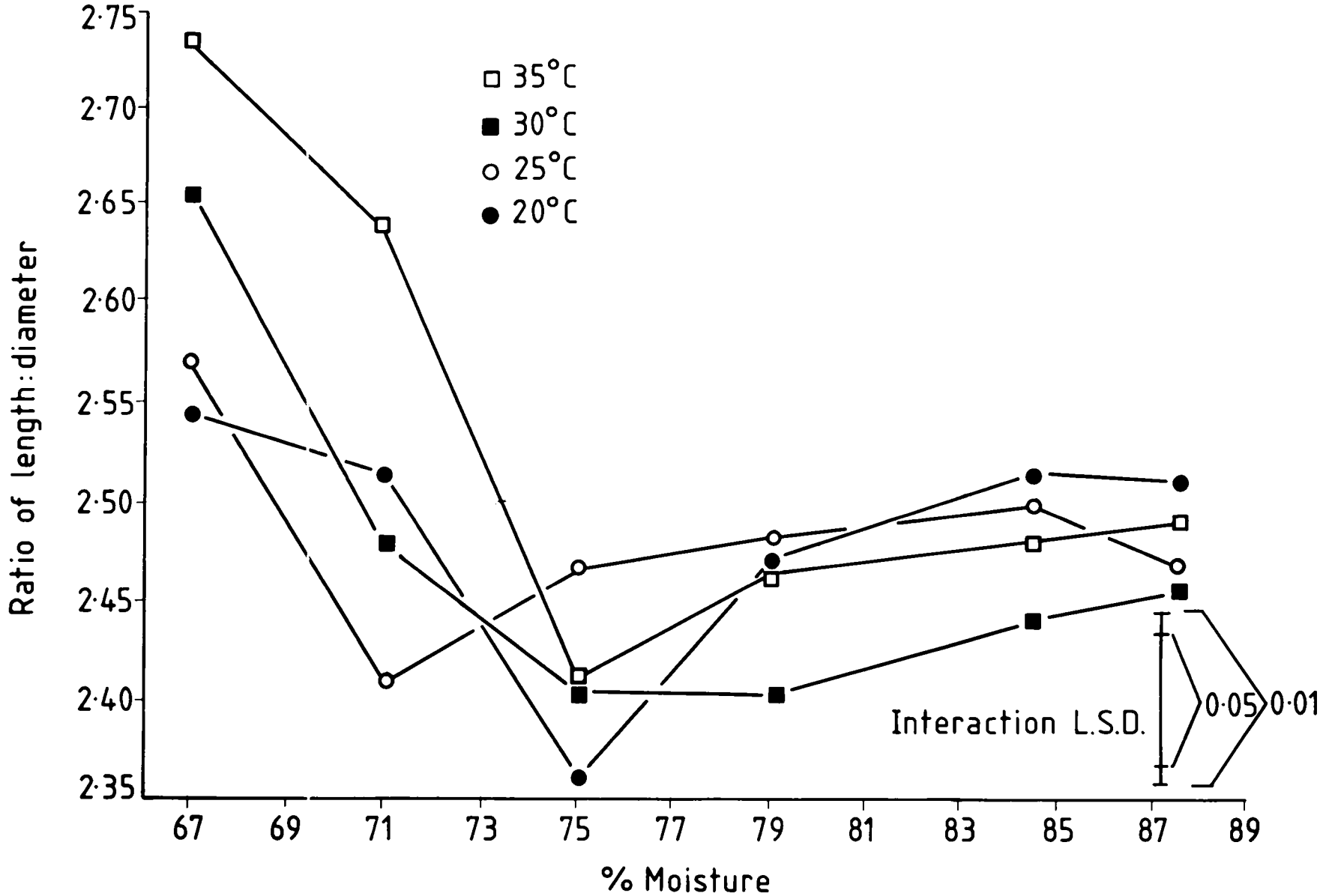

□ 35° C
■ 30° C
○ 25° C
● 20° C
√wt. (mg.)
1·6
1·5
1·4
1·3
1·2
1·1
1·0
0·9
67
69
71
73
75
77
79
81
83
85
87
89
% Moisture
Interaction L.S.D. 0·05 0·01

Figure 16. Treatment means and least significant differences (P=0.05; P=0.01) for puparial length : diameter ratio.


2·75
2·70
2·65
2·60
2·55
2·50
2·45
2·40
2·35
Ratio of length:diameter
35°C
30°C
25°C
20°C
Interaction L.S.D.
0·05
0·01
67
69
71
73
75
77
79
81
83
85
87
89
% Moisture

CHAPTER 4 : OTHER MORTALITY FACTORS

INTRODUCTION

Moisture and temperature are certainly not the only characteristics of dung which affect larval growth. Other factors considered here as potentially contributing to mortality were pH, osmotic concentration of dung fluid and the effects of prolonged immersion on egg hatch.

Under field conditions dung pads are likely to harbour large numbers of a wide range of invertebrate animals as well as dense microbial populations. Both physical and metabolic activity of these inhabitants would modify the chemical nature of the environment in the pad. Excretion of end-products is likely to modify the acidic nature of fluid in dung, and pH at extreme levels is a feature of the dung environment which might affect development of larvae.

As dung pads lose moisture by evaporation, the osmotic concentration of the remaining dung fluid solution would become more concentrated. Many insect larvae are dependent on a wet environment, and soft-bodied fly larvae may be particularly sensitive to desiccation such as would be caused by an increase in salt concentration in the surrounding medium.

In many areas where buffalo flies exist, periods of very heavy rainfall are experienced. Inundation of pads by surface water would be common during heavy rain, and immersion of pads and their inhabitants may be prolonged. Such events would produce sporadic but catastrophic results by the drowning of many dung-inhabiting animals, possibly including buffalo fly eggs. Unlike larvae, these are not

mobile, and are generally placed on the under-sides of pads, where even a few centimetres of surface water would immerse them.

In order to determine the possible significance of these factors, experiments were carried out to determine the effects of exposing buffalo flies of selected instars to potentially stressful levels of the above factors.

REVIEW OF LITERATURE

1. pH and osmolality

There is little in the literature concerning the possible effects of pH and osmolality on mortality among insects and their influence on buffalo flies has not been investigated. Morgan and Schmidt (1966) measured the effects of a range of pH levels in the larval breeding medium on survival of larvae of the closely-related horn fly *H. irritans irritans*. There was uniformly high survival to pupation and subsequent eclosion of adults, over the pH range 6.0 to 8.5. Weights of pupae were uniform over the range 6.0 to 9.0. The percentage of adults emerging was reduced but still high at a pH of 9.0. At pH values 5.5 and 9.5 no larvae successfully pupated. As larvae were placed in a medium of adjusted pH after hatching, there was no record of the effects of pH levels on survival of eggs.

2. Immersion of eggs

Windred (1933) observed, on the island of Java, that rainfall was important in limiting numbers of buffalo flies. In generally drier areas, fly numbers were higher during the wetter part of the year. However, in areas of high average rainfall, the drier winters were more favorable for flies, provided that the lower temperatures were not limiting: he deduced that very heavy precipitation could cause mortality among immature stages, and conducted experiments to test the effects of immersion on survival. Immersion for 24 hours at 26°C did not affect survival of eggs, larvae could not survive two hours immersion and pupae could tolerate up to 48 hours in water, depending on their age. Greenham (1970), in a description of the egg of the dung-breeding bushfly *M. vetustissima*, stated that eggs developed normally

if immersed in water, but not if covered with dung. Hinton (1959) observed that plastron respiration would operate in aerated water, but not if the egg were completely surrounded by dung. The eggs of the buffalo fly are placed on the lower surfaces of dung pads, but are not forced into the dung by the ovipositing female. The need for respiration under conditions of immersion would thus only arise after accidental immersion such as that caused by sporadic heavy or prolonged rain.

Plastron respiration, a mechanism to extract dissolved oxygen from water has been described for *Calliphora erythrocephala* Meigen (Diptera : Calliphoridae) and *L. sericata* (another Calliphorid) (Hinton, 1960a) and for several species of Muscinae (Diptera) including *H. stimulans* and *L. irritans* (Hinton, 1960b). The eggs of *M. vetustissima* and *H. irritans exigua* have been shown by scanning electron microscopy to possess plastron areas (Anon, 1978), and Hinton (1961) reviewed the general structure and function of the plastron for a number of genera, including the genus *Haematobia*.

ANALYTICAL METHODS

In all experiments described in this chapter, the effects of treatments were measured as the percentage of embryos, larvae or pupae which survived the treatment. Data were analysed as the inverse sines of the square roots of the measured proportions, expressed as radians. Where presented, confidence intervals were calculated using the above arcsine transformations and are thus asymmetrical about their respective means when retransformed to original percentage form.

All regression equations are presented in terms of the angular transform of Y. These equations were calculated by the step-up method of multiple regression, and significant terms were identified by testing coefficients for difference from zero, using a critical probability level of 0.05.

METHODS - pH

Larvae were reared on pads of surgical gauze soaked in a homogenate of dung and distilled water, as in the section describing techniques. The pH of the homogenate was adjusted by the addition of ammonium hydroxide or acetic acid (Morgan and Schmidt, 1966). Measurement of pH was made using a glass electrode "Radiometer" pH meter Model 22 (Copenhagen).

Preliminary earlier experiments were conducted to monitor the stability of the adjusted pH at 15, 25 and 35°C. The pH of dung homogenate was adjusted to a number of levels between 4.9 and 10.5 inclusive. Gauze pads soaked in these homogenate preparations were held at the stated temperatures, and the pH of each was measured at 48 hour intervals for 288 hours, a period longer than that at which larvae would be held during ensuing experiments. The results of these observations (Table 13) showed a considerable drift from all levels including the original unadjusted 7.35, at all temperatures, to a value of approximately 9. Consequently, in experiments involving eggs and larvae, five ml of homogenate, of appropriate pH, were added to each culture at 36 hours intervals. This maintained both the pH level and the nutrient status of the homogenate.

Eggs on paper towelling were placed on the soaked pads within 30 minutes of oviposition. When hatch was complete the numbers of hatched and unhatched eggs were counted. Pupae, when formed, were removed and held to await emergence of adults. There was thus a record of the effects of pH on hatch of eggs, survival of larvae to pupation, and survival of pupae to eclosion. Following removal of pupae, the pH of homogenate in each replicate was checked for drift away from the initial

adjusted level.

The initial investigation was restricted to effects of pH on hatch, over a range of temperatures. In the subsequent experiment, survival of eggs, larvae and pupae was measured over a range of pH values, at a constant temperature of $30^{o}C$.

RESULTS - pH

The results of the initial study of the effect of pH on hatching are shown in Table 14. At all three temperatures, a pH level of 10.5 caused total mortality prior to hatch. At lower pH values between 4.5 and 9.0 hatch percentage was high in all treatments. These data were analysed by two-way analysis of variance with replication. Neither temperature nor pH significantly affected the level of hatchability of eggs. ($P>0.50$ and $P>0.20$ respectively) No interaction between the two factors could be demonstrated.

A further experiment was conducted to assess the effect of pH on larval development. These observations were made at a constant temperature of $30^{o}C$. In addition to measurement of larval survival further data on the upper limit to hatching were provided by this experiment. This limit was observed to lie between the values of 9.0 and 10.5, in the experiment previously described. The survival of eggs and larvae, in homogenate of which the pH level ranged from 4.5 to 9.5 is recorded in Table 15. All survival data subsequently described were analysed using one-way analysis of variance.

Within the range 4.5 to 9.0 pH did not affect survival of embryos to hatching. ($P>0.50$). This was expected, considering the results of the previous experiment, and was confirmed by analysis of the present data. The upper lethal limit was found to lie between 9.0 and 9.5.

The numbers of larvae which pupated after successfully hatching were also recorded. Between pH values of 5.8 and 9.0, the percentage of larvae surviving from hatch to pupation did not vary ($P>0.50$). However, at pH value 4.5, no larva reached pupation,

despite the high prehatch survival in this treatment.

Pupae were removed from the cultures and the proportion of emergence of adults was recorded. Survival between pupation and eclosion was high, and not significantly different among pH treatments ($P>0.50$).

METHODS - OSMOLALITY

Experiments were conducted to investigate the effects of osmolality on survival of eggs and larvae. Hatching and rearing took place on homogenate-soaked cotton pads, in the same manner as used in the pH studies. Osmolality was adjusted by the addition of soluble salts to the dung homogenate and was measured in a Knauer osmometer. In early experiments, the salt used to adjust osmolality was ammonium sulphate. However, it was noted that within a day of the addition of the salt to the homogenate, a strong odour of ammonia was being given off. To avoid any possible effects of ammonia on survival, this series of experiments was abandoned and a new series begun using magnesium sulphate to adjust osmolality.

Eggs on paper-towelling were placed on the soaked cotton pads shortly after deposition. The proportion which hatched successfully was noted and the larvae were allowed to pupate. A record of the effects of osmolality on survival of eggs and larvae was made.

RESULTS - OSMOLALITY

The initial experiment involving the use of ammonium sulphate to adjust osmolality is reported in Table 16. The response of egg hatch to change in salt concentration is described by the equation

$Y = 1.2720 - 0.0013X$ (Fig. 17) $(A^2 = 73.91\%)$

Larvae which hatched successfully survived to pupation only in those cultures whose osmolality was measured to be 70mOsm, the natural level of the homogenate before adjustment. No ammonium sulphate had been added to these cultures. As the presence of ammonium sulphate appeared deleterious, this salt was not used further. In the experiments described below, magnesium sulphate was used to adjust osmolality. The results of these experiments appear in Table 17. Percentage hatch, percentage of hatched larvae reaching pupation and the percentage of pupae undergoing eclosion successfully were recorded. For each phase of the survival studies, results were submitted to one-way analysis of variance.

Hatching of eggs was not significantly influenced by osmolality ($P>0.50$) over the entire range 62.5 - 980 milliosmoles but in the 780 and 980 mOsm treatments, no pupation occurred, despite successful hatching. Within the range 62.5 - 510 mOsm, inclusive, the level of successful pupation of hatched larvae was uniform, there being no significant differences between treatments ($P>0.50$). Similarly, pupae formed in all treatments within this range produced uniform numbers of adults, regardless of the osmolality of the rearing medium ($P>0.50$).

METHODS - IMMERSION

Survival of eggs was measured after various periods of immersion in ordinary tap water. These experiments were conducted at a number of constant temperatures. Eggs were collected within fifteen minutes of oviposition and were immediately immersed in glass tubes of water which had previously been brought to the selected temperatures. The tubes were stoppered to prevent evaporative cooling of the water, and the stoppers were pierced with a fine needle, to avoid a build up of pressure in the tubes during application of the stoppers. At appropriate intervals, samples of eggs were withdrawn in a Pasteur pipette, drained on paper towelling and placed on damp filter paper in covered Petri dishes until hatching occurred. The range of periods of immersion varied from zero hours to almost the entire expected pre-hatch period. After removal of the final samples of eggs, all were then held for a further period which was equal to double the normal pre-hatch period for the temperature being applied. This was to allow for any possible retarding effect of immersion on the speed of development of the embryo. On expiration of this period, the numbers of hatched and unhatched eggs were counted.

RESULTS - IMMERSION

Table 18 and figure 18 describes the survival to hatch after immersion in water for varying periods, shortly after oviposition, at a number of constant temperatures. Survival was reduced after relatively short periods of immersion and diminished progressively as immersion time increased. At all temperatures, mortality rate was high, in some cases approaching complete, as the duration of immersion approached the total pre-hatch period.

Further treatment of the data allowed direct comparison of hatch levels at the various temperatures. Each period of immersion was transformed to a percentage of the total pre-hatch period for the particular temperature. Immersion periods for all temperatures were thus placed in the same relative time-scale. Polynomial equations of the form

$$Y = a + b_1x + b_2x^2 \quad \ldots\ldots\ldots + b_nx^n$$

were fitted to the data, using the least squares method. The contribution of the higher powers of the independent variable was determined by testing the coefficient for difference from zero. The regression coefficients together with the attendant values for A^2 appear in Table 19, with the predicted mortality after immersion for the entire normal pre-hatch period, and are presented graphically in Figure 19.

Raw data for the 35°C experiment appear linear. (Figure 18) This may be due to speed of development at this temperature masking the curvilinearity which appeared in lower temperature experiments, where development is slower. This apparent linearity of raw data at 35°C becomes curvilinear when the vertical scale is transformed (Figure 19). The raw data for lower temperatures, apparently curvilinear, generally

retain this characteristic when similarly transformed. At 25°C the cubic term was found to contribute significantly to the explanation of variance. This was considered to be biologically improbable and the illustrated equation is in quadratic form, as are those for the 20 and 30°C treatments.

The degree to which survival was depressed by immersion was shown to vary with temperature (Table 19). At 20°C, survival was lower than for 25 and 30°C. This may be explained by a differential effect of lowered temperature on developmental and metabolic rates. Development generally has a higher threshold temperature than metabolism. Thus at 20°C, although the relative immersion period of 100 per cent. pre-hatch is as for the other higher temperatures, the actual elapsed time immersed is much longer because of slower development rate. Metabolism, less affected by lower temperatures than development rate, thus proceeded for these extended periods of time over which eggs were immersed, to the relative detriment of eggs at lower temperatures, the result being higher mortality among eggs at these temperatures. At 35°C, eggs are near the upper lethal temperature limit. Further stress imposed by immersion may explain survival being lower than at the more favorable 25 and 30°C levels. The complete data matrix was subjected to step-up multiple regression analysis using the least squares method. The equation best describing the data (Figure 20) was

$$Y = -0.5927 - 0.9340X_1 + 0.1152X_2 - 0.0019X_2^2 \qquad (A^2 = 84.82\%)$$

where Y = percentage hatch (arcsin transformed)

X_1 = immersion period as percentage of pre-hatch

and X_2 = constant temperature.

The apparent interaction indicated by low survival at 20°C was not demonstrated in the multiple regression analysis, as the coefficient for the interaction term was not significantly different from zero ($P>0.10$).

DISCUSSION AND CONCLUSIONS

1. pH

Embryos and larvae of *H. irritans exigua* were shown to tolerate a wide range of pH levels in the rearing medium. Values between 4.5 and 9.0 produced no differential effect on hatch, which was uniformly high in all these treatments. The failure of larvae to develop in the pH 4.5 treatment demonstrated that they are less tolerant than eggs of low pH levels, possibly due to their feeding requirements, and the protection of embryos by the chorion. The pH levels at which larvae survived to pupation were generally similar to those shown suitable for survival of horn fly, *H. irritans*, by Morgan and Schmidt (1966). These authors introduced larvae to the pH treatments after hatch, thus restricting their observations to effects of pH on larval survival. The experiments described in this chapter involved the placing of eggs in experimental treatments shortly after oviposition. Results thus did not indicate whether exposure to pH 4.5 prior to hatch contributed to the complete mortality among larvae in this treatment, despite successful hatch. The generally similar patterns of larval survival for *H. irritans irritans* and *H. irritans exigua*, despite exposure of eggs of the latter to various pH levels, suggest that survival of larvae is, in practical terms, independent of pH variation during the pre-hatch period.

2. Osmolality

The possibility that the lethal effects of high concentrations of magnesium sulphate were due to factors other than osmolality was not investigated. Within the range of osmolality values tested, embryonic

survival was indifferent to osmotic concentration, whereas larval survival was not. When exposed to extreme osmotic values, eggs appeared to benefit from chorionic protection and from independence of the need to move and feed in the surrounding medium.

The potential of pH and osmolality of dung to affect fly numbers depends on their natural levels in dung. This matter is examined in Chapter 5.

3. Immersion

The fine structure of the buffalo fly egg has been investigated by scanning electron microscopy (Anon, 1978). This report interpreted the structures seen as enabling eggs to withstand prolonged inundation. These observations were supported by a study of egg survival, wherein eggs survived six hours inundation at 27°C. The report implied that eggs were newly-laid. Conditions were thus comparable to those described in this chapter, where, at 25°C, immersion for 6 hours reduced survival from 85.2 per cent. to 54.5 per cent; at 30°C, a 6 hour immersion reduced hatch from 77.4 per cent. to 36.1 per cent. These findings are also at variance with those of Windred (1933) alluded to earlier in this chapter, where eggs were reported to have survived immersion for 24 hours at 26°C. Eggs of the bushfly *M. vetustissima* are reported to be less susceptible to drowning if several hours elapse between oviposition and immersion (Anon, 1978). Should buffalo fly eggs behave similarly, discrepancies between results reported here and those of other workers may be explained.

The survival of eggs when immersed in dung fluid has not been exhaustively studied. Factors in addition to those applying when eggs are immersed in water are the presence of suspended and dissolved solids,

and microbial interference with the levels of dissolved oxygen. The osmotic character of dung fluid, influenced by dissolved matter, has been shown in Chapter 5 of this study to be unlikely to influence survival of eggs. However, gaseous exchange is essential for a developing embryo and a lowering of oxygen tension in dung fluid by microbial activity could interfere with embryonic development and thereby affect survival. It is thus likely that immersion in dung, or inundation with a mixture of rain water and dung fluid would induce mortality above the levels reported here for immersion in water alone.

The significance of drowning of embryos in the population dynamics of the buffalo fly depends on a number of factors, the most critical of which may be the duration of immersion. During prolonged rainfall, it is likely that eggs, placed habitually on the under side of dung pads, could be immersed for some hours. Duration of immersion could be further prolonged in areas with poor drainage. Data from this study suggest the possibility of significant mortality as a consequence. However, drying and hardening of the investments of the embryo before immersion may mitigate harmful effects; eggs used in this study were immersed shortly after oviposition. Secondary mortality might occur if eggs survive inundation, but were separated physically by water from the pad, thus denying the newly-hatched larvae an appropriate food source.

Temp. (°C)	Initial pH	Elapsed time (hours) 48	96	144	192	240	288
35	10.50	9.92 (0.13)	9.44 (0.09)	9.56 (0.02)	9.44 (0.04)	9.04 (0.09)	8.85 (0.13)
25		9.71 (0.14)	9.40 (0.12)	9.37 (0.13)	9.29 (0.15)	9.21 (0.17)	9.25 (0.17)
15		10.06 (0.04)	9.85 (0.13)	9.71 (0.06)	9.49 (0.04)	9.32 (0.01)	9.39 (0.02)
35	9.00	9.01 (0.02)	8.67 (0.03)	8.64 (0.10)	8.94 (0.19)	8.78 (0.03)	8.66 (0.04)
25		8.72 (0.14)	8.64 (0.14)	8.49 (0.19)	8.75 (0.06)	8.66 (0.09)	8.19 (0.15)
15		8.72 (0.07)	8.76 (0.12)	9.09 (0.07)	8.97 (0.07)	8.88 (0.01)	8.81 (0.04)
35	7.35*	8.73 (0.03)	8.58 (0.05)	8.76 (0.05)	8.84 (0.07)	8.57 (0.12)	8.54 (0.14)
25		8.70 (0.02)	8.60 (0.07)	8.22 (0.21)	8.27 (0.20)	8.32 (0.21)	8.47 (0.21)
15		8.20 (0.06)	8.68 (0.04)	8.97 (0.25)	8.86 (0.03)	8.58 (0.20)	8.73 (0.07)
35	7.00	8.56 (0.15)	8.61 (0.02)	8.85 (0.04)	8.75 (0.09)	8.73 (0.11)	8.68 (0.13)
25		8.60 (0.08)	8.64 (0.19)	8.62 (0.03)	8.69 (0.03)	8.68 (0.01)	8.75 (0.04)
15		8.27 (0.01)	8.27 (0.16)	9.06 (0.02)	8.93 (0.01)	8.58 (0.21)	8.77 (0.04)
35	4.90	6.62 (0.26)	8.35 (0.05)	8.81 (0.04)	8.97 (0.07)	8.93 (0.08)	8.82 (0.21)
25		5.11 (0.01)	7.85 (0.17)	8.67 (0.05)	8.85 (0.02)	8.72 (0.03)	8.83 (0.02)
15		5.10 (0.00)	5.15 (0.02)	5.18 (0.03)	6.81 (0.05)	7.93 (0.03)	8.70 (0.03)

TABLE 13. Levels of pH of dung homogenate and standard errors (n = 4) at various intervals after artificial adjustment of initial pH level, held at three constant temperatures.

*Initial unadjusted pH level of homogenate.

Temp. (°C)	pH level of homogenate 10.5	9.0	7.2*	5.8	4.5
35	0.00	81.33 (72.39-88.86)	84.48 (74.39-92.31)	80.67 (72.08-88.08)	80.23 (71.13-87.96)
30	0.00	77.23 (55.92-93.04)	77.09 (73.01-80.92)	80.23 (68.08-90.04)	81.74 (77.71-85.80)
25	0.00	80.37 (71.76-87.62)	82.54 (77.09-87.62)	84.61 (74.55-92.40)	84.35 (80.09-88.19)

TABLE 14. Percentages of eggs hatching, and 95 per cent. confidence intervals (n = 4), in homogenate cultures at selected constant pH levels, at three constant temperatures.

*Original unadjusted pH level of homogenate.

	pH level of homogenate				
	9.5	9.0	7.6*	5.8	4.5
% Egg Hatch	0.00	87.62 (81.47 - 92.77)	85.48 (78.10 - 91.45)	83.2 (61.40 - 88.53)	90.35 (74.39 - 99.08)
% Pupae from hatched eggs	**	65.62 (56.44 - 74.24)	71.45 (58.17 - 82.93)	70.66 (50.70 - 87.16)	0.00
% Adults from pupae	**	88.19 (81.19 - 93.73)	81.06 (73.16 - 87.85)	87.62 (75.45 - 95.96)	**

TABLE 15. Percentage survival of eggs, larvae and pupae reared at 30°C in homogenate at constant pH levels. Confidence intervals (95 per cent.) appear in brackets (n = 4).

*Original unadjusted pH level of homogenate.

**No record of survival available where previous instars failed to survive.

Osmolality (milliosmoles)	Mean % hatch	95% Confidence Interval
70*	90.76	86.92 - 93.98
265	70.97	68.73 - 72.85
320	55.92	29.34 - 80.65
400	51.05	29.82 - 72.85
540	25.76	16.80 - 36.05
720	17.98	9.55 - 28.55

TABLE 16. Percentages of eggs hatching, and 95 per cent. confidence limits (n = 4), in homogenate cultures of various constant osmolality levels, artificially adjusted by addition of ammonium sulphate.

*Original unadjusted level of homogenate.

	Osmolality (milliosmoles)							
	62.5*	140.0	170.0	220.0	295.0	510.0	780.0	980.0
% Eggs hatching	76.50 (65.39-86.09)	79.95 (76.20-83.33)	74.85 (67.76-81.19)	77.52 (50.88-90.86)	76.35 (68.08-83.72)	75.30 (61.59-86.69)	75.45 (65.78-84.10)	75.60 (73.16-77.67)
% Pupae from hatched eggs	72.54 (43.04-93.90)	71.13 (66.90-85.11)	60.57 (30.14-87.04)	67.26 (39.09-89.93)	60.22 (49.48-70.66)	64.45 (53.49-74.70)	0.00	0.00
% Adults from pupae	81.60 (66.00-93.21)	86.92 (72.54-95.17)	87.74 (77.38-95.17)	89.72 (67.43-99.81)	84.48 (74.24-92.59)	82.67 (74.24-89.72)	**	**

TABLE 17. Percentage survival of embryos, larvae and pupae reared at 30°C in homogenate of constant osmolality adjusted using magnesium sulphate. Confidence intervals (95 per cent.) appear in brackets (n = 4).

* Original unadjusted osmolality of homogenate.

** No record of survival abailable when previous instars failed to survive.

Immersion period (hrs)	35°C % hatch	Immersion period (hrs)	30°C % hatch
0	86.69 (78.25-91.45)	0	77.67 (65.78-87.62)
1	82.41 (78.10-86.33)	2	70.18 (61.42-78.25)
3	69.70 (66.28-73.01)	4	51.22 (47.56-54.88)
5	63.11 (50.70-74.70)	6	36.05 (31.27-40.97)
7	43.73 (31.43-56.61)	8	30.30 (18.26-43.91)
9	29.82 (15.02-47.21)	10	22.33 (13.67-32.41)
(Normal pre-hatch = 12.9 hrs)		12	16.41 (11.03-22.62)
		14	12.26 (10.71-13.91)
		16	7.69 (5.29-10.49)
		(Normal pre-hatch = 15.1 hrs)	

	25°C		20°C
0	85.60 (78.10-91.74)	0	85.60 (79.95-90.45)
3	67.59 (54.88-79.11)	4	63.11 (56.09-69.86)
6	54.53 (44.25-64.62)	8	28.24 (18.40-39.21)
9	55.57 (42.18-68.57)	12	30.95 (23.36-39.09)
12	48.26 (41.49-55.05)	16	12.26 (2.39 -20.33)
15	38.75 (31.76-45.99)	20	4.39 (0.99-10.07)
18	33.39 (25.91-41.32)	24	3.02 (2.03- 4.18)
(Normal pre-hatch = 21.3 hrs)		28	0.11 (-0.56-+2.08)
		(Normal pre-hatch = 29.3 hrs)	

TABLE 18. Mean percentages survival (n = 4) and 95 per cent. confidence intervals of eggs immersed in water for various period immediately (± 15 minutes) after oviposition, at selected constant temperatures.

Temperature (°C)	a	b_1	b_2	A^2	Predicted % Survival at 100% immersion
35	1.170 (0.8605E-1)	0.044E-1 (0.1865E-2)	-0.683E-4 (0.2151E-4)	92.45	2.20
30	1.103	-0.012	0.372E-4	94.17	9.04
25	1.193 (0.6541E-1)	-0.105E-1 (0.1845E-4)	0.524E-4 (0.2095E-4)	84.64	0.64
20	1.171 (0.7926E-1)	-0.202E-1 (0.1683E-2)	0.899E-4 (0.1698E-4)	95.61	2.40

TABLE 19. Regression coefficients and their standard errors for equations relating percentage hatch to duration of immersion at selected constant temperatures. Values of A^2, and predicted hatch after immersion for the complete pre-hatch period are included.

Figure 17. The effect of osmolality of dung homogenate on percentage hatch of eggs

0.2 radians = 3.91 per cent.

1.2 radians = 86.92 per cent.

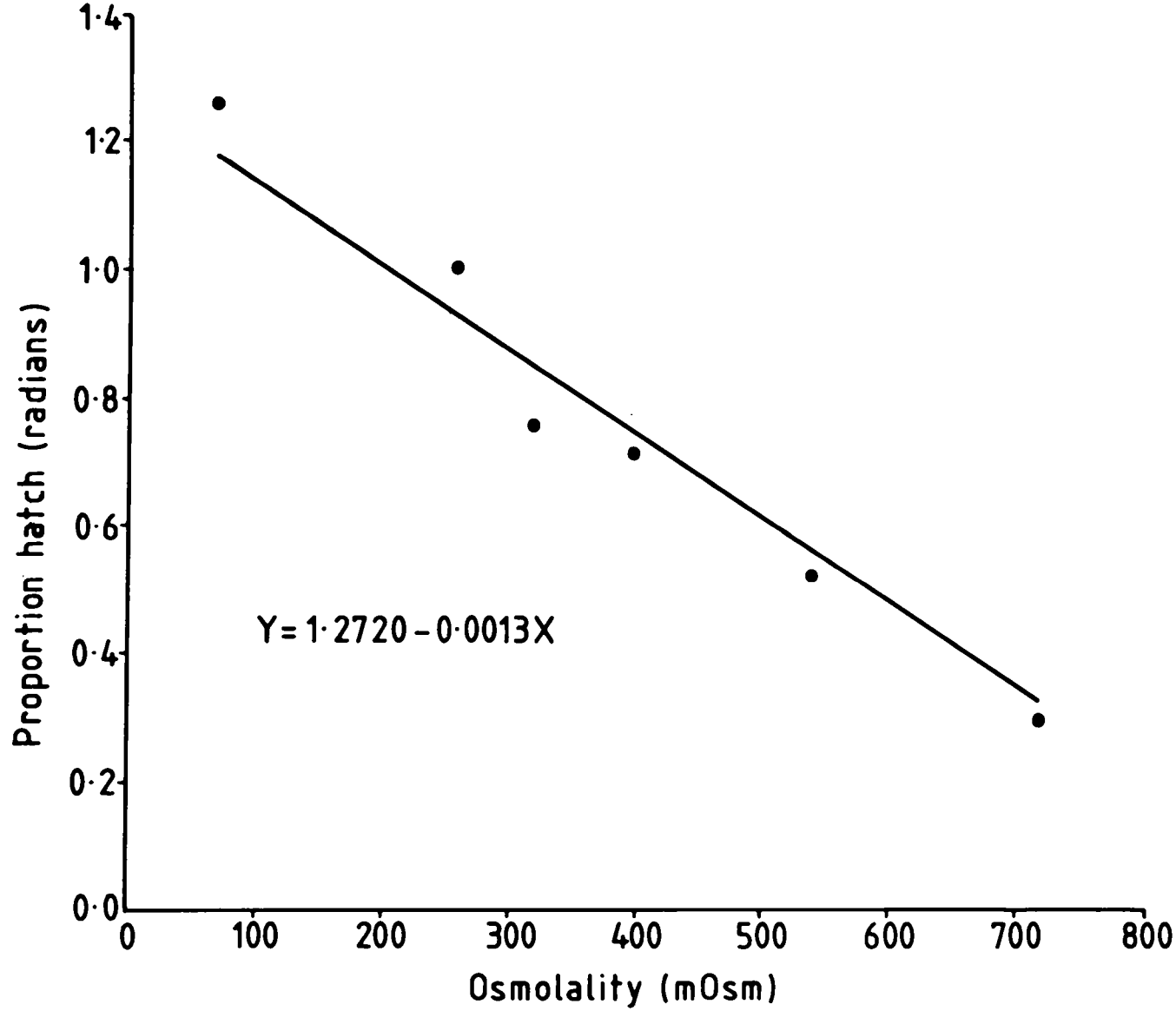

1·4
1·2
1·0
0·8
0·6
0·4
0·2
0·0
Proportion hatch (radians)
Y= 1·2720 - 0·0013X
0
100
200
300
400
500
600
700
800
Osmolality (mOsm)

Figure 18. The effects of various periods of immersion in water on percentage hatch of eggs at selected constant temperatures. Means and 95 per cent. confidence intervals are shown.

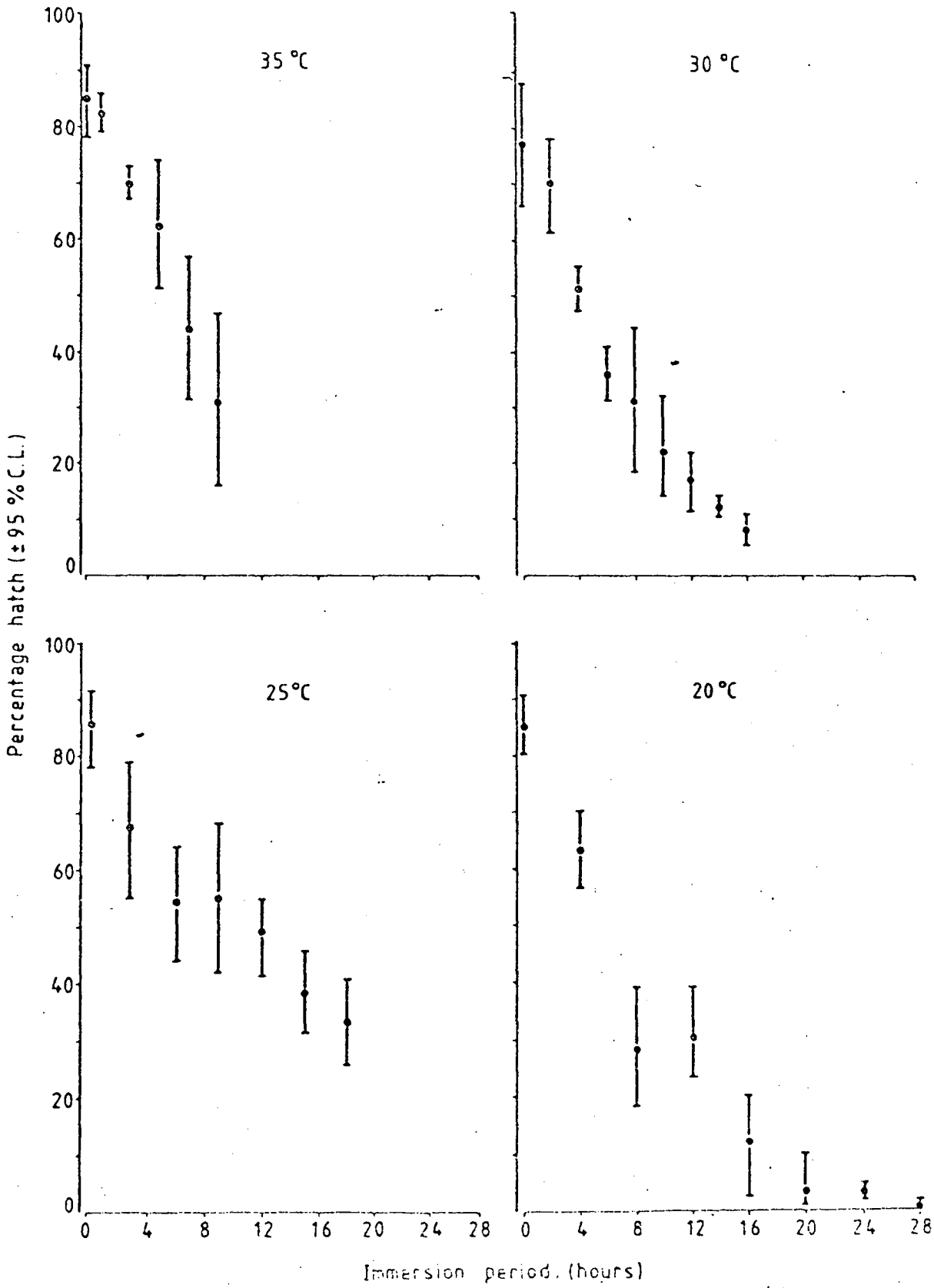

35 °C
30 °C
25°C
20 °C
Percentage hatch (± 95 % C.L.)
Immersion period. (hours)
0
20
40
60
80
100
0
4
8
12
16
20
24
28

Figure 19. Fitted curves showing the relationship between period of immersion (expressed as a percentage of total pre-hatch period at the particular temperature) and percentage hatch of eggs, at selected constant temperatures.

0.2 radians = 3.91 per cent.

1.2 radians = 86.92 per cent.

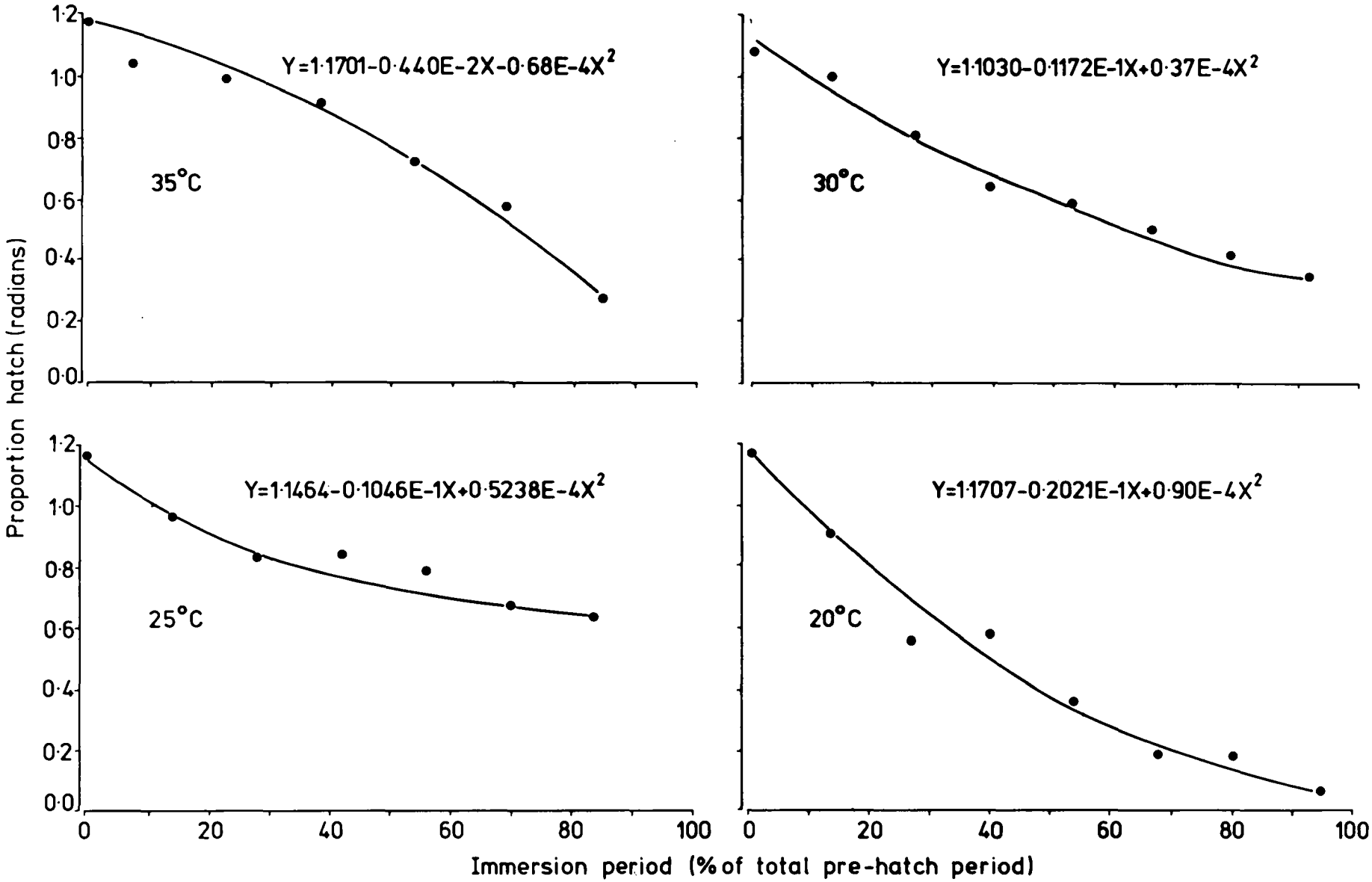
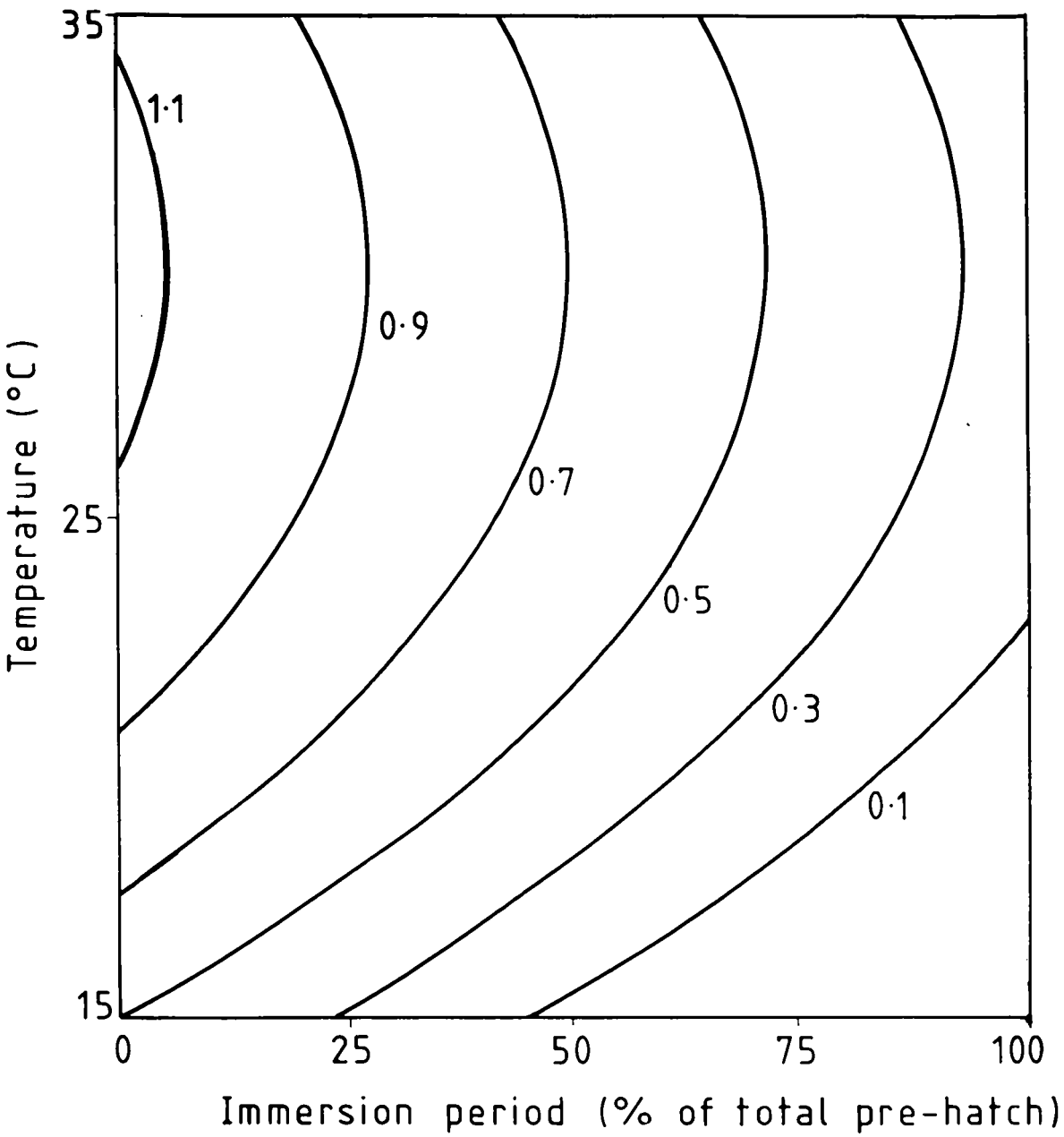

Y=1·1701-0·440E-2X-0·68E-4X²
35°C
Y=1·1030-0·1172E-1X+0·37E-4X²
30°C
Y=1·1464-0·1046E-1X+0·5238E-4X²
25°C
Y=1·1707-0·2021E-1X+0·90E-4X²
20°C
Proportion hatch (radians)
1·2
1·0
0·8
0·6
0·4
0·2
0·0
0
20
40
60
80
100
Immersion period (% of total pre-hatch period)

Figure 20. The combined effects of temperature (^{o}C) and duration of immersion (percentage of the total pre-hatch period) on the percentage hatch of eggs.

0.2 radians = 3.91 per cent.

1.2 radians = 86.92 per cent.


35
25
15
Temperature (°C)
1·1
0·9
0·7
0·5
0·3
0·1
0
25
50
75
100
Immersion period (% of total pre-hatch)

CHAPTER 5 : SUBSTRATE CHARACTERISTICS

INTRODUCTION

Preceding chapters have described the effects of various constant levels of moisture, temperature, pH and osmolality on eggs, larvae and pupae. For each of these factors upper and lower levels were found which caused death and in the cases of temperature and moisture, their effects on rates of growth were examined. To interpret field mortality the levels and normal degrees of variation of these factors must be understood.

Eggs are generally submitted to fresh-dung conditions, since females only oviposit on newly-dropped pads. However, dung is exposed to weather conditions for a number of days while larvae and pupae develop. Therefore application of knowledge of the above factors to a mortality study requires a monitoring of the nature of freshly-dropped dung, and of the way in which these factors change during pre-imaginal growth. To study the practical relevance of these factors, a series of three related sampling programmes was conducted in the following manner:

Programme 1. Measurement of moisture, pH and osmolality of freshly-dropped dung from three different pasture types, over a full seasonal cycle.

Programme 2. Measurement of progressive changes in moisture, pH and osmolality of dung at intervals after exposure.

Programme 3. Measurement of internal dung temperature and moisture loss under continuously monitored atmospheric conditions.

REVIEW OF LITERATURE

1. Moisture

The moisture content of dung depends on the nature of the diet (Dowe *et al.*, 1955, Greenham, 1972a), but is not a direct reflection of the moisture content of fodder. The phenomenon of scouring by cattle during the wet summer season in Northern Australia is due to elimination of body water as a consequence of the elimination of excess salts (Vercoe, personal communication). These excess salts are a feature of a diet of actively growing pasture. High dietary moisture intake in wet-season fodder is eliminated in urine, not in dung. The moisture content of fresh cattle dung generally lies between 70 and 90 per cent. (Church, 1970). Some effects of diet on moisture content were demonstrated by Dowe, *et al.*, (1955). These authors found that increasing proportions of grain concentrates in an alfalfa diet caused a decrease in dung moisture. Where equal parts by weight of alfalfa and corn were fed, dung moisture of six animals averaged 77.7 per cent. A ratio of five parts of corn and soybean meal to one of alfalfa reduced moisture to 73.1 per cent. Greenham (1972a) reported lower moisture contents of dung during dry seasons, from areas of either summer or winter rainfall, when pasture was more fibrous. Samples from Townsville, where buffalo flies are numerous for most of the year, varied in moisture content from a little over 70 per cent. during the dry spring and early summer, to nearly 90 per cent. at the end of the summer wet season.

2. Temperature

The effects of temperature on survival of immature stages of the buffalo fly have been reported in an earlier chapter. As atmospheric temperatures often exceed the upper lethal limits for eggs and larvae in areas where buffalo flies occur, the temperatures within dung pads were considered as a potentially important source of mortality. Greenham (1972b) observed that temperatures in a dung pad could exceed atmospheric maxima, once the early affects of evaporative cooling no longer operated. Minimum temperatures in the atmosphere and within the dung pads were similar. Atmospheric temperatures alone are thus of limited use in predicting mortality or speed of development, unless internal pad temperatures are known or can be calculated.

3. pH

Little is known of the behaviour of pH in dung subsequent to its dropping. Barnard (1973) described the vertebrate intestine as maintaining a buffered, slightly alkaline environment of pH level 7.0 - 8.5. Such values are compatible with those reported from fresh dung in Chapter 4. However, Van' T. Klooster (1967) reported pH values for freshly-dropped cattle dung of 6.6 - 6.9.

4. Osmolality

No reports of the osmotic character of bovine dung were located in the literature.

Plate 2. Native non-irrigated pasture at Brandon, Qld., during summer (2a) and winter (2b).

Plate 2a

Plate 2b

Plate 3. Improved non-irrigated pasture at Brandon, Qld., during summer (3a) and winter (3b).

Plate 3a

Plate 3b

Plate 4. Improved irrigated pasture at Brandon, Qld., during summer (4a) and winter (4b).

Plate 4a

Plate 4b

PROGRAMME 1 : THE NATURE OF FRESHLY DROPPED DUNG

MATERIALS AND METHODS

Freshly-dropped dung was collected on six occasions over the period 19/12/1975 - 12/10/1976, from the property of Pioneer Stations Pty. Ltd., a Brahman and Droughtmaster stud-breeding operation at Brandon, sixty-five kilometres south of Townsville. Samples were obtained from three separate paddocks, each of which carried a herd of pure or high-grade Brahman cows and calves for the duration of the sampling period. The paddocks chosen were all of similar soil type (a sandy loam), were in close proximity to each other, and carried different pasture types, viz. native, non-irrigated improved, and irrigated improved. The native pasture consisted mainly of a mixture of spear grass (*Heteropogon contortus*) and blue grass (*Dicanthium* spp); the non-irrigated improved pasture was a mixture of green panic (*Panicum maximum*) and a low component of the legume Siratro (*Macroptilium atropurpureum*), while the irrigated paddock contained Kazengulu Setaria (*Setaria anceps*), Siratro and Centro (*Centrosema pubescens*), another legume.

The pasture types described are shown in Plates 2, 3 and 4 during the summer wet season, and at the end of the dry winter. On each sampling day, four fresh pads were collected from each paddock. Only pads from adult female cattle were used, and these were collected within a few minutes of being dropped. Moisture, pH and osmolality were measured for each pad, using methods previously described.

RESULTS

Two-way analysis of variance was carried out on each of the pH, osmolality and moisture data sets to determine whether these properties of dung varied with pasture type or with the change of seasons. The probability levels of the F-values appear in Table 20. All of the factors showed very highly significant effects ($P<0.01$). The linear interactions between these two factors were also highly significant ($P<0.01$).

1. Osmolality

The osmolality levels of freshly-dropped dung from three pasture types, at different times of the year, appear in Table 21. There do not appear to be any consistent effects of season or pasture type on these values despite the results of the analysis of variance. There was no record for the herd running on the improved non-irrigated pasture for samples obtained on 20/8/1976. This herd had been fed a mixed grain supplement for four weeks prior to sampling. Sufficient moisture for measurement of osmolality could not be expressed from any of the four samples. Abnormalities among these samples also appeared during moisture and pH measurement.

2. pH

The pH levels of dung from all pasture types remained slightly alkaline with little variation over all seasons (Table 22) in contrast to the report of Van 'T Klooster (1967) that cattle dung was slightly acid. The only aberration occurred among the herd running on improved

non-irrigated pasture when sampled on 20/8/1976, after being fed grain supplement. This appears to have caused a depression of pH value. On cessation of grain feeding, shortly after this sampling, pH values returned to levels similar to those of the non-supplemented herds.

3. Moisture

Table 23 indicates moisture contents of dung samples from the three pasture types over a cycle of seasons. Dung moisture and daily and monthly rainfall recorded on the property where sampling was carried out are shown in Figure 21. Marsh and Campling (1970) reported a moisture level of 89 per cent. in dung from lush pasture. This level was only approached in this study by that from irrigated pasture in the December and January samples. Moisture levels from all pasture types fell during the dry winter, the greatest drop being in the irrigated pastures. Dung from native pasture, lower than the irrigated samples in the wet season, remained fairly constant until mid winter, then fell to a level close to that of the irrigated pasture. The improved non-irrigated pastures produced dung whose moisture content was erratic, being initially the lowest of the three before rising to be close to the native pasture level in February and March. The very low moisture level for the improved pasture in August is probably due to grain-feeding of this herd. As for pH values, moisture levels were closer to those of the other pastures when grain was withheld.

PROGRAMME 2 : CHANGES IN DUNG UNDER FIELD CONDITIONS

MATERIALS AND METHODS (Exposures were commenced on 24/11/1976 and 13/1/1977.

The dung used in these experiments was collected from cattle running under field conditions. On each occasion, the animals were being hand-fed on hay consisting of spear grass (*H. contortus*), and Townsville stylo (*Stylosanthes humilis*). The natural fodder available to them was a short new growth of native pasture which had re-grown after burning off. Collection was restricted to dung sufficiently recently dropped that no appreciable crust had formed. Dung thus collected was thoroughly mixed on a concrete floor. During this mixing the insecticide carbaryl as an 80 per cent. powder was incorporated at the approximate rate of 5 g/kg of wet dung. This was to avoid disturbance of dung by insects causing increased evaporation and possible destruction of pads.

Artificial pads were then formed, each weighing exactly two kilograms. A number of samples were taken from the mass to provide initial measurements of moisture, pH and osmolality. These pads were shaped in a round plastic box 20 cms in diameter. When being exposed each pad was placed on a 25 x 24 cm square of 16 gauge fibreglass gauze. This was to allow the pad near-normal contact with the ground, and to facilitate its later collection. The soil on the exposure site was hard, dry and compacted with a high clay content. Four pads were collected at intervals after exposure, and moisture, pH and osmolality were measured. Only the moist central areas of the pads were used in the sampling. It was considered probable that fly larvae would congregate

in these zones as the outer layers dried and hardened. No rainfall occurred during these exposures.

RESULTS

Osmolality, pH and moisture values for exposed pads appear in Tables 24 and 25. The osmolality of dung fluid increased during the course of both exposures, probably as a result of moisture loss. The regression equation relating the two parameters in this particular experiment was calculated to be

$$Y = 811.3148 - 8.3346X \qquad (A^2 = 78.31\%)$$

where Y = osmolality in milliosmoles

and X = percentage of dung moisture.

The relationship is linear. This equation is unlikely to describe the relationship between osmolality and dung moisture where the initial nature of dung or atmospheric conditions during exposure were different. There is, however, a clear indication of a rise in osmotic concentration as dung loses moisture.

Values for pH appeared to rise slightly but fairly consistently during the first exposure, but this did not recur in the second. At no stage did pH levels approach those previously found to be harmful to eggs or larvae. There were highly significant correlations between pH and both moisture and osmolality in Exposure 1. ($n = 32$, $r = 0.6230$, $P<0.001$, and $r = 0.5229$, $P<0.002$ respectively). As there is no evidence of dependence between pH and these two characteristics, regression equations for possible predictive use were not calculated.

Moisture levels were recorded only to relate to accompanying changes in pH and osmolality, and to provide data for the experiment

described in Appendix 3. The rate of drying of dung, as affected by atmospheric conditions, was investigated in Programme 3 reported below.

PROGRAMME 3 : MOISTURE CONTENT AND INTERNAL TEMPERATURE OF DUNG

MATERIALS AND METHODS

Continuous records were kept of atmospheric shade temperature and relative humidity, and the internal temperatures of either ten, eleven or twelve dung pads, over a number of days. All pads were periodically weighed to monitor moisture loss. Exposures were made on three occasions. In the second, dung moisture was varied by adding different amounts of water. All pads in the first two exposures were placed in direct sunlight. In the third, five pads were in sunlight, and five were shaded by a piece of particle board 2 cm thick, whose upper surface was sheathed with white laminex, and whose lower surface was painted black, to reduce transmission of radiant heat to pads. The board was supported 30 cms above the ground by concrete blocks at each corner, to allow air movement over the pads. The shade was such that direct sunlight only affected the shaded pads in the very early morning and late afternoon.

Internal dung temperatures were obtained using a 12-channel potentiometric dotted-line recorder (Siemens Industries Ltd. Kompensograph, Type M 73812) which provides a colour-coded imprint for each of the twelve channels at approximately one minute intervals. One thermocouple was inserted vertically in the centre of the underside of a dung pad, and the other immersed in an ice-water bath. The printed record was represented in millivolts, and converted to temperature by the formula

$$T^{\circ}C = E(1.549E + 25.702)$$

where E = millivolts, and

$T^{o}C$ = degrees Celsius in the dung pad minus the reference temperature of the icewater bath.

The temperature in the water bath was recorded continuously on a Thies revolving drum Thermograph. The temperature in the dung pad was thus obtained by calculating the $T^{o}C$ value above, and adding the recorded ice-water bath temperature. Careful time-checking was required to enable coordination of temperatures of dung-pads and of the ice-water bath. Atmospheric shade temperature and relative humidity were measured at ground level using a Thies recording thermohygrograph.

The descriptions of each exposure appear in Table 26. Duration of each was generally dictated by availability of equipment.

RESULTS

1. Internal temperature

Maximum and minimum levels were used to compare atmospheric temperatures with those within dung pads. Comparisons at other times would not be valid because of time lags between atmospheric temperatures and corresponding ones within the pad. Only days when the moisture content of the pads was known were used in the analyses described below. Measurements were made on twelve unshaded pads for the first two exposures, and on five for the final exposure. Where shaded and unshaded pads were compared (in the final exposure) measurements were carried out on five pads in each category. Since pads could not be sampled for zoned moisture content without being destroyed, moisture was calculated using the weight of the whole pad.

Tables 27 and 28 record maximum and minimum temperatures respectively for the unshaded pads of the first two exposures, accompanied by corresponding atmospheric levels. Tables 29 and 30 show maximum and minimum temperatures respectively for the shaded and unshaded pads in exposure 3, and include corresponding atmospheric temperatures.

1.1 Minimum temperature The effect of atmospheric temperature and dung moisture on internal minimum temperature of exposed pads were best described by the equation

$$Y = 7.1868 + 0.6036X_1 + 0.0208X_2 \qquad (A^2 = 48.30\%)$$

where X_1 = atmospheric minimum temperature

X_2 = percentage dung moisture

and Y = minimum temperature reached within the pad.

This relationship is presented graphically in Figure 22. The equation coefficients were calculated using the least squares method. Use of higher order terms in X_1 and X_2 did not improve the fit. The equation demonstrates that internal minimum temperatures exceed atmospheric minima, the relative disparity becoming less as atmospheric minima increase. The contribution of moisture to the value of internal minimum temperatures is small and positive.

The minimum temperatures reached in the shaded pads and accompanying unshaded pads in the third exposure appear in Table 30. There were no significant differences between the two groups of pads ($P>0.50$).

1.2 Maximum temperatures The relationship between atmospheric maximum temperature, dung moisture and internal maximum temperature of

unshaded pads was best described by the equation

$$Y = 48.0630 - 3.7469X_1 - 0.5897X_2 + 0.1007X_1^2 - 0.0167X_1X_2 \qquad (A^2 = 51.85\%)$$

where X_1 = atmospheric maximum temperature

X_2 = percentage dung moisture

and Y = maximum temperature reached within the pad (Fig. 23).

The step-wise regression analysis was carried out by the least squares method. The relationship is more complex than that of the minimum temperatures; high moisture content appears to result in some cooling effect but maximum temperatures in dung pads still generally exceeded atmospheric maxima, the difference in observed data reaching 10.4°C in one case, when the atmospheric maximum was 32.2°C.

Shaded pads, as anticipated, reached maximum temperatures significantly ($P<0.01$) lower than those of unshaded pads (Table 29). Shaded maxima also fell below atmospheric maxima, probably due to some continuing effect of evaporative cooling even after crust formation.

1.3 Time lag Table 31 presents details of the time interval between atmospheric and internal temperature limits, both maximum and minimum. At minimum temperatures average lags for shaded and exposed pads, and their ranges, were 1.3 (-1.0 to 2.5), and 1.2 (-1.0 to 2.5) respectively. Corresponding values for maximum temperatures were 3.4 (1.8 - 5.4) and (0.8 - 2.6).

2. Accumulated Temperatures

2.1 Calculation, raw data and basic statistics When linear relationships between temperature and growth rate are verified or assumed, a single thermal constant (Time x Temperature above threshold) can be used to predict the time development will take, unless the temperature regime goes beyond the lethal limit. However, the relationship between speed of development of buffalo fly eggs and temperature was found to be non-linear, in Chapter 1. Consequently, asymptotic equations of the form

$$Y = A + BR^X$$

where Y is elapsed time and X is constant temperature were derived to describe these relationships for all instances. Since these are non-linear regressions, prediction of duration of stadia under measured conditions of fluctuating temperatures cannot be conducted using simple accumulation of hour degrees. The accumulation of temperatures in this study was carried out in terms of the proportion of total development which would occur in 24 hours in a dung pad; this was calculated as the sum of proportions of development for each hour, since there was an hourly record available of the internal temperatures of the dung pads in the three series of exposures described above.

Proportions were estimated in terms of female development, since this would be the most important interval in prediction of generations. The equation for the duration of the total pre-imaginal development was reported in Chapter 1 to be

$$Y = 154.6582+0.7687E4*0.8606^X$$

The hourly proportion of development is thus represented by the expression

$$\frac{1}{154.6582 + 0.7687E\text{-}4 * 0.8605^{X}} .$$

The accumulated proportions of development in dung pads in exposures 1, 2 and 3 appear in Tables 32, 33 and 34 respectively. Atmospheric temperatures are included similarly transformed, for comparison.

2.2 The effects of moisture on accumulation Moisture content of dung was shown above to have a small but significant effect on maximum and minimum daily temperatures reached in pads (Figures 22 and 23). It was thus desirable to assess the effects of moisture on temperature accumulation. Data from exposures 1 and 2 were combined, using those cases where both accumulation and moisture levels were available. The moisture levels were based on a weighing within the 24-hour period which provided the accumulation estimate. Atmospheric temperature was expressed as accumulated hour-degrees. Internal accumulation was in terms of the proportion of pre-imaginal development in a 24-hour period. Regression analysis, using atmospheric accumulation and dung moisture as independent variables and within-pad accumulation as the dependent variable showed no contribution of dung moisture to temperature accumulation, the coefficient being not significantly different from zero ($P>0.10$). Further analyses were therefore restricted to the relationships between internal and external temperature without reference to dung moisture.

The relationships between atmospheric temperature, in untransformed day-degrees and speed of development for unshaded and shaded pads respectively were

$$Y = -0.1581 + .0004X \qquad (A^2 = 44.00\%)$$

and

$$Y = -0.0675 + 0.0002X \qquad (A^2 = 56.55\%)$$

where X = atmospheric day-degrees, and

Y = proportion of pre-imaginal development.

These equations are presented in Figures 24 and 25. When X-values were transformed to the same units of proportion of development, the relationship for unshaded pads was

$$Y = -0.0472 + 1.9736X \qquad (A^2 = 33.18\%)$$

This equation and that described below linking development rates in shaded and exposed pads were used in Chapter 6 to estimate speed of development under field conditions.

The behaviour of shaded pads can be described in terms of comparison with exposed pads under otherwise similar conditions. A regression was calculated from the data in exposure 3, having exposed and shaded pad temperatures as independent and dependent variables respectively (Fig. 26). Both were transformed to units of proportional development. The equation best fitting the data was

$$Y = -0.0059 + 0.9048X \qquad (A^2 = 87.41\%)$$

3. Moisture loss Moisture loss was monitored by periodic weighing of pads. Since pad moisture was likely to affect the rate of water loss, only intervals of 4.5 hours or less between weighings were considered when calculating the regression of moisture loss on pad moisture and atmospheric saturation deficit. Pad moisture for each case was taken to be the moisture content at the beginning of the period over which water loss was measured. Saturation deficit and moisture loss were expressed as mean units per hour, since the duration of observations varied. Measurements on exposed pads were

made during exposures 2 and 3 on 19 occasions, there being on each occasion a maximum of twelve (exposure 2) or five pads (exposure 3). Any broken pads were discarded. Moisture loss in five shaded pads was monitored only in exposure 3, on nine occasions.

The equations relating pad moisture and hourly saturation deficit to hourly moisture loss were calculated to be

$$Y = 2.8020 + 0.2428X_1 - 0.1310X_2 + .0015X_2^2 - 0.0031X_1X_2 \qquad (A^2 = 21.05\%)$$

for exposed pads (Fig. 27) and

$$Y = -22.6087 + 1.2654X_1 + 0.2992X_2 - 0.0025X_1^2 - 0.0155X_1X_2 \qquad (A^2 = 24.43\%)$$

for shaded pads (Fig. 28) where

X_1 = hourly saturation deficit (mm Hg) and

X_2 = percentage dung moisture

The dependent variable Y represents moisture loss per hour expressed as a percentage of moisture present in the pad. The A^2 values for these equations are not high; however the coefficients of all terms were tested and found to be significantly different from zero ($P<0.05$). The equations are shown in the above figures only within the limits of actual observations made. Extrapolation beyond the range of the data gives erroneous predictions, so that the range within which the above equations apply are: 40-90 per cent. pad moisture, and 0-30 mean hourly saturation deficit.

In both shaded and exposed pads, higher saturation deficits increased moisture loss. The effect was more marked at low levels of pad moisture. Between pad moisture levels of 40 and 50 per cent. moisture loss increased at an even and rapid rate as saturation deficit increased. Above 50 per cent. pad moisture the horizontal

distance between contours increases, indicating that moisture loss continues to increase with pad moisture, but that the effect of increasing saturation deficit is lessened. A possible explanation is that in wetter pads there is more passage of free moisture to the pad surface, particularly to the pad/soil interface; as the pad dries, there is less facility for the passage of free water, and moisture loss is then more dependent on diffusion of water vapour. The divergence in curvature of contours for both experiments is unexplained, and probably requires a more detailed understanding of water movement in the dung/soil/air system.

The loss of moisture from exposed and shaded pads is shown as raw data and basic statistics in Table 35. The rate of moisture loss in shaded pads is lower than that in exposed pads. The implications of this phenomenon, with regards to survival of immature flies, are discussed in Chapter 6.

DISCUSSION

1. Osmolality

The osmolality of freshly-dropped dung, investigated in Programme 1, varied widely, from a low value of 136.3 mOsm to a maximum of 221.25 mOsm. There does not appear to be any pattern to differences recorded, since they are not consistently related to pasture type or seasonal conditions. Explanation of these variations would require extensive monitoring of other factors, such as pasture condition and composition and the salt balance of the animals which was outside the scope of this work.

Previous experiments showed that eggs survived osmotic levels of 980.0 mOsm., and that larvae survived the lower level of 510.0 mOsm. (Table 17). The results of the sampling in Programme 1 suggest that the osmotic levels of freshly-dropped dung would not approach these levels, in spite of the variation discussed above. Thus, the osmotic character of fresh dung should not affect the survival of eggs and newly-hatched larvae. The long-term behaviour of osmolality, as demonstrated in the first exposure of Programme 2, depends on moisture (Tables 29 and 30).

Early experiments showed that larvae would not survive a moisture level of 63.0 per cent. (Table 10). These dung samples would have an osmotic concentration of approximately 270 mOsm at this moisture level. Observations recorded in Table 17 indicate successful development of larvae to pupation above and below this osmolality. Thus, larvae would have been killed by drying of the medium while levels of osmotic concentration were still quite suitable for growth.

2. pH

The initial values of dung pH, measured in Programme 1, show no systematic variation with pasture or season (Table 22). The only remarkable value recorded (5.69) was that from the grain-fed herd, where osmolality and moisture were also affected. This low value was still too high to reduce hatch or larval development. (Table 15). In all other measurements on freshly-dropped dung, values were slightly alkaline, and none exceeded 8.0. Thus the pH levels in fresh dung should not contribute to mortality among eggs and young larvae.

The pH of older dung pads is not likely to affect survival of larvae (Tables 24 and 25). The first experiment showed a possible tendency of pH to drift to higher levels, as moisture content dropped. A similar drift occurred in earlier laboratory experiments (Table 13) where moisture content was prevented from varying. Thus the shift in pH is probably independent of dung moisture. The drift to higher levels was not evident in the second exposure (Table 25), where only non-systematic variation occurred. In no pad sampled did the pH value approach a level likely to kill eggs or larvae. Assuming a general similarity in reactions of other closely-related muscid species, the above findings would reinforce the claim of Valiela (1969) that pH of dung would probably not influence survival of larvae of the face fly *M. autumnalis*.

3. Internal temperature

3.1 Maxima and minima. The regression equation relating maximum atmospheric and internal temperatures in unshaded pads confirms that

dung temperatures rise above those of the atmosphere. The experimental upper constant temperature thresholds for eggs, larvae and pupae were found to be 38, 35 and 35°C respectively. Whether dung temperatures above these levels kill flies depends on duration of exposure. In tropical areas atmospheric temperatures commonly remain high enough to produce internal temperatures equal to lethal constant ones for several hours. Further work to relate duration of exposure above lethal levels to mortality is needed, but high temperature appears a likely source of mortality among larvae. Some behavioural protection may be afforded eggs and pupae, as discussed in Chapter 2.

Minimum temperatures reached in dung pads are similar to atmospheric temperatures, particularly at levels commonly occurring in buffalo fly areas, where nightly levels do not often approach zero. Such temperatures are unlikely to cause mortality. Where minima are lower, in southern and western areas of eastern Australia, low temperatures may kill flies, for instance if freezing is involved. The contributions of moisture to internal maximum and minimum temperatures are small, although statistically significant (Figures 22 and 23). Across the range of temperatures involved, minimum levels in pads are almost linearly related to atmospheric temperature, there being little modifying effect of moisture.

Maximum internal temperatures are closely related to atmospheric levels, with moisture having little effect, near the upper threshold for larvae. The effects of moisture are more marked at lower temperatures. Moisture content, although it has some

buffering effect on temperature, will not ameliorate mortality caused by extreme temperatures to any substantial extent.

3.2 Accumulated temperatures Where response of growth rate to temperature is linear, the use of heat units expressed as hour- or day-degrees above threshold are easily used in determining generation time, and are usually calculated using only records of daily maximum and minimum temperatures. Such methods assume close approximation of the area under the daily skewed temperature curve to that under the sine curve. Arnold (1960) presented this technique in its simplest form, using the mean of maximum and minimum daily temperatures, and estimating daily accumulation, in day degrees, to be

$$\frac{\text{Maximum} + \text{Minimum}}{2} - (\text{Development threshold}) \times 1 \text{ (day)}$$

This is a valid estimate, providing the daily minimum exceeds the development threshold. Where this was not the case, Arnold proposed that the accumulated temperature above threshold be estimated by a triangle whose base equalled the internal between intersections of the threshold, and whose height equalled the distance of the maximum above the threshold.

Baskerville and Emin (1969) expanded on the above system of provision for thresholds, by presenting a series of integration equations which allowed for both lower and upper thresholds.

In a field situation, disparity between successive minima must be recognised in applying estimations of the type described above. Techniques to achieve this were developed by Allen (1976), Stinner *et al.*, (1974) and Frazer and Gilbert (1976).

If note is to be taken of non-linear relationships between temperature and growth the techniques above for estimating generation times are inappropriate, and continuous accumulation of proportional development over successive short intervals e.g. hourly is necessary. With the advent of modern data-logging systems this technique is no longer so tedious as to be impracticable. The regressions calculated above to relate atmospheric temperature to that within a dung pad allow the use of atmospheric temperature measurements to calculate speed of development of larvae and pupae. Studies of the microclimate in which eggs hatch, on the wet surface of the pad, are necessary before similar relationships between atmospheric conditions and those on the pad surface can be established.

4. Moisture

It was proposed (Chapter 2) that dung moisture probably had little effect on the survival of eggs and pupae. The present discussion is therefore restricted to the influence of moisture content on larvae survival.

4.1 Initial moisture Initial dung moisture levels reported here (Table 23) are above those at which development ceases (Table 10). Apart from the low moisture level apparently caused by the feeding of grain to cattle on improved non-irrigated pasture during August, initial dung moisture remained above 80 per cent. of wet weight throughout the year, regardless of pasture type. A much lower moisture was reported by Greenham (1972a) in dung from the Townsville area during an earlier dry season, when initial levels fell almost to 70 per cent. There is, however, no record of initial dung moisture being so low that larval development cannot proceed.

4.2 Moisture loss Larval development ceased when dung moisture level was adjusted to 64 per cent. (Table 10). Reduced larval survival and pupal size at 67 per cent. indicate that the moisture content required for development is probably nearer 67 than 64 per cent. Rapid drying of dung, particularly during seasons when the development rate of flies is slow, may therefore be a significant mortality factor among fly larvae. This contrasts with the opinion of Valiela (1969a) that dung moisture probably does not affect survival of the related face fly *M. autumnalis*.

Although freshly-dropped dung appears generally to be moist enough to support larvae, initial moisture levels will influence the time taken for pad moisture to fall to unfavorable levels. There may thus be an indirect effect of initial dung moisture on the survival of larvae, regardless of initial favorable moisture content.

Other factors which may modify the drying rate of dung are rainfall, dew and shade. The effects of repeated heavy dew-fall were not investigated here, but could conceivably influence the net moisture loss from pads. Similarly rainfall could effect a reconstitution of dung moisture, unless heavy enough to break up the pads. However, rainfall during winter periods, when fly survival is low, is generally light and sporadic in areas where buffalo flies occur. Thus any replenishment of dung moisture levels, and resulting improvement of the environment in pads, is likely to be sporadic and short-lived.

Shade has a twofold effect on the environment within dung pads. Internal temperatures were shown above to be modified by shade,

with a subsequent lowering of predicted development rate of larvae. The experiments described here also show that shading reduces the rate of moisture loss from pads. The relative magnitudes of these two factors would determine whether larval survival is increased or decreased by shading of dung pads. This concept is discussed in Chapter 6.

	pH	Osmolality	Moisture
Time effect	<0.001	<0.001	<0.001
Pasture effect	<0.001	<0.001	<0.001
Interaction	<0.01	<0.001	<0.002

TABLE 20. Probabilities of F-values for time, pasture and interaction effects on levels of pH, osmolality and dung moisture.

Date	Pasture type		
	Improved (Irrigated)	Improved (Non-Irrigated)	Native (Non-Irrigated)
19.12.75	175.8 (162.9-188.8)	174.2 (170.6-177.8)	165.8 (162.2-169.4)
10.1.76	179.4 (172.6-186.2)	168.1 (145.8-190.5)	159.4 (150.6-168.2)
8.3.76	170.6 (146.9-194.4)	183.8 (167.4-200.1)	172.5 (159.9-185.1)
9.6.76	161.25 (153.6-168.9)	193.8 (169.1-218.4)	221.2 (198.4-244.1)
20.8.76	136.3 (126.2-146.3)	No Record	155.0 (143.7-166.3)
12.10.76	153.8 (141.8-165.7)	153.8 (133.9-173.7)	146.3 (138.6-153.9)

TABLE 21. Means and 95 per cent. confidence intervals (n = 4) for the osmolality (mOsm) of freshly-dropped dung from three different pasture types at various times of the year.

Date	Pasture type		
	Improved (Irrigated)	Improved (Non-Irrigated)	Native (Non-Irrigated)
19.12.75	7.26 (7.07-7.45)	7.23 (7.16-7.31)	7.18 (7.04-7.33)
10.1.76	7.25 (7.14-7.36)	7.21 (7.08-7.35)	7.08 (7.03-7.12)
8.3.76	7.15 (6.98-7.32)	7.14 (6.92-7.36)	7.20 (7.14-7.26)
9.6.76	7.65 (7.56-7.74)	7.08 (6.92-7.23)	7.48 (7.40-7.55)
20.8.76	7.23 (7.18-7.28)	5.69 (5.15-6.22)	7.15 (7.09-7.21)
12.10.76	7.23 (6.99-7.46)	7.20 (6.91-7.49)	7.13 (6.85-7.40)

TABLE 22. Means and 95 per cent. confidence intervals (n = 4) for pH levels of freshly-dropped dung from three different pasture types, at various times of the year.

Date	Pasture type		
	Improved (Irrigated)	Improved (Non-Irrigated)	Native (Non-Irrigated)
19.12.75	88.64 (88.53-88.75)	82.40 (81.87-82.93)	84.98 (84.23-85.72)
10.1.76	88.41 (86.57-90.04)	84.48 (81.47-87.16)	84.98 (82.01-87.85)
8.3.76	84.10 (82.41-85.72)	86.09 (84.10-87.96)	85.48 (80.78-89.50)
9.6.76	83.46 (81.06-85.85)	83.84 (82.93-84.73)	84.98 (82.80-87.16)
20.8.76	81.74 (79.39-83.84)	76.05 (74.09-77.81)	81.47 (80.51-82.27)
12.10.76	83.84 (80.23-87.04)	82.01 (80.09-83.97)	84.61 (82.01-87.04)

TABLE 23. Means and 95 per cent. confidence intervals (n = 4) of the moisture content, expressed as a percentage of wet weight, of freshly-dropped dung from three different pasture types, at various times of the year.

Day	pH	Osmolality	Moisture %
0	7.33 (7.28 - 7.37)	116.25 (112.27 - 120.23)	80.92 (80.51 - 81.47)
1	7.34 (7.28 - 7.40)	155.00 (153.34 - 156.66)	78.82 (78.54 - 79.25)
2	7.48 (7.38 - 7.57)	168.75 (166.55 - 170.95)	78.10 (77.67 - 78.39)
3	7.58 (7.49 - 7.66)	171.25 (167.63 - 174.87)	76.79 (76.35 - 77.38)
4	7.80 (7.73 - 7.87)	187.50 (176.61 - 198.38)	75.75 (75.30 - 76.35)
5	7.63 (7.49 - 7.78)	198.75 (194.77 - 202.73)	74.85 (73.93 - 75.90)
6	7.63 (7.51 - 7.74)	201.92 (196.70 - 207.14)	72.54 (72.08 - 73.01)
7	7.67 (7.57 - 7.76)	189.75 (185.78 - 193.72)	73.16 (72.85 - 73.63)

TABLE 24. Means and 95 per cent. confidence intervals (n = 4) of pH, osmolality and moisture (as a percentage of wet weight) of dung at exposure and after various intervals (first of two exposures).

Day	pH	Osmolality	Moisture (%)
0	7.46 (7.42 - 7.50)	165.00 (155.26 - 174.74)	76.94 (75.15 - 78.54)
1	7.19 (7.07 - 7.31)	178.13 (170.60 - 185.65)	76.50 (74.70 - 78.25)
2	7.21 (7.11 - 7.31)	190.00 (184.37 - 195.63)	77.38 (76.79 - 78.10)
3	7.21 (6.98 - 7.44)	206.88 (203.07 - 210.68)	76.20 (74.24 - 77.96)
4	7.28 (7.12 - 7.43)	214.38 (207.58 - 221.17)	77.23 (75.90 - 78.54)
5	7.34 (7.16 - 7.51)	221.25 (216.12 - 226.38)	75.60 (73.93 - 77.38)
8	7.53 (7.39 - 7.66)	189.38 (184.37 - 194.38)	73.63 (72.85 - 74.24)
11	7.49 (7.18 - 7.79)	165.63 (143.51 - 187.74)	72.39 (70.97 - 73.93)

TABLE 25. Means and 95 per cent. confidence intervals (n = 4) of pH, osmolality and moisture (as a percentage of wet weight) of dung at exposure and after various intervals (second of two exposures).

Exposure No.	Date Exposed	Duration (days)	Temperature Record	Initial Moisture (%)	Shade	Weighing Frequency
1	19.6.74	20	First 13 days only	80.6-82.8 (all pads)	All exposed	2-daily 1 per day
2	16.7.74	17	First 14 days only	79.6-80.0 (3 pads)	All exposed	3-4 daily 3-4 per day
				82.0-82.1 (3 pads)		
				83.2-83.4 (3 pads)		
				83.9-84.0 (3 pads)		
3	19.9.74	15	First 10 days only	84.5-84.7	5 shaded 5unshaded	2-3 daily 3-4 per day

TABLE 26. Details of three separate exposures of dung pads, designed to show the relationship between atmospheric conditions, temperatures inside dung pads, and rate of moisture loss.

Day (Exposure 1)	Atmosphere	Pads
1	30.6	32.4 (31.1 - 33.7)
3	30.0	31.8 (31.0 - 32.6)
5	30.0	33.2 (32.5 - 33.9)
7	31.7	36.4 (35.4 - 37.4)
9	31.1	31.9 (30.7 - 33.1)
11	32.8	36.0 (35.2 - 36.8)
(Exposure 2)		
1	30.6	31.8 (31.2 - 32.4)
2	30.0	33.9 (33.3 - 34.5)
7	33.2	35.6 (34.6 - 36.6)
9	31.0	30.2 (29.5 - 30.9)
11	34.0	37.6 (36.4 - 38.8)

TABLE 27. Maximum temperatures (^{o}C) reached in the atmosphere, and in unshaded pads (means, with 95 per cent. confidence intervals, for ten or eleven pads) in exposures 1 and 2.

Day (Exposure 1)	Atmosphere	Pads
1	16.7	16.7 (15.9 - 17.5)
3	17.2	19.5 (19.2 - 19.8)
5	16.1	18.1 (17.8 - 18.4)
7	18.3	20.3 (20.0 - 20.6)
9	16.1	18.1 (17.8 - 18.4)
11	16.1	15.7 (15.3 - 16.1)
(Exposure 2)		
1	15.6	18.6 (18.2 - 19.0)
2	15.6	18.2 (17.9 - 18.5)
7	22.0	21.6 (21.1 - 22.1)
9	21.0	20.1 (19.9 - 20.3)
11	18.0	20.1 (19.9 - 20.3)

TABLE 28. Minimum temperatures (^{o}C) reached in the atmosphere, and in unshaded pads (means, with 95 per cent. confidence intervals, for twelve pads) in exposures 1 and 2.

Day	Atmosphere	Unshaded pads	Shaded Pads
2	27.8	30.3 29.0 - 31.6	25.2 24.8 - 25.6
4	27.8	34.1 30.6 - 37.6	25.3 24.7 - 25.9
6	32.8	40.3 39.1 - 41.5	30.4 29.6 - 31.2
8	35.6	42.5 40.0 - 45.0	30.6 29.7 - 31.5
10	32.2	40.5 39.0 - 42.0	31.2 29.9 - 32.5

TABLE 29. Maximum temperatures (^{o}C) reached in the atmosphere, and in shaded and unshaded pads (means, with 95 per cent. confidence intervals, for five pads) in exposure 3.

Day	Atmosphere	Unshaded pads	Shaded Pads
2	13.3	16.4 16.1 - 16.7	17.6 17.1 - 18.1
4	20.6	21.5 21.4 - 21.6	21.2 20.8 - 21.6
6	15.0	18.7 17.8 - 19.6	20.0 19.6 - 20.4
8	15.6	21.3 20.7 - 21.9	23.0 22.6 - 23.4

TABLE 30. Minimum temperatures (^{o}C) reached in the atmosphere, and in shaded and unshaded pads (means, with 95 per cent. confidence intervals, for five pads) in exposure 3.

Days after exposure	Minimum		Maximum	
	Shaded	Unshaded	Shaded	Unshaded
1	-1.0	-1.0	4.2	1.7
2	2.5	2.5	5.4	2.6
3	1.9	1.9	3.8	2.3
4	0.8	0.8	2.5	1.0
5	0.9	1.4	2.4	1.4
6	2.4	2.4	1.8	0.8
7	0.9	0.9	4.1	2.1
8	1.8	1.3	2.9	1.9

TABLE 31. Time lapse in hours after atmospheric minimum and maximum temperatures for the internal temperatures of shaded and unshaded pads to reach corresponding minima and maxima.

Day	Atmosphere	Exposed Pads
1	0.0538	0.0486 (0.0454 - 0.0516)
2	0.0596	0.0603 (0.0566 - 0.0640)
3	0.0587	0.0677 (0.0636 - 0.0718)
4	0.0616	0.0676 (0.0657 - 0.0695)
5	0.0632	0.0722 (0.0691 - 0.0753)
6	0.0579	0.0684 (0.0660 - 0.0708)
7	0.0590	0.0772 (0.0756 - 0.0788)
8	0.0642	0.0790 (0.0777 - 0.0807)
9	0.0603	0.0735 (0.0716 - 0.0754)
10	0.0561	0.0663 (0.0639 - 0.0687)
11	0.0587	0.0691 (0.0666 - 0.0716)
12	0.0570	0.0603 (0.0571 - 0.0635)

TABLE 32. Accumulated daily temperatures (means and 95 per cent. confidence intervals) of exposed dung pads, and corresponding atmospheric accumulations, expressed as proportions of the total pre-imaginal development time for females (Exposure 1).

Day	Atmosphere	A	B	C	D
1	0.0584	0.0676 (±0.0042) (78.2)	0.0647 (±0.0064) (80.0)	0.0647(±0.0052) (81.3)	0.0639 (±0.0052) (82.1)
2	0.0611	0.0764 (±0.0012) (76.8)	0.0733 (±0.0054) (78.5)	0.7137 (±0.0009) (79.9)	0.0711 (±0.0039) (80.6)
3	0.0598	0.0819 (±0.0007)	0.0791 (±0.0050)	0.0799 (±0.0027)	0.0796 (±0.0052)
4	0.0612	0.798 (±0.0014)	0.0776 (±0.0045)	0.0771 (±0.0017)	0.0779 (±0.0057)
5	0.0639	0.0814 (±0.0018)	0.0780 (±0.0045)	0.0792 (±0.0040)	0.0794 (±0.0060)
6	0.0620	0.0811 (±0.0020)	0.0810 (±0.0035)	0.0778 (±0.0035)	0.0774 (±0.0077)
7	0.0771	0.0757 (±0.0117) (64.0)	0.0754 (±0.0027) (63.0)	0.0736 (±0.0114) (63.5)	0.0706 (±0.0087) (60.0)
8	0.0817	0.0754 (±0.0191)	0.0768 (±0.0025)	0.0733 (±0.0164)	0.0735 (±0.0102)
9	0.0778	0.0675 (±0.0134)	0.0686 (±0.0055)	0.0684 (±0.0064)	0.0652 (±0.0079)
10	0.0781	0.0724 (±0.0119)	0.0725 (±0.0057)	0.0735 (±0.0055)	0.0697 (±0.0069)
11	0.0734	0.0742 (±0.0144)	0.0777 (±0.0062)	0.0776 (±0.0072)	0.0747 (±0.0094)
12	0.0795	0.0701 (±0.0166)	0.0753 (±0.0037)	0.0761 (±0.0040)	0.0723 (±0.0089)

TABLE 33. Accumulated daily temperatures (mean and 95 per cent. confidence limits) and moisture levels of four groups of dung pads, with corresponding atmospheric accumulations, expressed as proportions of the total pre-imaginal development times for females (Exposure 2).

Day	Atmosphere	Shaded Pads	Unshaded Pads
1	0.0538	0.0513 (0.0492-0.0534)	0.0609 (0.0594-0.0624)
2	0.0539	0.0592 (0.0574-0.0610)	0.0773 (0.0744-0.0802)
3	0.0668	0.0692 (0.0662-0.0722)	0.0873 (0.0851-0.0895)
4	0.0694	0.0693 (0.0668-0.0718)	0.0881 (0.0861-0.0901)
5	0.0753	0.0868 (0.0847-0.0889)	0.1032 (0.1011-0.1053)
6	0.0699	0.0784 (0.0765-0.0803)	0.0871 (0.0847-0.0895)
7	0.0616	0.0755 (0.0721-0.0789)	0.0863 (0.0837-0.0889)
8	0.0644	0.0847 (0.0821-0.0873)	0.0972 (0.0955-0.0989)

TABLE 34. Accumulated daily temperatures (means and 95 per cent. confidence intervals) of shaded and unshaded dung pads, and corresponding atmospheric accumulations, expressed as proportions of the total pre-imaginal development times for females (Exposure 3).

Day	Mean loss of moisture per hour (g)	
	Shaded pads	Unshaded pads
1	32.2 (16.6 - 47.8)	71.8 (65.0 - 78.6)
1	49.2 (40.2 - 58.2)	61.2 (48.6 - 73.8)
4	12.2 (6.4 - 18.0)	17.8 (15.0 - 20.6)
4	13.0 (8.4 - 17.6)	23.8 (21.3 - 26.3)
4	20.2 (12.8 - 27.6)	26.5 (23.1 - 29.9)
6	11.8 (9.5 - 14.1)	17.8 (14.5 - 21.1)
6	19.2 (17.6 - 20.8)	27.3 (25.3 - 29.3)
6	15.8 (14.8 - 16.8)	20.3 (18.4 - 22.2)
8	11.2 (8.2 - 14.2)	16.3 (14.0 - 18.6)
8	16.6 (15.2 - 18.0)	24.2 (18.9 - 29.5)
8	15.0 (11.6 - 18.4)	15.0 (13.2 - 16.8)

TABLE 35. Mean moisture loss (g) per hour and 95 per cent. confidence interval from shaded and unshaded pads, measured several times daily on various days after exposure (n = 5).

FIGURE 21. Dung moisture (as a percentage of wet weight) from three different pasture types at various times of the year. Daily and monthly rainfall are shown.

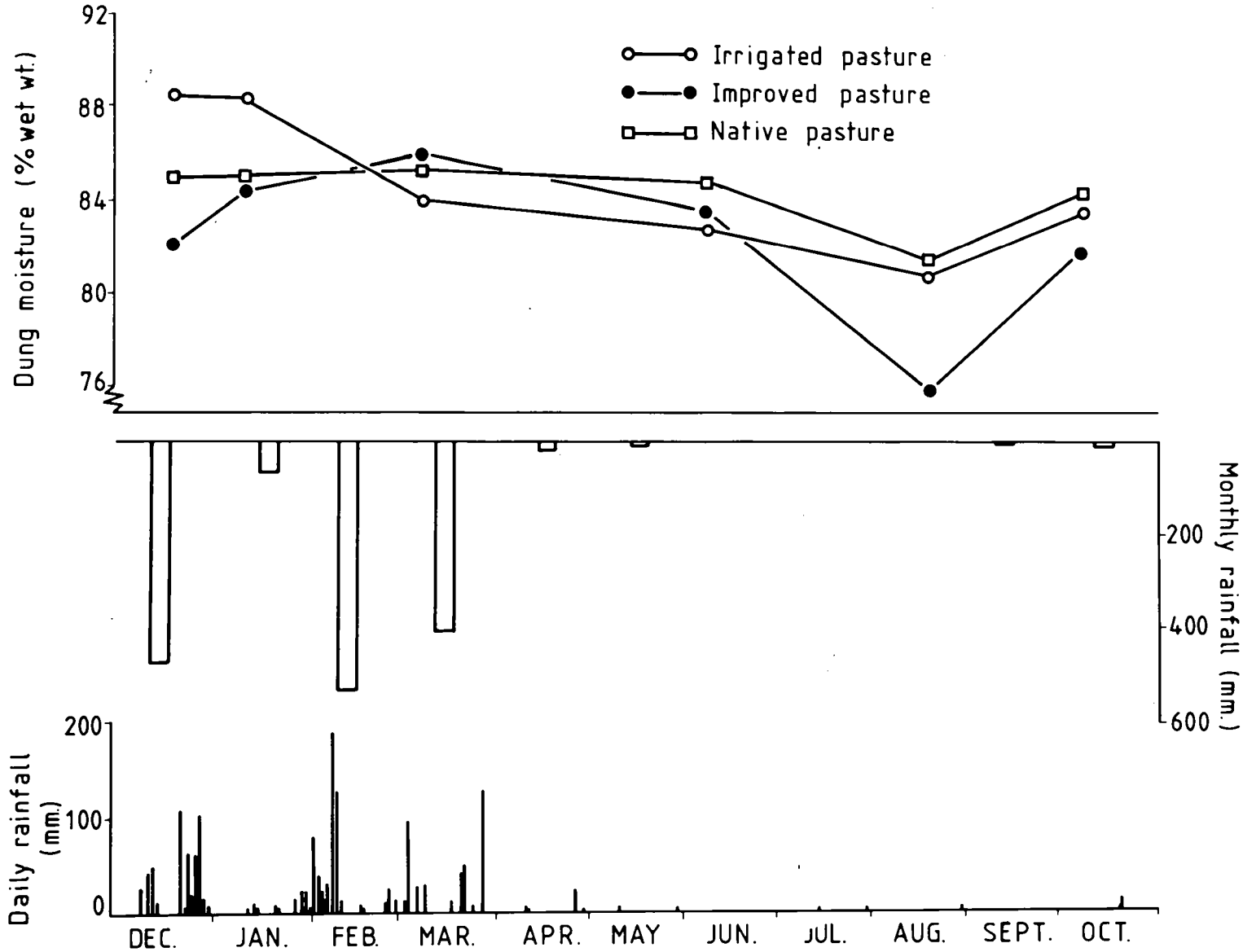

Irrigated pasture
Improved pasture
Native pasture
Dung moisture (% wet wt.)
92
88
84
80
76
Monthly rainfall (mm.)
200
400
600
Daily rainfall (mm.)
200
100
0
DEC.
JAN.
FEB.
MAR.
APR.
MAY
JUN.
JUL.
AUG.
SEPT.
OCT.

FIGURE 22. The effects of minimum daily atmospheric temperature and dung moisture content on the daily minimum temperatures reached in dung pads.

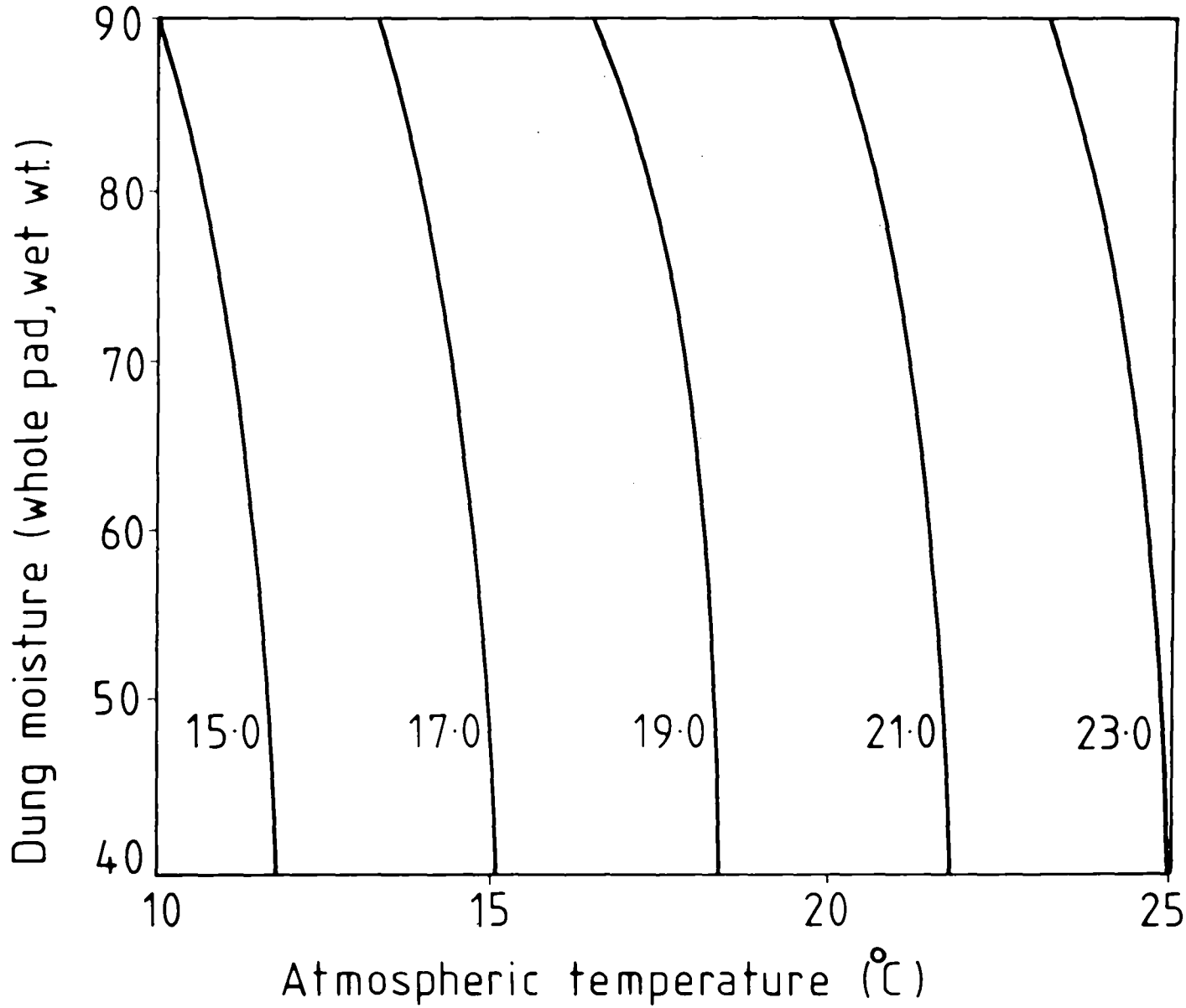

90
80
70
60
50
40
Dung moisture (whole pad, wet wt.)
15·0
17·0
19·0
21·0
23·0
10
15
20
25
Atmospheric temperature (°C)

FIGURE 23. The effects of daily maximum atmospheric temperature and dung moisture content on the daily maximum temperatures reached in dung pads.

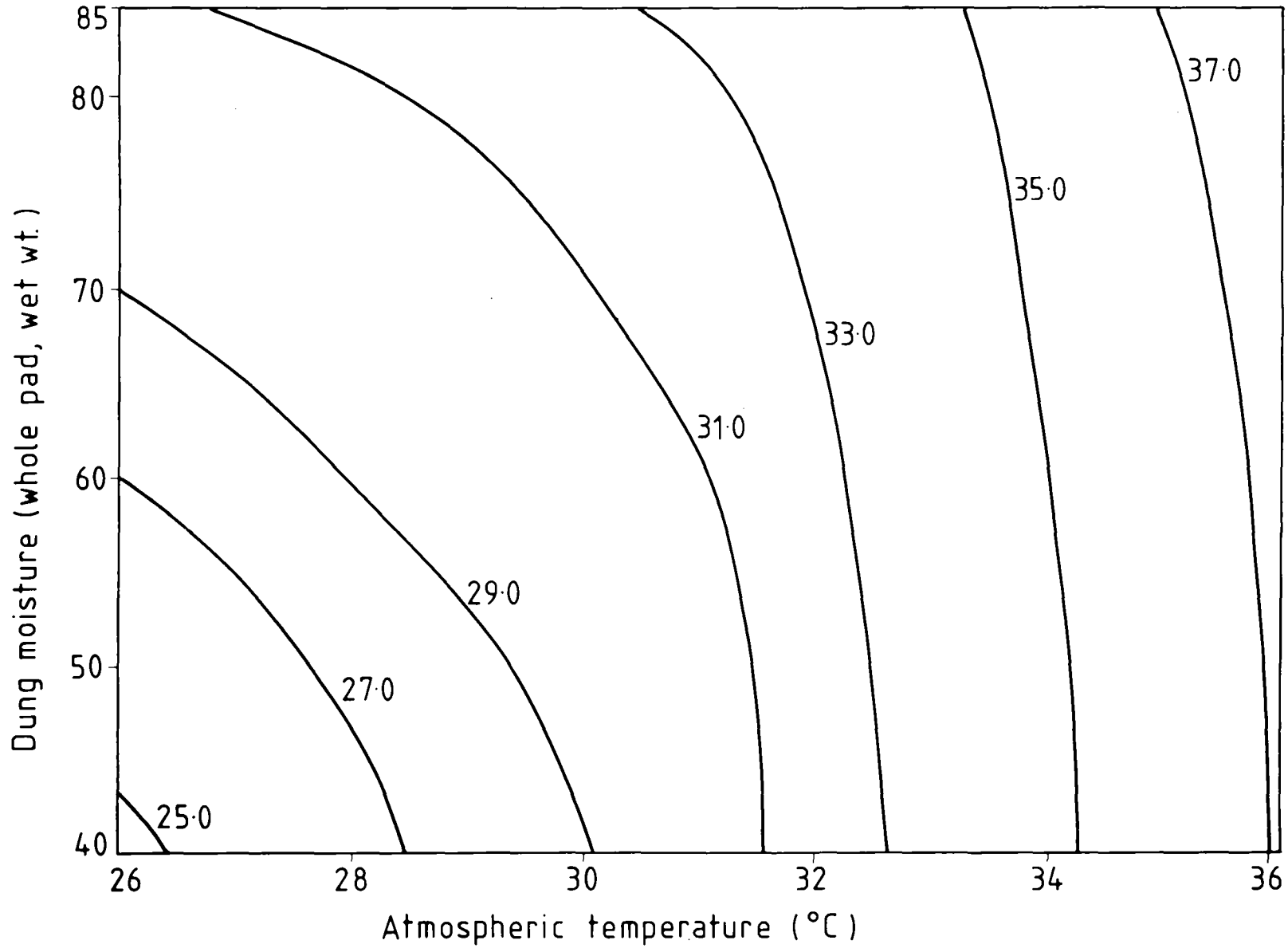

85
80
70
60
50
40
Dung moisture (whole pad, wet wt.)
25·0
27·0
29·0
31·0
33·0
35·0
37·0
26
28
30
32
34
36
Atmospheric temperature (°C)

FIGURE 24. The effect of accumulated temperature on the proportion of growth of immature female buffalo flies in exposed pads. Temperatures and proportions of development were calculated as the total of 24 x 1-hourly estimates.

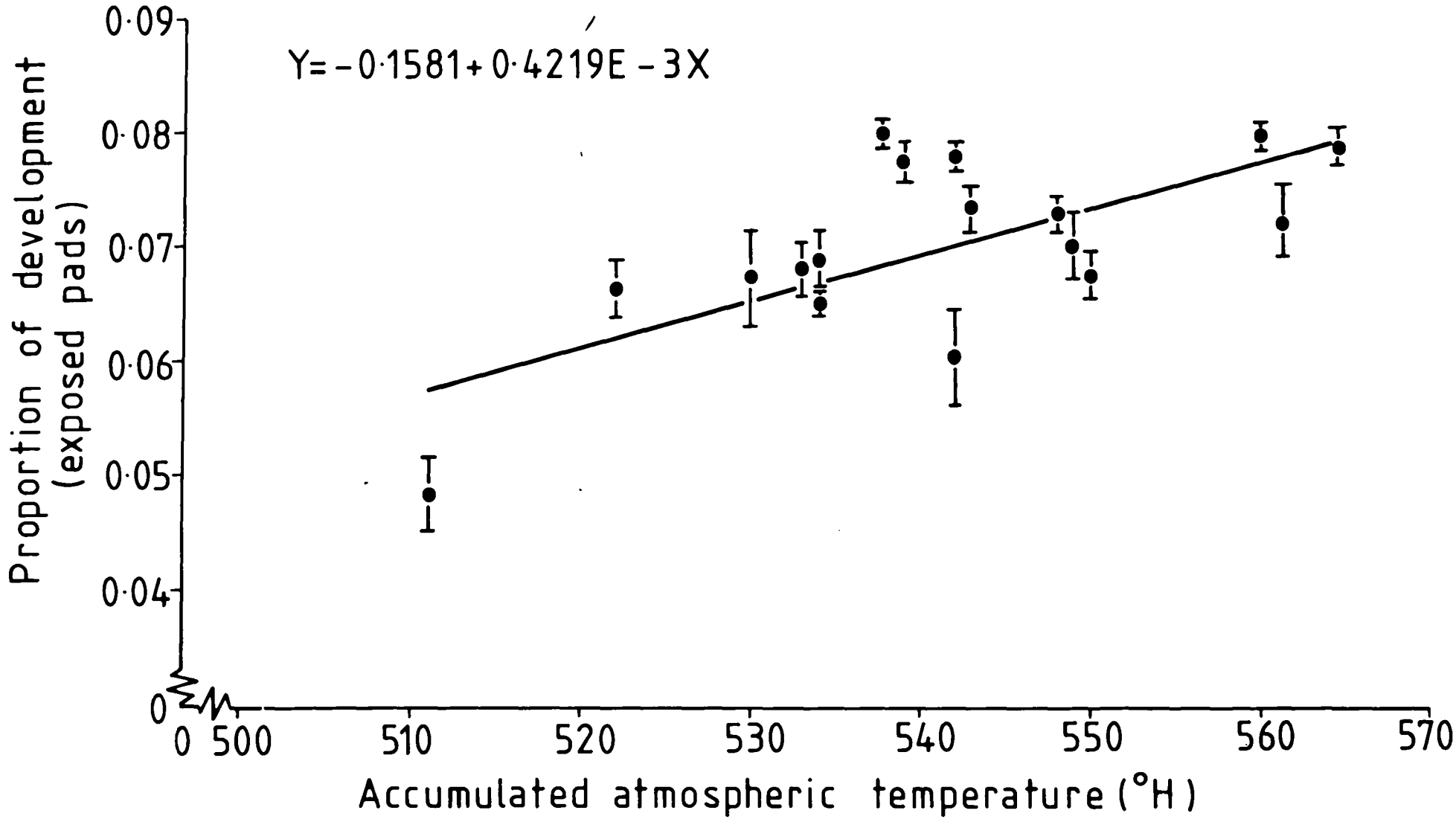

Y= -0·1581+ 0·4219E - 3X
Proportion of development (exposed pads)
0·09
0·08
0·07
0·06
0·05
0·04
0
0 500
510
520
530
540
550
560
570
Accumulated atmospheric temperature (°H)

FIGURE 25. The effect of accumulated temperature on the proportion of growth of immature female buffalo flies in shaded pads. Temperatures and proportions of development were calculated as the total of 24 x 1- hourly estimates.

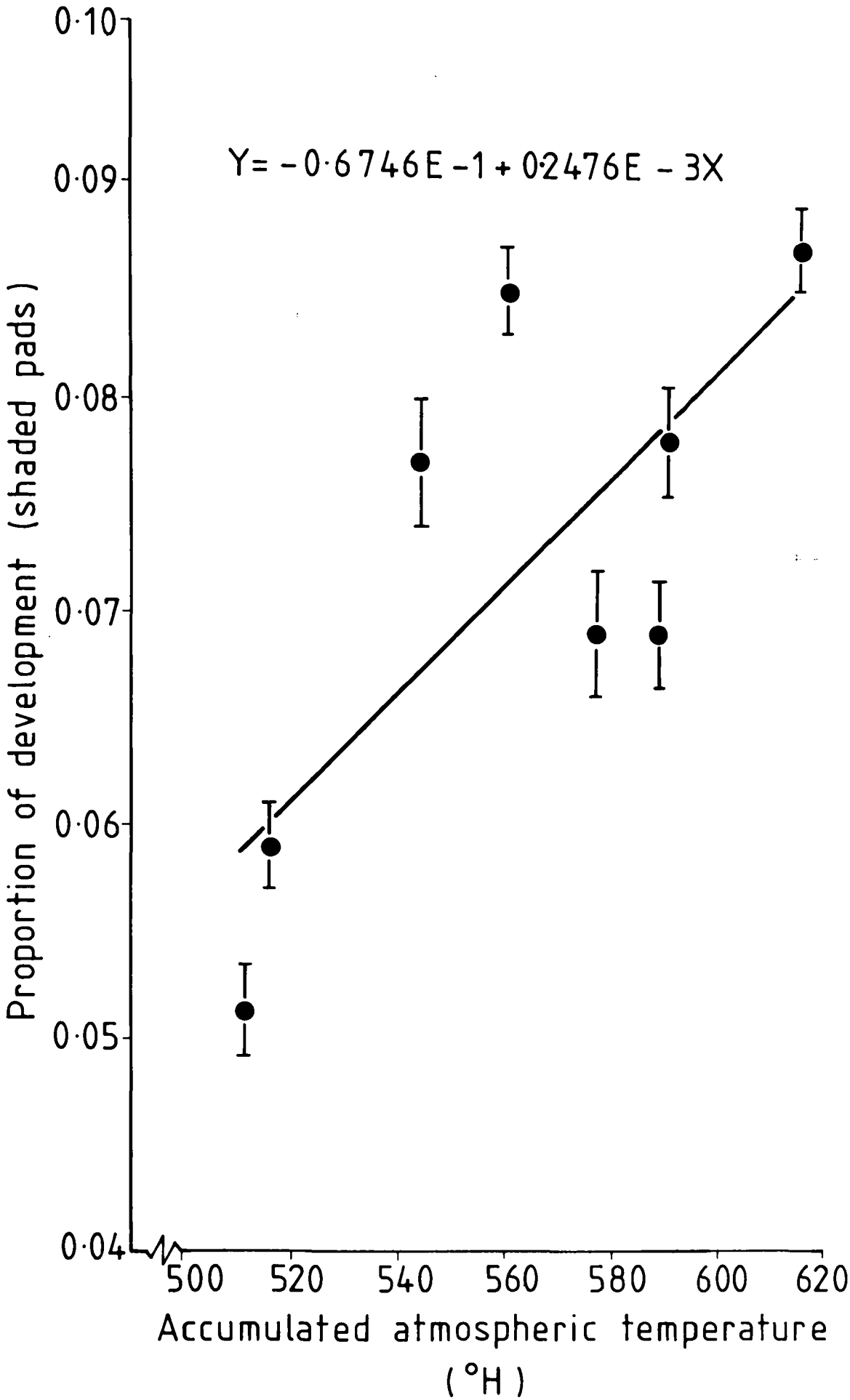

Y= -0·6746E-1 + 0·2476E-3X
0·10
0·09
0·08
0·07
0·06
0·05
0·04
Proportion of development (shaded pads)
500
520
540
560
580
600
620
Accumulated atmospheric temperature
(°H)

FIGURE 26. The proportions of growth achieved simultaneously in unshaded pads (X axis) and in shaded pads (Y axis).

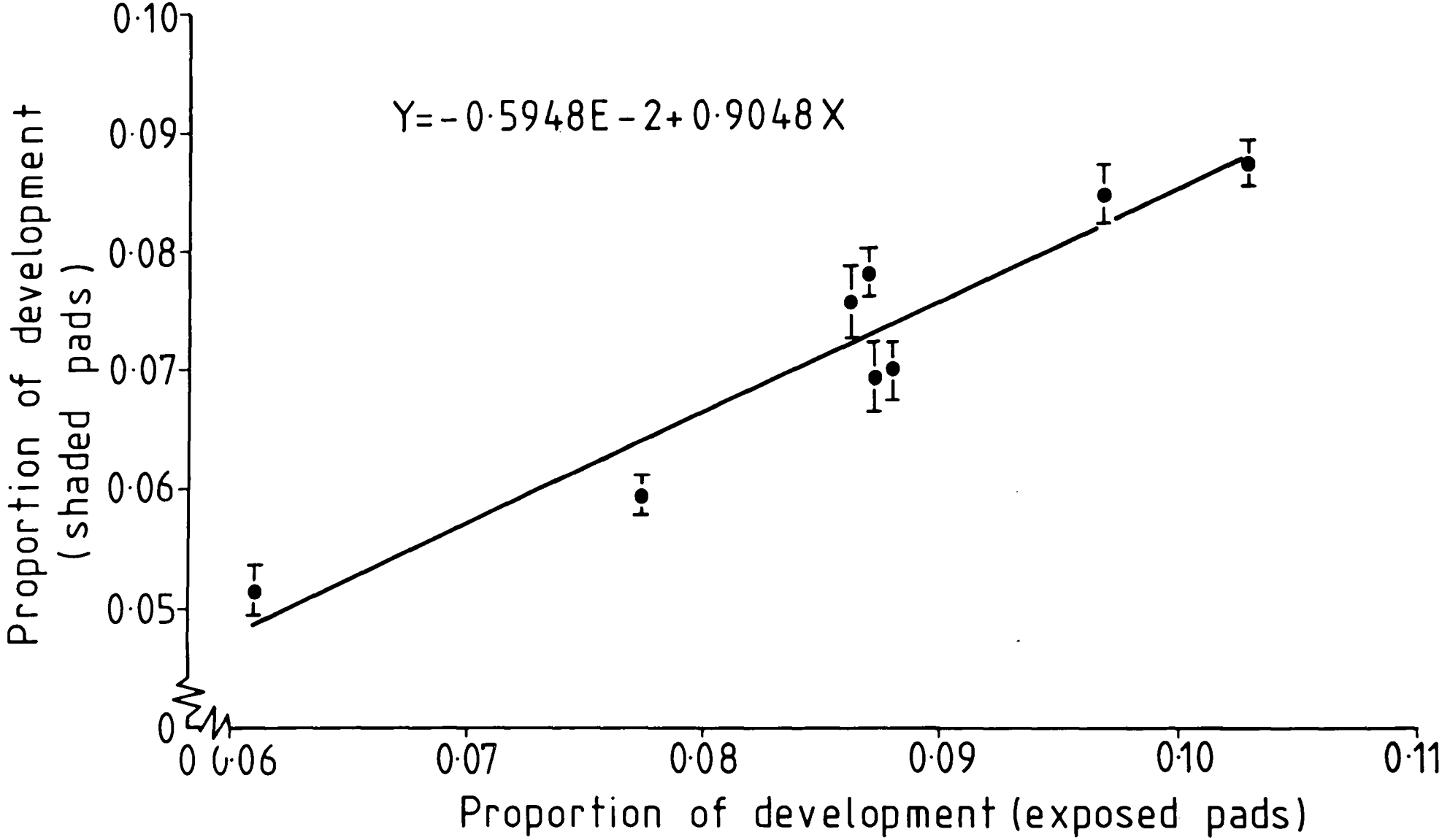

Y=-0·5948E-2+0·9048X
Proportion of development (shaded pads)
0·10
0·09
0·08
0·07
0·06
0·05
0
0 0·06
0·07
0·08
0·09
0·10
0·11
Proportion of development (exposed pads)

FIGURE 27. The effects of atmospheric saturation deficit and dung moisture content on the rate of moisture loss from shaded pads, estimated hourly as a percentage of the moisture present in the pad at the commencement of the observation period.

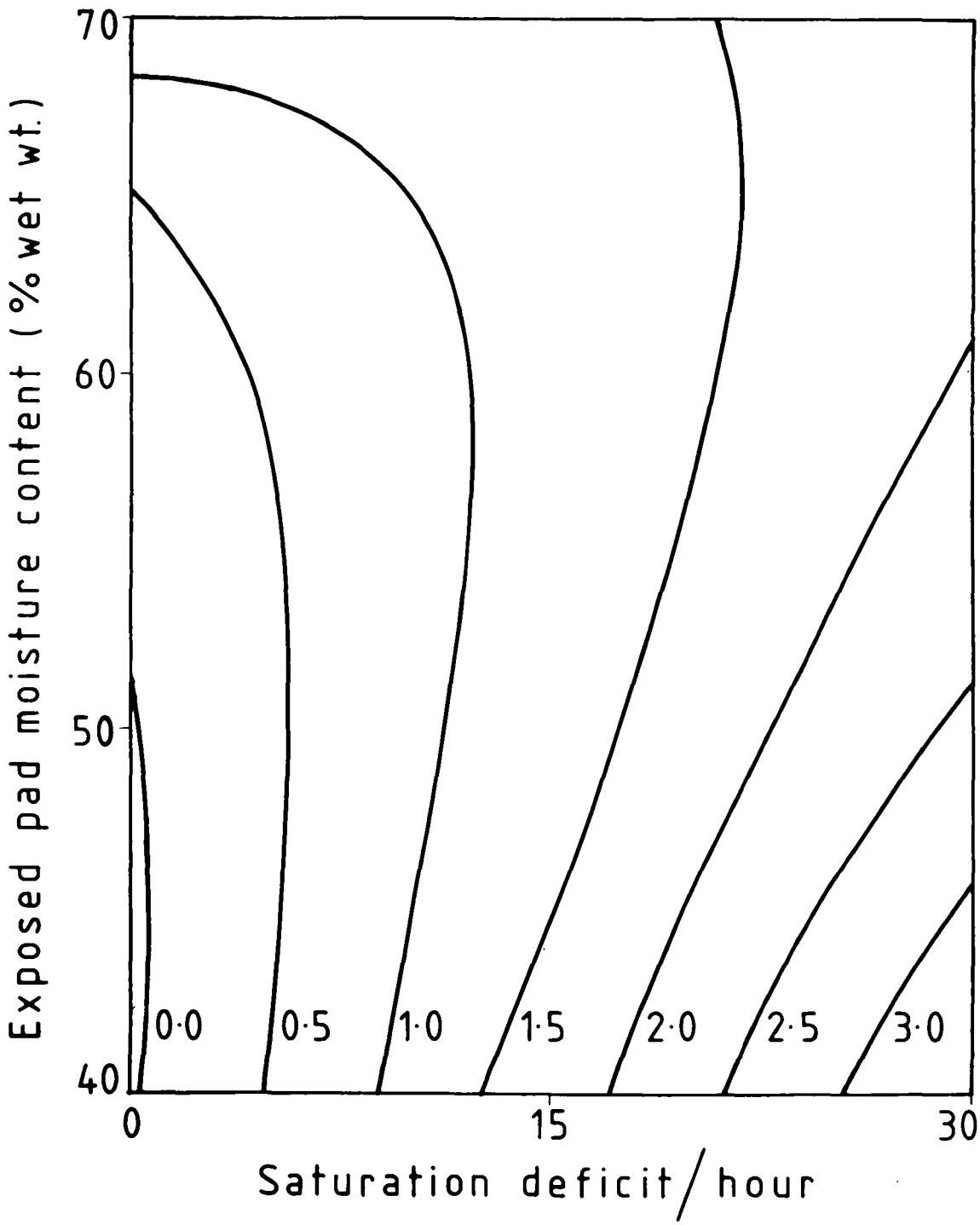
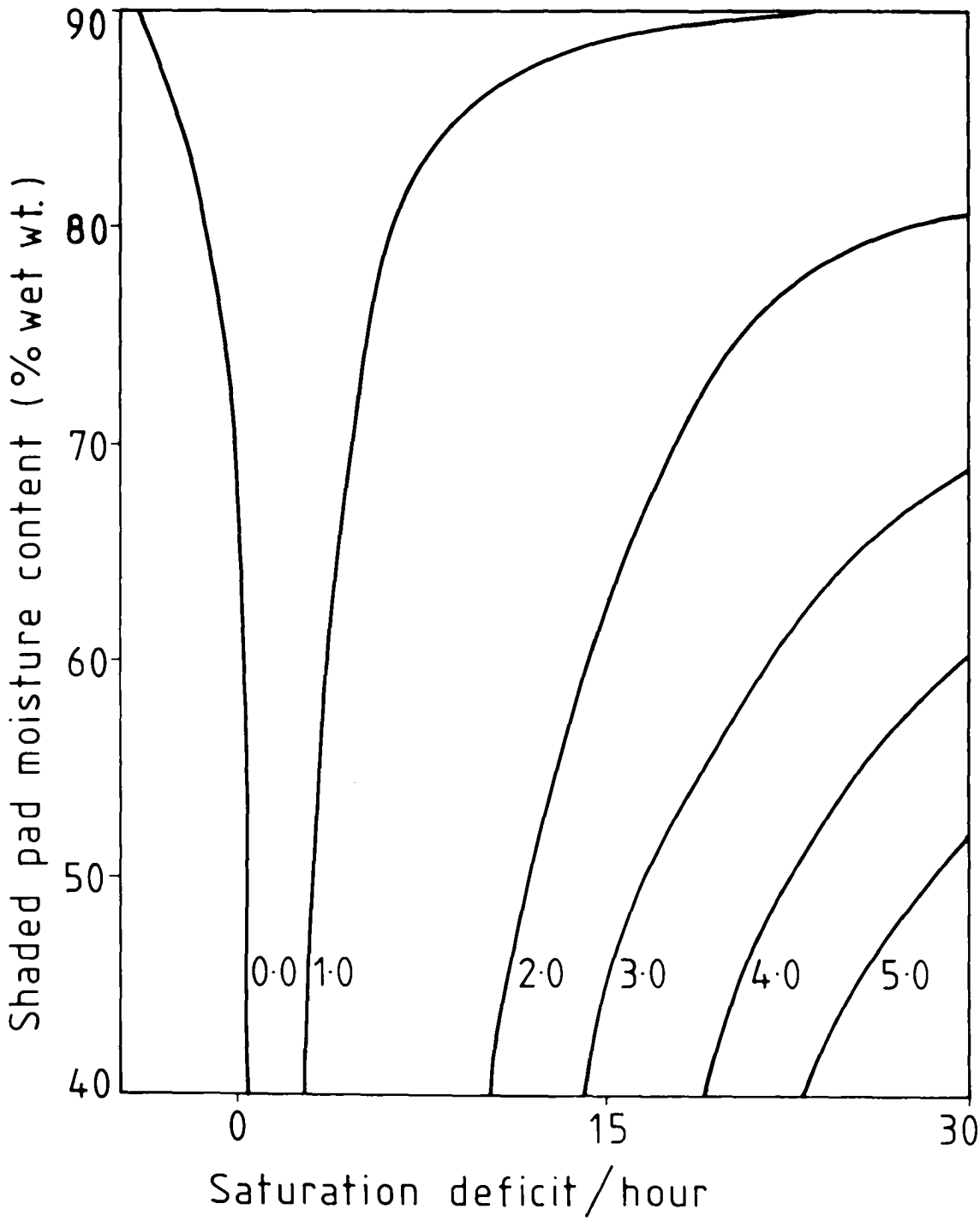

70
60
50
40
Exposed pad moisture content (% wet wt.)
0·0
0·5
1·0
1·5
2·0
2·5
3·0
0
15
30
Saturation deficit / hour

FIGURE 28. The effects of atmospheric saturation deficit and dung moisture content on the rate of moisture loss from unshaded pads, estimated hourly as a percentage of the moisture present in the pad at the commencement of the observation period.


90
80
70
60
50
40
Shaded pad moisture content (% wet wt.)
0·0
1·0
2·0
3·0
4·0
5·0
0
15
30
Saturation deficit / hour

CHAPTER 6: INTERPRETATION OF SEASONAL POPULATION CHANGES

INTRODUCTION

Mid-winter temperatures in the coastal areas near Townsville are not normally likely to have a direct lethal effect on immature buffalo flies, at either upper or lower limits. In spite of this, fly populations in the mid-winter months in this area are usually so low as to appear virtually absent. In 1972 and 1973 weekly estimates of buffalo fly populations were made on a C.S.I.R.O. field station near Rockhampton in an attempt to monitor seasonal population changes. In both years, fly numbers fell to very low levels in winter, and increased sharply in early summer. An attempt to interpret these changes is reported in this chapter. This interpretation makes use of the results of experiments presented in earlier chapters in which factors influencing mortality and speed of development of immature buffalo flies, and the variation of these factors in dung under field conditions, were investigated.

REVIEW OF LITERATURE

1. Seasonal fluctuations

Buffalo flies in tropical Australia generally are found to be more numerous in summer than in winter. In contrast this pattern is not followed in the areas from which flies were introduced to Australia. Handschin (1933a) reported that fly abundance in Java was dictated by rainfall and that temperature régimes in these near-equatorial areas only affected fly populations when modified by high altitude. Sharper contrasts would occur between summer and winter temperatures in Australia, being further from the equator, and the wet season in fly-infested areas is strictly confined to a few mid-summer months. Dry and cool conditions during the north Australian winter are accompanied by a marked decline in fly numbers. The mechanisms responsible for this decline have not been reported in the literature. Handschin (1933a) stated that flies would not live below 20°C, but did not define whether this temperature was a mean or an extreme. Experiments reported in Chapter 2 showed that all immature stages survive a constant temperature of 17.5°C. It was also shown that an atmospheric temperature of 20°C would produce a higher temperature within dung pads. Thus an atmospheric temperature régime reaching a daily maximum of 20°C would not limit severely the survival of immature stages by direct effect, unless a lower lethal minimum temperature was reached. Such a temperature has not yet been defined. In Chapter 2, larvae were shown not to survive a constant temperature of 15°C. It is likely however, that they would tolerate a much lower temperature for short periods.

METHODS

Flies were counted weekly on three herds of cattle grazing in separate but adjoining paddocks. Initially the three herds comprised one of Hereford - Shorthorn cows (H-SH) and two of Africander X H-SH cross cows (AX1 and AX2). Counts were made on these three herds from December 1971 to November 1972, at which time herd AX1 was replaced by a herd of similarly bred AX steers. All herds grazed at the same stocking rate of one beast per hectare, on the same pasture mixture of green panic and siratro.

Herds were mustered separately into cattle yards, and the animals were held in single file in a narrow race. The number of flies on one side were estimated by counting, using two or three of five available observers. Counts were always conducted on the same sides of the animals, and at approximately the same time of day, about mid-morning. During the course of these observations there was no insecticidal control of buffalo flies nor of any other ectoparasites.

RESULTS

1. Analysis of herd differences

The period from May to November 1972 included a sharp fall in numbers in early winter, several months during which very few flies were seen, and a sharp increase in numbers commencing in late October and continuing through November. This period was chosen for close inspection of data because of these characteristics, and because of the change in herd composition in December 1972 described above. Details of counts during this period appear in Table 36. These counts represent estimates of the total number of flies present in each of the three herds; the estimates are in terms of counts made on only one side of each animal, usually by two or three observers. If necessary, an estimate of the actual number of flies present could be made by doubling these counts. As the same observers were not always involved in each count analysis of observer error was not attempted. The standard errors of the mean of the estimates for two or three observers are generally low (Table 36), and these means are thus taken as a reliable index of changes in population size on each herd.

Herd totals for each counting day were analysed to test for differences in fly counts between herds, attributable to either breed or paddock effects. For each count, the three herds were ranked from 1 (lowest count) to 3 (highest count). Flies were counted weekly for 30 weeks during this period. The data were analysed using the non-parametric Friedman two-way analysis of variance by ranks (Siegel, 1956). As expected, fly numbers were significantly different over time, but there were no differences between herds (chi-square = 0.15,

$P > 0.90$). Thus counts from all herds have been combined to give a single estimate of fly numbers.

2. Seasonal patterns

Estimates of fly populations for the period from December 1971 to August 1973 are shown in Figure 29. Counts appear as the mean per cow for all cattle from the three herds, one side only, and are means of estimates by two or three observers. The general pattern of infestations which are high in summer and low in winter is illustrated.

The fluctuations within summer peaks, as shown during February-March, 1972, and December-January, 1973/74, cannot be attributed directly to atmospheric conditions, which are uniformly warm and humid during these months. These fluctuations may be generated by high population density effects such as larval competition for food or space, or disease. Interspecific competition with other coprophagous species may be high during summer months, and heavy rainfall during the summer wet season may affect eggs or larvae (Krijgsman and Windred, 1933).

In an attempt to explain the gross changes in fly counts in June and October, 1972, estimates of speed of development and the drying rate of dung during the months of April to November, 1972, were made using results of experiments described in previous chapters.

3. Rate of moisture loss from dung

From Rockhampton meteorological data, supplied by the Australian Bureau of Meteorology, an estimate of average hourly

saturation deficit was derived for each of the above months. For each day, the saturation deficit at maximum and minimum temperature was calculated using an accompanying record of relative humidity. From these figures the daily average arithmetic mean saturation deficit was calculated. The hourly mean for each month was estimated from the total of daily estimates. These values appear in Table 37.

The equations described in Figures 27 and 28 (Chapter 5) relate hourly saturation deficit and dung moisture content to the rate of loss of moisture from unshaded and shaded dung pads. Rate of drying was calculated using theoretical initial dung moisture contents of 75, 80 and 85 per cent. Calculations of hourly moisture loss were conducted over successive hours until the estimated dung moisture content reached the lower lethal limit for larvae, between 64 and 67 per cent.

The dung moisture terms in the above equations are expressed as the moisture content of the whole pad without reference to zonation. The experiment described in Appendix 3 related whole-pad moisture to the moisture content in the wet interval zones of dung pads. Experiments in Chapter 3 determined that larval growth ceased at a dung moisture of 64 per cent. Using the equation derived in Appendix 3 this level in the wet zone of a dung pad equates to a whole-pad moisture of approximately 33 per cent. The drying intervals described here are the times estimated for pad moisture to reach this level. These intervals, with accompanying durations of pre-imaginal stadia described below are shown in Tables 38 and 39 for unshaded and shaded pads respectively.

4. Rate of pre-imaginal development

The effects of temperature on the generation time of buffalo flies were studied using an estimate of mean maximum and minimum temperatures for each month. A continuous temperature record, made in Townsville during July 1974 was used to describe the rate of hourly temperature change over a 24-hour period. From the results of this experiment hourly temperatures were calculated from the maximum and minimum temperature data from Rockhampton, on the assumption that daily temperature marches in these two localities are similar. Using the equations derived in Chapter 1, relating development rate and temperature, an hourly estimate of average female pre-imaginal growth for each of the months was then made. Inversion of this estimate gave the corresponding development time for combined pre-imaginal development. Any hour when the temperature was below the larval threshold of 15°C was not included in the calculation. The estimates of proportions of growth were conducted using the equation

$$Y = -0.0472 + 1.9736X$$

where X = Atmospheric temperature converted to the corresponding proportion of development time, and

Y = proportion of development within the pad.

This equation was derived in Chapter 5, and applies to pads exposed to sunlight. Proportions of development in shaded pads were estimated from the equation

$$Y = -0.0059 + 0.9048X$$

where X = proportion of development in exposed pads, and

Y = proportion of development in shaded pads.

This equation was also derived in Chapter 5.

Table 38 presents estimates of pre-imaginal stadia, and the corresponding time estimated for dung moisture to fall to lethal levels, for unshaded dung pads. Data for shaded pads appear in Table 39.

DISCUSSION

The pre-imaginal intervals estimated in Tables 38 and 39 include the pupal stadia. Pupae are less likely than other instars to be affected by dung moisture, and larvae are the most vulnerable. The pre-pupal stadia occupy approximately half of the total pre-imaginal interval (Table 2) and the relationships between these stadia and dung moisture are discussed here.

In all cases reported in Tables 38 and 39 pre-pupal stadia (*i.e.* approximately half of the estimated total pre-imaginal period) occupy much longer intervals than do the corresponding drying times for dung, in any month. However, flies were proliferating rapidly in October and November (Figure 29). This indicates that the relative estimated magnitudes of dung drying times and development intervals are not realistic; the autumn decrease and spring increase in fly numbers in the field are not exactly explained by the results of experiments reported here. Further studies of the speed of larval development under fluctuating temperatures may give a more realistic estimate of the behaviour of field populations, and a more detailed study of the temperature and moisture characteristics of dung pads would further contribute to accurate predictions of growth and survival of immature buffalo flies. The values of Fisher's A^2 for equations relating moisture loss from dung pads to atmospheric saturation deficits (Chapter 5) were relatively low. Factors such as insolation, wind speed and the texture and moisture of soil were not measured during these experiments, and probably contributed to the rate of moisture loss.

However, the data reveal important characteristics both of moisture loss in dung and of the growth of immature buffalo flies. The drying power of the air, as measured by saturation deficit in Table 44 and reflected in dung drying rates in Tables 45 and 46, varied little over the study period. The effects of lower winter temperatures on evaporation are offset by reduced humidity. In contrast, the speed of development of immature flies responds sharply to change of season, there being an estimated five to six-fold increase in development time of pre-imaginal stages in exposed pads, and nearly a ten-fold increase in shaded pads from April to June. Even considering the error in the estimates presented here, it is most unlikely that dung moisture would remain high enough during winter to support growth to pupation. If low dung moisture content kills eggs and larvae in most pads, fly populations during winter must be maintained in pads protected in some way from drying, perhaps with reduced effects of winter temperatures on speed of growth of immature flies. There is no evidence of true diapause in buffalo flies. Rapid long-range reinfestation in early summer is not likely, considering that adult flies held humidified at 24^{o}C in the laboratory for egg collection died in 24 hours.

CONCLUSIONS

The basis of a study of the population dynamics of a species comprises an understanding of the rate at which a generation develops, and of those factors which control mortality and fecundity. Experiments described in Chapter 1 provided some knowledge of the way temperature affects the speed of development of immature buffalo flies, and established the upper and lower thresholds for constant temperature, beyond which development ceased. Extension of this work to define the effects of daily variations in temperature would provide more exact information on the rate at which immature flies grow under field conditions. The influence of temperature fluctuations on growth should be defined, and the response to intermittent periods of temperatures beyond the thresholds also needs to be investigated. Prediction of growth rate would further be enhanced by greater understanding of the effects of dung moisture. This was demonstrated to be of probable importance in Chapter 1 but was not studied in depth.

Mortality may result from several different influences. That caused by moisture and temperature was found to be potentially important. Atmospheric temperatures in excess of the lethal upper limits for eggs, larvae and pupae commonly occur in summer in tropical Australia; towards the southern limits of distribution, and in inland areas further north, minimum daily temperatures frequently fall below zero during winter. These extremes are theoretically capable of causing catastrophic mortality of immature stages over wide areas, and may contribute substantially to short-term population changes.

Atmospheric humidity is unlikely to cause death directly. Eggs are deposited on fresh dung, and hatch quickly; larvae and pupae, within the dung pad or soil beneath it, are not exposed directly to the air. Eggs and larvae are susceptible to immersion, and the destruction of pads by heavy rain would deprive larvae of their food source. Thus mortality caused by rainfall is likely to be severe, but sporadic.

Dung moisture was found always to be initially suitable for larval growth. The lowest level described in the literature (70 *per cent.* of wet weight) was found (Chapter 2) to be suitable for survival of larvae although size and fecundity of the ensuing adults may be reduced (Chapter 3). The moisture content of dung would thus only limit survival if pads dried out to lethal levels before pupation.

The other physical attributes of dung studied were pH and the osmotic concentration of dung fluid. Upper (pH and osmolality) and lower (pH only) extremes of these, produced artificially, were accompanied by heavy mortality (Chapter 4). However, initial and progressive natural levels in dung did not ever approach these values, and these factors may thus be discounted as being relevant to field mortality.

Calculations described in Chapter 6 are an attempt to estimate generation time at different times of the year. They are accompanied by estimates of the rates at which dung dries out under field conditions. The calculations of rate of development are probably reasonably accurate, although the modifying effects of fluctuating temperatures and changes in dung moisture have not been allowed for. The estimates predict that larvae could not survive

during a period when field observations indicate that they did. The most likely source of error is in the calculation of loss of dung moisture. The relationships between dung moisture, the pad and the nature of the surrounding air and soil are undoubtedly complex and further study is needed to define the micro-climate in which larvae exist.

Day (1972)	Herd								
	H-SH (19 animals)			AX1 (19 animals)			AX2 (20 animals)		
4.5	2025 ±	75.7	(3)	1885 ±	82.2	(3)	1963 ±	67.5	(2)
11.5	785	2.0	(2)	1768	117.5	(2)	1670	90.0	(2)
18.5	1042	66.4	(3)	1273	89.9	(3)	1723	27.4	(3)
25.5	1358	80.2	(3)	1010	46.5	(3)	998	35.6	(3)
1.6	1560	10.0	(2)	1933	43.6	(3)	1595	49.3	(3)
7.6	874	26.0	(3)	1603	56.4	(3)	612	10.7	(3)
14.6	1210	25.7	(3)	1400	46.5	(3)	1515	5.0	(2)
19.6	633	30.7	(3)	1170	25.0	(2)	1195	21.8	(2)
27.6	219	7.7	(3)	156	4.9	(3)	252	9.8	(3)
6.7	57	5.0	(3)	55	1.7	(3)	29	3.0	(3)
13.7	197	14.0	(2)	45	2.0	(3)	109	6.7	(3)
20.7	156	14.7	(3)	204	17.9	(3)	200	3.0	(2)
26.7	158	4.0	(2)	48	1.5	(2)	198		(1)
2.8	35	3.7	(3)	15	1.2	(3)	32	2.6	(3)
10.8	9	2.7	(3)	7	1.5	(2)	4	0.0	(2)
16.8	6	0.9	(3)	0		(3)	2	0.6	(3)
24.8	6	1.0	(2)	0		(3)	2	0.6	(3)
1.9	7	0.3	(3)	1	0.3	(3)	1	0.7	(3)
7.9	4	0.7	(3)	3	0.3	(3)	4	0.9	(3)
14.9	0			5	0.3	(3)	3	0.9	(3)
20.9	13	1.2	(3)	10	1.7	(3)	17	2.4	(3)
27.9	18	0.0	(3)	19	1.5	(3)	23	1.2	(3)
3.10	3	0.0	(3)	22	2.3	(3)	11	1.5	(3)
11.10	24	1.5	(2)	11	0.3	(3)	20	3.0	(2)
18.10	9	0.6	(2)	36	3.5	(3)	50	4.4	(3)
26.10	422	5.4	(3)	289	29.0	(2)	268	12.5	(2)
1.11	631	10.5	(2)	827	2.0	(2)	644	28.9	(3)
8.11	1715	60.0	(3)	3775	105.4	(3)	1377	24.9	(3)
16.11	2455	105.0	(2)	2737	57.5	(2)	2295	79.4	(3)
22.11	4435	355.0	(2)	4420	260.0	(2)	4115	228.3	(3)
29.11	4198	382.5	(2)	No record			3942	20.9	(3)

TABLE 36. Total fly counts on three herds of Hereford-Shorthorn (H-SH) or Africander-cross (AX) cows. Counts are the means, and their standard errors, of two or three observers.

Month (1972)	Days (n)	Mean Saturation Deficit
April	30	12.0 (11.4 - 12.6)
May	31	8.0 (7.2 - 8.8)
June	30	7.4 (6.8 - 8.0)
July	31	9.4 (8.4 - 10.4)
August	31	10.8 (9.6 - 12.0)
September	30	11.1 (10.5 - 11.7)
October	31	13.9 (12.7 - 15.1)
November	30	13.1 (11.3 - 14.9)

TABLE 37. Mean hourly saturation deficit, and 95 per cent. confidence interval, for the months April to November inclusive, 1972, in Rockhampton.

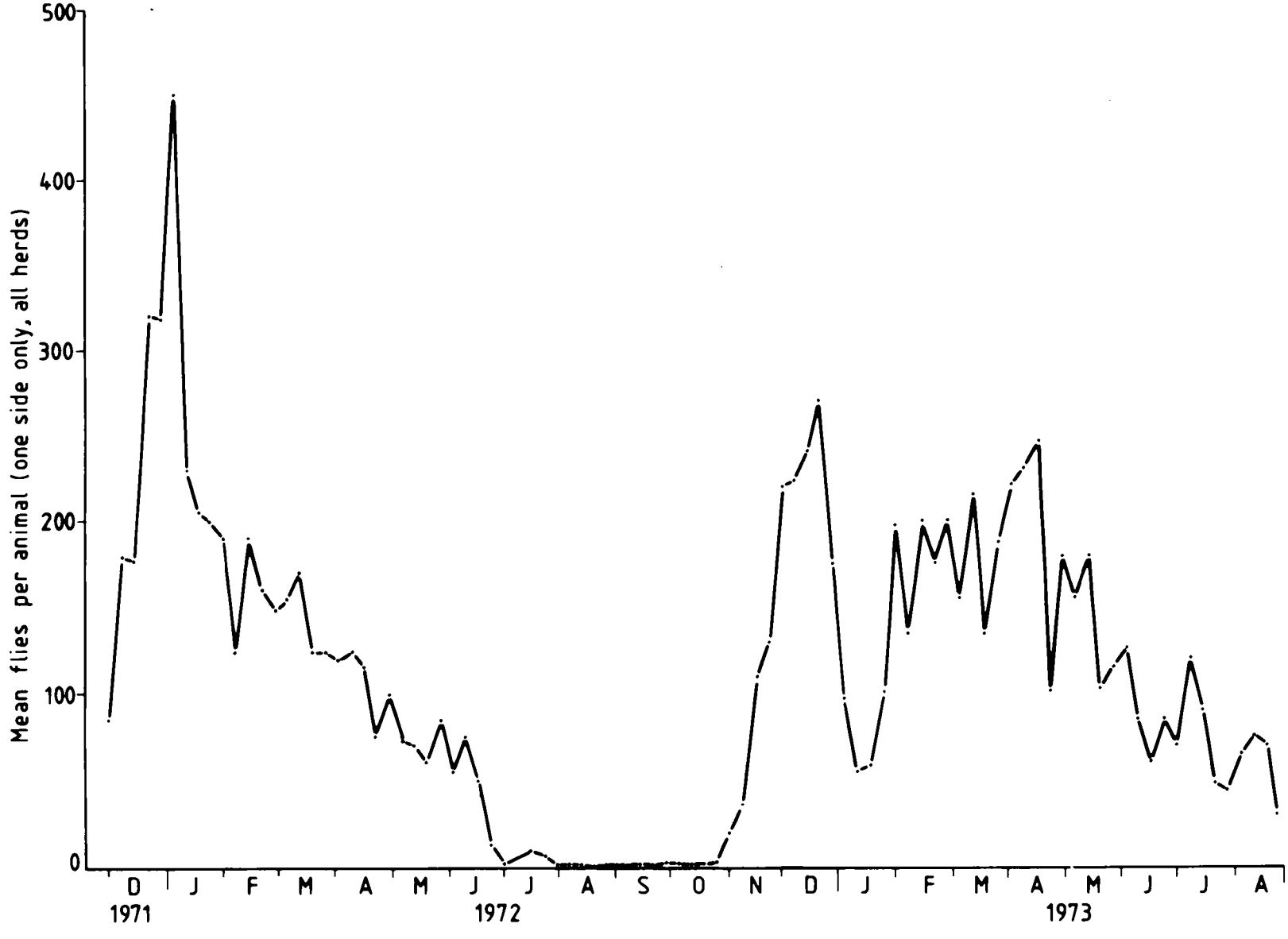
	Drying period (hours)			Development period (hours)
	75%	80%	85%	
April	65	72	78	310
May	77	83	89	761
June	80	85	91	1646
July	72	78	84	2477
August	69	75	81	1486
September	67	73	79	360
October	62	67	74	288
November	63	69	75	240

TABLE 38. Time required for unshaded dung pads of three different initial moisture contents to reach lower lethal moisture limits for buffalo fly larvae, and corresponding combined pre-imaginal stadia for the period April to November, 1972, in Rockhampton.

	Drying period (hours)			Development period (hours)
	75%	80%	85%	
April	130	171	197	374
May	153	221	214	1060
June	156	194	217	3293
July	144	183	207	4899
August	137	176	75	2798
September	136	175	200	439
October	124	164	191	361
November	127	167	193	283

TABLE 39. Time required for shaded dung pads of three different initial moisture contents to reach lower lethal moisture limits for buffalo fly larvae, and corresponding combined pre-imaginal stadia for the period April to November, 1972, in Rockhampton.

Figure 29. Mean fly counts per cow (one side only) for the period December 1971 to August 1973 in Rockhampton. Counts are from all three herds combined and are means for two or three observers.


Mean flies per animal (one side only, all herds)
500
400
300
200
100
0
D
J
F
M
A
M
J
J
A
S
O
N
D
J
F
M
A
M
J
J
A
1971
1972
1973

BIBLIOGRAPHY

ANON. 1928. *J. Coun. scient. Res. Aust.* The buffalo fly pest. 289-291.

ANON. 1929. The buffalo fly problem. *J. Coun. scient. Ind. Res. Aust.* 2 : 184.

ANON. 1930. Buffalo fly investigations. *J. Coun. scient. Ind. Res. Aust.* 3 : 66.

ANON. 1931. The buffalo fly problem - recent developments. *J. Coun. scient. Ind. Res. Aust.* 4 : 126.

ANON. 1931. Buffalo fly problem - eastern boundary of infestation. *J. Coun. scient. Ind. Res. Aust.* 4 : 194.

ANON. 1934. The buffalo fly - its occurrence in Southern China. *J. Coun. scient. Ind. Res. Aust.* 7 : 55-6.

ANON. 1944. Buffalo fly investigations. *J. Coun. scient. Ind. Res. Aust.* 17 : 305-6.

ANON. 1977. *Rep. Div. Ent. C.S.I.R.O. Aust.*, 141pp.

ANON. 1978. *Rep. Div. Ent. C.S.I.R.O. Aust.*, 96pp.

ALLEN, J.C. 1976. A modified sine wave method for calculating degree days. *Envir. Ent.* 5 : 388-396.

ARNOLD, C.Y. 1960. Maximum-minimum temperature as a basis for computing heat units. *Proc. Am. Soc. hort. Sci.* 76 : 682-692.

BACHELOR, J.S. and BRADLEY, J.R. Jr. 1975. Effect of temperature on development and mortality of the boll weevil egg stage. *Envir. Ent.* 4 : 319-320.

BARNARD, E.A. 1973. Comparative biochemistry and physiology of digestion. pp. 133-139 *in* Prosser, C.L. (ed.) *Comparative Animal Physiology*, 3rd ed. W.B. Saunders Co. Philadelphia.

BASKERVILLE, G.L. and EMIN, D. 1969. Rapid estimation of heat accumulation from maximum and minimum temperatures. *Ecology* 50 : 514-517.

BAY, D.E., PITTS, C.W. and WARD, G. 1968. Oviposition and development of the face fly in feces of six species of animals. *J. econ. Ent.* 61 : 1733-1736.

BAY, D.E., PITTS, C.W. and WARD, G. 1969. Influence of moisture content of bovine feces on oviposition and development of the face fly. *J. econ. Ent.* 62 : 41-44.

BAY, D.E., PITTS, C.W. and WARD, G. 1970. Face fly larval development in relation to bovine sex and quantity of feces. *J. econ. Ent.* 63 : 1973.

BLUME, R.R., KUNZ, S.E., HOGAN, B.F. and MATTER, J.J. 1970. Biological and ecological investigations of horn flies in central Texas: influence of other insects in cattle manure. *J. econ. Ent.* 63 : 1121-1123.

BODENHEIMER, F.S. 1925. On predicting the development cycles of insects. I. *Ceratitis capitata* Wied. *Bull. Soc. ent. d'Egypte*: 149-157.

BORNEMISSZA, G.F. 1968. Studies on the histerid beetle *Pachylister chinensis* in Fiji, and its possible value in the control of buffalo fly in Australia. *Aust. J. Zool.* 16 : 673-688.

BORNEMISSZA, G. 1970. Insectary studies on the control of dung breeding flies by the activity of the dung beetle *Onthophagus gazella* F. (Coleoptera : Scarabaeinae). *J. Aust. entomol. Soc.* 9 : 34-41.

BUTLER, G.D. 1966. Development of several predaceous Hemiptera in relation to temperature. *J. econ. Ent.* 59 : 1306-1307.

BUTLER, G.D. Jr. and RITCHIE, P.L. Jr. 1970. Development of *Chrysopa carnea* at constant and fluctuating temperatures. *J. econ. Ent.* 63 : 1028-1030.

BUTLER, G.D. Jr. and DICKERSON, W.A. 1972. Life cycle of the convergent Lady beetle in relation to temperature. *J. econ. Ent.* 65 : 1508-1509.

CAMPBELL, T.G. 1938. Recent investigations on the buffalo fly (*Lyperosia exigua* de Meijere) and its parasites in north Australia. *J. Coun. scient. Ind. Res. Aust.* 11 : 77-82.

CHENG, T. 1958. The effect of biting fly control on weight gain in beef cattle. *J. econ. Ent.* 51 : 275-278.

CHIANG, H.C. and SISSON, V. 1968. Temperature relationships of the development of northern corn rootworm eggs. *J. econ. Ent.* 61 : 1406-1410.

CUTKOMP, L.K. and HARVEY, A.L. 1958. The weight responses of beef cattle in relation to control of horn and stable-flies. *J. econ. Ent.* 51 : 72-75.

DAVIDSON, J. 1942. On the speed of development of insect eggs at constant temperatures. *Aust. J. exp. Biol. med. Sci.* 22 : 233-239.

DAVIDSON, J. 1944. On the relationship between temperature and rate of development of insects at constant temperatures. *J. Anim. Ecol.* 13 : 26-38.

DEPNER, K.R. 1961. The effect of temperature on development and diapause of the horn fly, *Siphona irritans* (L.) (Diptera : Muscidae). *Can. Ent.* 93 : 855-859.

DEPNER, K.R. 1962. Continuous propagation of the horn fly *Haematobia irritans* (L.) (Diptera : Muscidae). *Can. Ent.* 94 : 893-895.

DOWE, T.W., MATSUSHIMA, J. and ARTHAUD, V. 1955. The effects of the corn-alfalfa hay ratio on the digestibility of the different nutrients by cattle. *J. Anim. Sc.* 14 : 340-349.

DUNBAR, D.M. and BACON, O.G. 1972. Influence of temperature on development and reproduction of *Geocoris atricolor, G. pallens* and *G. punctipes* (Heteroptera : Lygaeidae) from California. *Envir. Ent.* 1 : 596-599.

ECKENRODE, C.J. and CHAPMAN, R.K. 1971. Effect of various temperatures upon rate of development of the cabbage maggot under artificial conditions. *Ann. Ent. Soc. Am.* 64 : 1079-1083.

FERRAR, P. 1969. Colonisation of an island by the buffalo fly *Haematobia exigua*. *Aust. vet. J.* 45 : 290-292.

FERRAR, P. 1973. The C.S.I.R.O. dung beetle project. *Wool Technol. Sheep Breed.* 20 : 73-75.

FERRAR, P. 1975. Disintegration of dung pads in north Queensland before the introduction of exotic dung beetles. *Aust. J. exp. Agric. Anim. Husb.* 15 : 325-329.

FERRIERE, C. 1933. Note sur les parasites de *Lyperosia exigua* de Meijere. *Révue suisse Zool.* 40 : 637-643.

FRAZER, B.D. and GILBERT, N. 1976. Coccinelids and aphids : a quantitative study of the impact of adult ladybirds (Coleoptera : Coccinellidae) preying on field populations of pea aphids (Homoptera : Aphididae). *J. ent. Soc. Br. Columbia.* 73 : 33-56.

GEE, R.W. 1970. Australia and exotic animal diseases - a reappraisal. *Aust. vet. J.* 26 : 129-137.

GRANETT, P. and HANSENS, E.J. 1957. Further observations on the effect of biting fly control on milk production in cattle. *J. econ. Ent.* 50 : 332-336.

GREENHAM, P.M. 1970. Some ecological studies on the bush fly *Musca vetustissima* Walker (Diptera : Muscidae). Ph.D. Thesis, Australian National University, 141pp.

GREENHAM, P.M. 1972(a). The effects of the variability of cattle dung on the multiplication of the bush fly (*Musca vetustissima* Walk.). *J. Anim. Ecol.* 41 : 153-165.

GREENHAM, P.M. 1972(b). The effect of the temperature of cattle dung on the rate of development of the larvae of the Australian bushfly, *Musca vetustissima* Walker (Diptera : Muscidae). *J. Anim. Ecol.* 41 : 429-437.

HANDSCHIN, E. 1932. A preliminary report on investigations on the buffalo fly (*Lyperosia exigua* de Meij.) and its parasites. *Pamph. Coun. scient. Ind. Res. Aust.* No. 31, 24pp.

HANDSCHIN, E. 1933(a). Beitrage zur Biologie der Buffel fliege (*Lyperosia exigua* de Meij) und ihren parasiten. *Révue suisse Zool.* 40 : 187-200.

HANDSCHIN, E. 1933(b). Studien an *Lyperosia exigua* de Meijere und ihren parasiten. I. *Lyperosia exigua* in Java und Nord Australien. *Révue suisse Zool.* 40 : 449-528.

HANDSCHIN, E. 1934(a). Studien an *Lyperosia exigua* de Meijere und ihren parasiten. II Die naturliehen Feinde von *Lyperosia*. *Révue suisse Zool.* 41 : 1-77.

HANDSCHIN, E. 1934(b). Studien an *Lyperosia exigua* de Meijere und ihren parasiten. III Die anzietung *Spalangia* zu ihrem wirte. *Révue suisse Zool.* 41 : 267-297.

HARRIS, R.L. and FRAZAR, E.D. 1970. Intake of blood by adult horn flies reared in the laboratory. *Ann. ent. Soc. Am.* 63 : 1475-1476.

HILL, G.F. 1917. Some notes on the bionomics of the buffalo fly (*Lyperosia exigua* de Meijere). *Proc. Linn. Soc. N.S.W.* 41 : 763-768.

HINTON, H.E. 1959. Plastron respiration in the eggs of *Drosophila* and other flies. *Nature, Lond.* 184 No. 4682 : 280-281.

HINTON, H.E. 1960(a). Plastron respiration in the eggs of blow flies. *J. Insect. Physiol.* 4 : 176-183.

HINTON, H.E. 1960(b). The chorionic plastron and its role in the eggs of the Muscinae (*Diptera*). *Q. Jl. microsc. Sci.* 101 : 313-332.

HINTON, H.E. 1961. How some insects, especially the egg stages, avoid drowning when it rains. *South London Entomological and Natural History Society Proceedings*. 1960 : 138-154.

HOELSCHER, C.E. and COMBES, R.L. 1971. The horn fly. III. Sex ratio and factors affecting adult emergence. *Ann. Ent. Soc. Am.* 64 : 912-919.

HUFFAKER, C.B. 1944. The temperature relations of the immature stages of the malarial mosquito, *Anopheles quadrimaculatus* Say, with a comparison of the developmental power of constant and variable temperatures in insect metabolism. *Ann. ent. Soc. Am.* 37 : 1-27.

HURWOOD, I.S. 1968. Methoxychlor residues in the meat and milk of cattle following limited applications of methoxychlor for the control of buffalo fly (*Haematobia exigua*). *Aust. vet. J.* 44 : 377-381.

IHEAGWAM, E.U. 1978. Effects of temperature on development of the immature stages of the cabbage whitefly, *Aleyrodes proletella* (*Homoptera* : *Aleyrodidae*). *Entomologia exp. appl.* 23 : 91-95.

JENKINS, C.F.H. 1945. Entomological problems of the Ord River Irrigation Area. *J. Dep. Agric. West. Aust.* 22 : 131-145.

JOHNSON, T.H. and BANCROFT, M.J. 1920(a). Experiments with certain Diptera as possible transmitters of bovine onchocerciasis. *Proc. R. Soc. Qd.* 32 : 31-57.

JOHNSTON, T.H. and BANCROFT, M.J. 1920(b). The life history of *Habronema* in relation to *Musca domestica* and native flies in Queensland. *Proc. R. Soc. Qd.* 32 : 61-88.

KITCHING, R.L. 1976. On the prothoracic spiracles of the first instar larvae of calyptrate Cyclorrhapha (Diptera). *J. Aust. ent. Soc.* 15 : 233-235.

KRIJGSMAN, B.J. and WINDRED, G.L. 1933. Investigations on the buffalo fly *Lyperosia exigua* de Meij. Pamph. No. 43, *Coun. scient. ind. Res. Aust.* 40pp.

KUNZ, S.E., BERRY, I.L. and FOERSTER, K.W. 1977. The development of the immature forms of *Stomoxys calcitrans*. *Ann. ent. Soc. Am.* 70 : 169-172.

LARSEN, E.B. 1943. The influence of humidity on life and development of insects. *Vidensk. Meddr. dansk naturh Foren.* 107 : 127-184.

MACKERRAS, I.M. 1933. The taxonomy of *Lyperosia exigua* de Meijere (Diptera : Muscidae). *Ann. Mag. nat. Hist.* 10 ser. v.11 : 58-64.

MACKERRAS, I.M. 1947. Recent developments in the control of cattle ticks and buffalo fly. *Aust. vet. J.* 23 : 185-189.

MACQUEEN, A. 1975. Dung burial activity and fly control potential of *Onthophagus nuchicornis* (Coleoptera : Scarabaeinae) in British Columbia. *Can. Ent.* 107 : 1215-1220.

MALLOCH, J.R. 1932. Exotic Muscaridae (Diptera). *XXXVI Ann. Mag. Nat. Hist.* 10(a) : 377-405, 421-447, 501-518.

McEACHRAN, J. and HILL, G. 1915. Bulletin of the Commonwealth Dept. of Trade and Customs, 8pp.

McLINTOCK, J. and DEPNER, K.R. 1954. A review of the life-history and habits of the horn fly, *Siphona irritans* (L.) (Diptera : Muscidae). *Can. Ent.* 86 : 20-33.

MELVIN, R. and BECK, D.E. 1931. Length of the developmental stages of the horn fly *Haematobia irritans* (Linné), at constant temperature. *J. econ. Ent.* 24 : 330-331.

MESSENGER, P.S. and FLITTERS, N.E. 1959. Effect of variable temperature environments on egg development of three species of fruit flies. *Ann. ent. Soc. Am.* 52 : 191-204.

MESSENGER, P.S. 1964. The influence of rhythmically fluctuating temperatures on the development and reproduction of the spotted alfalfa aphid, *Therioaphis maculata*. *J. econ. Ent.* 57 : 71-76.

MILLER, R.W., PICKENS, L.G., MORGAN, N.O., THIMIJAN, R.W. and WILSON, R.L. 1973. Effect of stable flies on feed intake and milk production of dairy cows. *J. econ. Ent.* 66 : 711-713.

MORGAN, H.O. and GRAHAM, O.H. 1966. Influence of cattle diet on survival of horn fly larvae. *J. econ. Ent.* 59 : 835-837.

MORGAN, N.O. and SCHMIDT, C.D. 1966. The pH tolerance of horn fly larvae. *J. econ. Ent.* 59 : 222-223.

NOLAN, J. and ANNAND, A.M. 1963. An *in vitro* comparison of the effectiveness of DDT and Sevin against buffalo fly. *Aust. vet. J.* 39 : 227-230.

NORRIS, K.R. 1946. The testing of the American horn fly trap, and a new type of trap involving the use of DDT against the buffalo fly, *Siphona exigua* (de Meijere). *J. Coun. scient, ind. Res. Aust.* 19 : 65-76.

NORRIS, K.R. 1947. The use of DDT for the control of the buffalo fly (*Siphona exigua* (de Meijere)). *J. Coun. scient. ind. Res. Aust.* 20 : 25-42.

PFADT, R.E., LLOYD, J.E. and SHARAFI, G. 1975. Pupal development of cattle grubs at constant and alternating temperatures. *J. econ. Ent.* 68 : 325-328.

PHILIPP, J.S. and WATSON, T.F. 1971. Influence of temperature on population growth of the pink bollworm, *Pectinophora gossypiella* (Lepidoptera : Gelichiidae). *Ann. ent. Soc. Am.* 64 : 334-340.

PONT, A.C. 1973. Studies on Australian Muscidae (Diptera). IV. A revision of the subfamilies Muscinae and Stomoxyinae. *Aust. J. Zool. suppl.* Series No. 21 : 265-273.

PRADHAN, S. 1945. Insect population studies. II. Rate of insect-development under variable temperature of the field. *Proc. Indian Acad. Sci.*: 74-80.

ROBERTS, F.H.S. 1952. Insects Affecting Livestock. Australian Agricultural and Livestock series. Angus and Robertson, Sydney and London, pp.267.

SANDERS, D.P. and DOBSON, R.C. 1969. Contributions to the biology of the horn fly. *J. econ. Ent.* 62 : 1362-1366.

SARDEY, M.R. and THAKARE, V.K. 1977. Effect of temperature and humidity on the life-cycle of buffalo fly, *Lyperosia exigua* (de Meijere, 1903). *Indian J. Anim. Sci.* 47 : 561-565.

SCHEERPELTZ, O. 1934. Zwei neue Arten der Gattung *Aleochara* Gravh. (Coleopt., Staphylinidae) die aus Puppen von *Lyperosia* (Dipt.) als Parasiten) gezogen worden. *Révue suisse Zool.* 41 : 131-147.

SCHMIDT, C.D., HARRIS, R.L. and HOFFMAN, R.A. 1967. Mass rearing of the horn fly, *Haematobia irritans* (Diptera : Muscidae) in the laboratory. *Ann. ent. Soc. Am.* 60 : 508-510.

SCHMIDT, C.D., HARRIS, R.L. and HOFFMAN, R.A. 1968. New techniques for rearing horn flies. *Ann. ent. Soc. Am.* 61 : 1045-1046.

SEDDON, H.R. 1967. Diseases of domestic animals in Australia. Part 2: Arthropod infestations. Second ed. Commonwealth of Australia, Dept. of Health. Service Publications No. 6, pp.152.

SIDDIQUI, W.H. and BARLOW, C.A. 1972. Population growth of *Drosophila melanogaster* (Diptera : Drosophilidae) at constant and alternating temperatures. *Ann. ent. Soc. Am.* 65 : 993-1001.

SIEGEL, S. 1956. Non-parametric statistics for the behavioural sciences. International Student edition. McGraw-Hill Kogakusha Ltd., pp.312.

SNYDER, F.M. 1965. Insects of Micronesia Diptera : Muscidae. *Insects Micronesia.* 13 : 311-313.

SPING LIN, HODSON, A.C. and RICHARDS, A.G. 1954. An analysis of threshold temperatures for the development of *Oncopeltus* and *Tribolium* eggs. *Physiol. Zool.* 27 : 287-311.

STEELMAN, C.D., WHITE, T.W. and SCHILLING, P.E. 1972. Effects of mosquitoes on the average daily gain of feedlot steers in Southern Louisana. *J. econ. Ent.* 65 : 462-466.

STEELMAN, C.D., WHITE, T.W. and SCHILLING, P.E. 1976. Efficacy of Brahman characters in reducing weight loss of steers exposed to mosquito attack. *J. econ. Ent.* 69 : 499-502.

STEELMAN, C.D. and SCHILLING, P.E. 1977. Economics of protecting cattle from mosquito attack relative to injury thresholds. *J. econ. Ent.* 70 : 15-17.

STINNER, R.E., GUTIERREZ, A.P. and BULTER, G.D. 1974. An algorithm for temperature-dependent growth rate simulation. *Can. Ent.* 106 : 519-524.

TESKEY, H.J. 1969. On the behaviour and ecology of the face fly, *Musca autumnalis* (Diptera : Muscidae). *Can. Ent.* 101 : 561-576.

STRONG, F.E. and APPLE, J.W. 1958. Studies on the thermal constants and seasonal occurrence of the seed-corn maggot in Wisconsin. *J. Econ. Ent.* 51 : 704-707.

TILLYARD, R.J. 1931. The buffalo fly in Australia. *J. Coun. scient. ind. Res. Aust.* 4 : 234-243.

TOBA, H.H., KISHABA, A.N., PANGALDAW, R. and VAIL, P.V. 1973. Temperature and the development of the cabbage looper. *Ann. ent. Soc. Am.* 66 : 965-974.

TSITSIPIS, J.A. and MITTLER, T.E. 1976. Development, growth, reproduction and survival of apterous virginoparae of *Aphis fabae* at different temperatures. *Entomologia exp. appl.* 19 : 1-10.

VALIELA, I. 1969. An experimental study of the mortality factors of larval *Musca autumnalis* de Geer. *Ecol. Monogr.* 39 : 199-225.

VAN' T KLOOSTER, A.Th. 1967. De Toestand van calcium, magnesium en enkele andere mineralen in darmn houd en mest var herkauwers in wer band met hun resorptie. *Meded LandbHoogesch. Wageningen.* 137pp.

WEAST, R.C. (ed.) 1971. Handbook of chemistry and physics. The Chemical Rubber Company, Ohio. pp.2283.

WINDRED, G.L. 1933. The influence of moisture on the larvae of *L. exigua*. Pamph. No. 43. *Coun. scient. ind. Res. Aust.* 40pp.

ZUMPT, F. 1973. The stomoxyine biting flies of the world: Diptera: Muscidae. Taxonomy, biology, economic importance and control measures. Stuttgart, Gustav Fischer, pp.175.

APPENDIX 1: EFFECTS OF CONSTANT ATMOSPHERIC TEMPERATURES ON THE INTERNAL TEMPERATURES OF WHOLE-DUNG CULTURES

INTRODUCTION

When the effects of temperature on mortality and speed of development were being studied, the monitoring of actual dung temperatures was not practicable. The air temperatures of the incubators were used to estimate the temperature within the dung. An experiment was conducted to investigate possible disparity in temperature regimes between the atmosphere and the dung cultures.

MATERIALS AND METHODS

The experiment was conducted at three constant temperatures, *viz.* 17.5, 25.0 and 35.0°C. These represented low, medium and high levels of the range at which most of the fly-rearing was carried out. Dung was placed in containers in the manner described for normal rearing, and a mercury thermometer was placed permanently in each culture. The moisture content of the dung was 85 per cent. calculated on the wet weight. Six cultures were assigned to each temperature level; three of these were inoculated with approximately fifty buffalo fly eggs and three were not. Readings of dung and atmospheric temperatures were made at various intervals, continuing over a period similar to that required for development to the adult stage at the particular temperature.

RESULTS

The temperatures in the air and in dung cultures are recorded in Table 40. Two-way analysis of variance of the resulting data showed the expected difference due to temperature ($P < 0.001$) but there were no significant differences between temperatures in dung and the atmosphere at any one temperature, whether flies were present or not ($P > 0.50$).

DISCUSSION AND CONCLUSIONS

Since there were no detectable differences between air and dung temperatures, air temperatures were taken to be equal to dung temperatures in all experiments involving incubation at constant temperatures.

Time (Days)	Atmosphere	Flies present	Flies absent
1	34.5	33.2 (0.3)	35.0 (-)
2	35.0	34.3 (0.6)	34.5 (0.5)
3	35.0	34.5 (0.5)	34.7 (0.3)
4	35.0	34.7 (0.3)	34.5 (-)
5	34.5	34.8 (0.3)	34.8 (0.3)
6	34.5	34.7 (0.3)	35.0 (-)
7	35.0	35.0 (-)	34.7 (0.3)
8	35.0	34.7 (0.3)	35.0 (-)
9	34.0	35.3 (0.6)	35.3 (3.6)
1	24.0	23.5 (0.5)	23.7 (0.6)
2	24.0	23.8 (0.3)	24.2 (0.3)
3	24.0	24.0 (0.5)	23.7 (0.3)
4	24.0	24.2 (0.3)	24.2 (0.3)
5	24.0	24.2 (0.3)	24.0 (-)
6	24.0	24.2 (0.3)	24.0 (-)
7	24.0	24.0 (-)	24.2 (0.3)
8	24.0	24.2 (0.3)	24.0 (-)
9	24.0	24.3 (0.3)	24.2 (0.3)
10	24.0	24.2 (0.3)	24.3 (0.3)
11	24.5	24.2 (0.3)	24.0 (-)
12	24.5	24.3 (0.3)	24.2 (0.3)
1	17.5	17.5 (0.9)	18.0 (-)
5	17.5	17.7 (0.6)	18.0 (0.5)
9	18.0	17.7 (0.3)	17.5 (0.5)
13	18.0	17.3 (0.3)	17.8 (0.3)
17	17.5	18.2 (0.3)	17.7 (0.8)
21	18.0	18.2 (0.3)	18.2 (0.3)
26	18.0	17.5 (-)	17.8 (0.3)
31	17.5	17.7 (0.3)	17.7 (0.5)

TABLE 40 Temperatures in the atmosphere and within dung cultures with and without fly larvae in three different constant-temperature cabinets. Estimates of dung temperatures are the means of three replicates, and their standard errors.

APPENDIX 2: EFFECTS OF TEMPERATURE AND MOISTURE OF THE LARVAL BREEDING MEDIUM ON PUPARIAL FORM IN THE BUFFALO FLY.

TREATMENTS AND SAMPLE STATISTICS FOR THE MEASURED VARIABLES.

Treatment			Length (mm)		Diameter (mm)		Weight (mg)	
No.	$\%H_2O$	T(^oC)	$\bar{y}$	sd	$\bar{y}$	sd	$\bar{y}$	sd
1	67	20	2.54	0.085	1.00	0.059	1.2	0.2
2	67	25	2.46	0.148	0.96	0.081	1.2	0.3
3	67	30	2.40	0.146	0.91	0.076	1.0	0.3
4	67	35	2.64	0.154	0.97	0.066	1.1	0.2
5	71	20	2.76	0.119	1.10	0.077	1.8	0.5
6	71	25	2.70	0.133	1.12	0.069	1.6	0.3
7	71	30	2.59	0.126	1.05	0.075	1.4	0.3
8	71	35	2.75	0.089	1.04	0.056	1.4	0.2
9	75	20	3.02	0.093	1.28	0.041	2.3	0.4
10	75	25	2.97	0.094	1.21	0.085	2.1	0.5
11	75	30	2.85	0.107	1.19	0.089	2.0	0.4
12	75	35	2.83	0.061	1.18	0.077	1.9	0.3
13	79	20	3.19	0.109	1.29	0.037	2.7	0.3
14	79	25	3.34	0.131	1.35	0.066	3.1	0.5
15	79	30	3.12	0.111	1.30	0.048	2.2	0.9
16	79	35	3.31	0.159	1.35	0.092	2.7	0.6
17	84.5	20	3.37	0.104	1.34	0.056	2.9	0.4
18	84.5	25	3.54	0.122	1.42	0.077	3.7	0.5
19	84.5	30	3.39	0.106	1.39	0.056	3.2	0.4
20	84.5	35	3.32	0.139	1.34	0.060	2.7	0.6
21	87.5	20	3.21	0.126	1.28	0.051	2.6	0.6
22	87.5	25	3.53	0.122	1.43	0.058	3.8	0.5
23	87.5	30	3.31	0.092	1.35	0.051	3.0	0.5
24	87.5	35	3.37	0.156	1.36	0.070	2.9	0.5

APPENDIX 3: COMPARISON OF MOISTURE CONTENTS OF WHOLE PADS AND OF MOIST CENTRAL ZONES

INTRODUCTION

Estimates of moisture loss and internal temperatures of dung pads in Chapter 5 were made sequentially on the same pads, which could therefore not be sub-sampled for zonation of moisture content. Pads were weighed periodically during exposures, and dry weight of each was determined at the end of the experiment. This allowed an estimate of the total water content of a pad at any weighing time, but gave no measure of its spatial distribution. An experiment was conducted to establish the relationship between total moisture content and that in the moist central areas of dung pads.

MATERIALS AND METHODS

Artificial pads weighing two kilograms were formed and exposed in the manner described in Chapter 5. Initial moisture levels were determined gravimetrically, and from this the dry weight of pads was determined. Four pads were collected on each sampling day. They were weighed to determine total moisture content. The pads were then broken up, and sub-samples from the moister internal area were taken. The moisture content of these sub-samples was then determined.

RESULTS

Whole-pad moisture levels, and the accompanying moist-zone estimates, are shown in Table 41. The regression equation for this relationship is

$$Y = 51.0340 + 0.3910X \quad (A^2 = 96.54\%)$$

where Y = moist-zone moisture content and

X = whole-pad moisture content, both expressed in terms of the wet weight.

DISCUSSION AND CONCLUSIONS

The equation above fits the experimental data very closely; however it is not implied that the regression describes the general relationship between total and zonal moisture contents of dung pads. Factors such as varying weather conditions, the texture of dung and the nature of substrate on which the pad is lying may alter considerably the moisture patterns in pads. The equation was used to interpret seasonal field counts of flies in Chapter 6. It should be recognized that dung type, soil type and climate during the counting period were different from those applying during the above experiment.

Day	Whole pad moisture (% wet weight)	"Moist zone" moisture (% wet weight)
0	83.3	83.3
2	77.3	81.5 (80.5-82.5)
5	73.4	78.5 (77.5-79.5)
7	65.0	76.4 (75.0-77.5)
9	61.1	74.9 (73.9-75.6)
12	51.6	71.3 (70.2-72.4)
14	49.6	70.5 (69.4-71.6)

TABLE 41 Means and 95 per cent. confidence intervals of dung moisture, based on whole pads, and on samples from the "moist zones" of pads. Percentages are expressed in terms of wet weight.