STUDIES ON THE TOXICITY OF PHOSPHINE AND METHYL BROMIDE TO FOUR MOTH PESTS OF STORED PRODUCTS WITH SPECIAL EMPHASIS ON THE INFLUENCE OF DIAPAUSE

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ABSTRACT

Moth pests of stored products included in this work were Ephestia elutella (Hübner), Ephestia kuehniella Zeller, Ephestia cautella (Walker), and Plodia interpunctella (Hübner). Information was gathered on the physical limits of the four species, on the properties of cultures set up in the laboratory, and on the factors involved in the induction and termination of diapause. Diapause was induced by low temperature and short daylength in fully grown larvae of E. elutella and P. interpunctella. Light intensities below 1 lux affected the induction of diapause in both species. In P. interpunctella, another factor inducing diapause was high population density. Attempts to produce an arrest in the development of E. cautella were unsuccessful. Termination of diapause was hastened by such factors as long photoperiod, high temperature, chilling periods, and fumigation.

Diapausing larvae of E. elutella were tolerant to methyl bromide, requiring concentration time (CT) products for 99% kill of 281 and 164 mg h/l at 15 and 25°C respectively. Diapausing larvae of P. interpunctella required CT products of 225 and 67 mg h/l at 10 and 25°C respectively for 99% kill. In the absence of diapause all stages were susceptible to methyl bromide, succumbing to a CT product of 64 mg h/l at 15°C.

With phosphine, the egg stage of all four species was highly tolerant for the first 40% of the developmental period. Eggs exposed beyond this period showed a marked increase in susceptibility, and were controlled by less than 0.04 mg/l in 4-day exposures at 25°C. Longer exposures of phosphine were more efficient than shorter ones of similar CT product, and Haber's rule could not be applied.

Factors controlling diapause in laboratory and field stocks were different, and a correlation was observed between diapause intensity and tolerance to fumigation. Further information was gained by fumigating the high intensity diapausing stages of Pieris brassicae (L) and Bombyx mori (L).

C O N T E N T S

INTRODUCTION

Although gases and volatile liquids have been used for many years to control pests infesting stored products, most information on the toxicity of fumigants to insects has been gathered for beetles, and little for moths, the second largest insect group of pests. Some moth species enter a diapause, which increases their tolerance to fumigation. The aim of the present work was to study the toxicity of the two most widely used fumigants, phosphine and methyl bromide, to all life stages of moths under controlled laboratory conditions. Where possible, experiments were designed to simulate storage conditions in choice of equipment, fumigation procedure and in the organisation and presentation of insects for test. Hence in the majority of experiments on the toxicity of phosphine, exposure periods included the time required for the evolution of gas from the commercial formulation used. Experimental conditions were carefully controlled with particular emphasis on fumigation temperature and the physiological state of the pest. Insects were reared and fumigated on a standardised food medium, and the relative humidity chosen for fumigation was one which would not adversely affect survival. All tests were conducted at atmospheric pressure.

Many insects living in temperate regions, including some which have become pests of stored products, have evolved a facultative diapause which enables them to survive the winter, and the onset of this condition is determined by the environmental conditions prevailing at a sensitive phase in the life cycle. In the present work, the importance of diapause in raising tolerance levels entailed much research into the factors governing the induction, maintenance and termination of diapause. For stocks reared continuously in laboratories, diapause serves no obvious function, so there is selection against it. To obtain a rapid turnover of individual cultures, successive generations are started with the first adults to appear, and individuals in diapause are removed from the breeding line. In addition, laboratory stocks are isolated from natural climatic changes such as fluctuations in temperature and daylength which govern the induction of diapause.

For studies on diapause, fresh samples were obtained from natural infestations, and cultures bred from these were maintained at the laboratory in sites subject to the influence of the outside environment so that breeding and development were controlled by the seasons.

REVIEW

FUMIGATION AND INSECT CONTROL

Fumigation is a very ancient practice. According to the writings of Homer, the fumes of burning sulphur were used for the disinfection of houses in Greece as early as the 12th century B.C. Until quite recently, sulphur dioxide was used to control bed bugs in houses, and it has also been used against pests in ships and warehouses. Sulphur dioxide never became popular in the milling industry because of the fire hazard of burning sulphur, the corrosive action on metals, and the adverse effect on grain and flour. The first compound to gain general acceptance as a grain fumigant was carbon disulphide. Its potential as a fumigant was reported in 1854 by Lazare Garreau, but it did not come into general use until 1879 (Cotton, 1956).

Hydrogen cyanide appeared on the scene in 1877 when it was used to kill Dermestid beetles infesting a cabinet of insect specimens, and in 1886 it was used to control scale on citrus trees under tents (Cotton, 1956). Later, hydrogen cyanide was accepted for treatment of a very wide range of commodities, including grain and flour.

It has been realized for over 40 years that phosphine and methyl bromide are suitable for use as insect fumigants. Nevertheless, these two compounds remain the most recent additions to the handful of fumigants in widespread use today.

For successful control by fumigation, both exposure period and gas concentration

are of great importance. The product of these two factors has proved a useful way of expressing fumigant dosage, as, within certain limits, long exposures at low concentrations, and short exposures at high concentrations, in general give comparable results. Because there are two factors involved, there are two thresholds for mortality, a minimum concentration below which no kill is achieved, regardless of length of exposure, and a minimum exposure below which no concentration, however high, will result in mortality (Knight, 1925). A relationship thus operates where a given concentration-time (CT) product approximately achieves a particular level of mortality only over a restricted range of concentrations and exposures. In spite of this and other limitations which are extensively discussed by Sun (1947), the relationship, which is widely known as Haber's formula or rule, (Peters and Ganter, 1935; 1935a; Busvine, 1938; Page and Lubatti, 1939), remains an international method for expressing fumigant dosage. The units of CT products are generally quoted as mg x hours per litre (mg h/l).

The toxicity of fumigants to insects is influenced by many factors before and after fumigation as well as during the actual exposure. In general, susceptibility if increased by subjection to high temperature, low pressure, or extremes of humidity. The nature of the infested commodity and the condition of the pest insects themselves are additional factors of great importance.

TOXICITY OF METHYL BROMIDE TO INSECTS

Historically, methyl bromide was first used as a fire depressant with inflammable fumigants such as ethylene oxide. In testing such a mixture against several species, including the Granary weevil Sitophilus granarius (Linnaeus), Le Goupils (1932) noticed an increased insecticidal action, and after conducting toxicity tests with methyl bromide alone, he proposed that the compound should be used as an insect fumigant. Le Goupils' first

experiments were performed under partial vacuum, a technique first investigated in America, just before the first world war, and one which had become widely practiced in France by the time the insecticidal properties of methyl bromide were discovered (Lepigre, 1949).

Work on the toxicity of methyl bromide to insects gained momentum through the 30's, and the fumigant was put to use in the horticultural and fruit industries. In Morocco during February, 1934, Francolini (1935) conducted the first methyl bromide fumigation of a grain store. The grain was infested with weevils then identified as the Rice weevil Sitophilus oryzae (Linnaeus), and a few S. granarius. As a result of the treatment, complete control was obtained. More specific data on the toxicity of methyl bromide to storage pests were presented by Shepard and co-workers (Shepard et al., 1937; Fisk and Shepard, 1938; Shepard and Buzicky, 1939). Tests were conducted at atmospheric pressure with 5-hour exposures at 25°C.

In the United Kingdom today, methyl bromide is by far the most commonly used fumigant. It is suitable for the control of rodents and micro-organisms as well as insects, and is used in treating a wide range of materials in warehouses, factories, mills, silos, ships, trucks, vacuum chambers, glasshouses, poultry houses, or anywhere under gas-proof sheeting. The properties and usage of methyl bromide were fully reviewed by Thompson (1966).

CT products required for adequate control vary widely with temperature. On the basis of work done at what is now the Pest Infestation Control Laboratory, Brown (1959) estimated the minimum CT products required for a 99.9% kill of a range of beetle pests of stored products at various temperatures (table 1).

TABLE 1. Estimates of minimum CT products giving at least 99.9% kill of a variety of insect species. (Brown, 1959)

Temp °C	CT product : mg x hr per litre
10	200
15	180
20	150
25	100
30	70

These estimates form the basis of the current dosages recommended by the U.K. Ministry of Agriculture, Fisheries and Food for methyl bromide fumigations of foodstuffs (Thompson, 1970). Higher dosages are recommended for controlling infestations of mites, or of the Khapra beetle Trogoderma granarium Everts. A CT product of 290 mg h/l is required for a 99.9% kill of T. granarium larvae at 10°C (Reynolds, 1956). Dosages are also varied according to the nature of the commodity treated to allow for differences of sorption and penetration. The threshold concentration for mortality with methyl bromide is about 2 mg/l (Brown, 1959), and care is taken to prevent gas concentrations falling near this level during treatments. In specifications recommended to the European Plant Protection Organisation (Thompson, 1970), the total input of methyl bromide is calculated as the sum of two temperature dependent values, a dosage per cubic metre of the enclosure, and a dosage per ton of the particular commodity under treatment. Dosages required for control of more tolerant species at low temperatures may taint certain commodities, and in these cases methyl bromide is not a suitable fumigant.

The first account of the effectiveness of methyl bromide against Pyralid stored product pest species appeared in 1936 in the Annual Report of the United States Bureau of Entomology and Plant Quarantine, in which complete control of the Raisin moth Ephestia figulilella Gregson*, and the Indian-meal moth Plodia interpunctella (Hübner), was claimed after a treatment of 25 lb boxes of packed raisins. In tests on a number of storage species, Lepesme (1938) found that a dose of 20 mg/l of methyl bromide in a 90 minute exposure at a lowered pressure of 50 mg Hg, was effective against the Mediterrancan flour moth Ephestia kuehniella Zeller. Shepard and Buzicky (1939) included P. interpunctella among twelve test species, and quoted LC 50 values of 5.0 and 3.1 mg/l respectively for larvae and adults in 5-hour fumigations at 25°C.

* Taxonomy of Pyralid moths after Whalley (1970).

Since the Second World War, little work has been done on the relative toxicity of methyl bromide to moth pests of stored products, although effective control has been reported in a wide range of trials conducted in the field. Phillips et al. (1959) conducted laboratory tests exposing all stages of the Warehouse moth Ephestia elutella (Hübner) in columns packed with cocoa beans, and obtained complete kill with CT products of about 50 mg h/l at 26.7°C. Harein and Press (1966), fumigating P. interpunctella larvae in peanuts at 25-28°C, 60% ± 10% relative humidity (RH), obtained LD 50 and LD 95 values of 2.35 - 3.91 mg/l and 6.19 - 6.41 mg/l respectively in 24-hour exposures. More recently Mostafa et al. (1972), working on eggs at 26°C, 65% RH , obtained LD 50 values for E. kuehniella ranging from 10.7 - 15.4 mg/hl, depending on age and length of exposure. He concluded that with methyl bromide, concentration was the more important factor in the CT product, but the longest exposure period tested was only 7 hours.

Contradictory results have been reported for the susceptibility of diapausing larvae of P. interpunctella. Reynolds (1961) noted only 84% mortality of diapausing larvae which had developed on Rhodesian white maize from a CT product of 100 mg h/l at 25°C, indicating very high tolerance. In contrast, Sardesai (1968; 1972), working on larvae reared on a food medium comprising five parts wheatmeal to one part glycerol, obtained an LD 95 of only 58 mg h/l with 4-hour exposures at 26.7°C.

The possibilities of using methyl bromide in mixture with other gases have been investigated for many years. Jones (1938) investigated the effect of various carbon dioxide concentrations on fumigations of the Rust-red flour beetle Tribolium castaneum (Herbst). He found that carbon dioxide enhanced fumigant efficiency, but to a lesser extent with methyl bromide than with methyl formate or ethylene oxide. High carbon dioxide concentrations reduced the kill of insects, and the best results were obtained for methyl bromide with 10%

carbon dioxide. More recent work (Calderon and Carmi, 1971; 1973) has demonstrated that the presence of carbon dioxide can assist the distribution of methyl bromide in vertical bins loaded with grain.

Fumigation trials have been conducted with varying degrees of success on many other gas mixtures containing methyl bromide. Mixtures of methyl bromide with hydrogen cyanide and with ethylene dibromide have been reported to exhibit synergism (Johnson, 1939; Dawson, 1952). Today, methyl bromide applied commercially quite often contains 2% by weight chloropicrin. This is used as a lachrymatory agent to provide warning of leakages, as methyl bromide is highly toxic to man, and cannot be detected by smell at the concentrations normally met with in practice (Brown, 1950; Watkins, 1964). The small amounts of chloropicrin present would be unlikely to affect insect mortality, and similar results with or without the additive would be expected. In the present work, pure methyl bromide was used.

TOXICITY OF PHOSPHINE TO INSECTS

The insecticidal properties of phosphine have been known since the early twenties (Gunn, 1959), and formulations releasing the gas were first prepared under the name of 'Delicia' by Chemische Fabrik Delitia of Delitzsch, now in East Germany. The formulation consisted of various metal phosphides, notably those of barium, calcium, aluminium and magnesium (Freyberg and Freyberg, 1937), which decompose in the presence of moisture to produce phosphine gas and the metal hydroxide. The phosphides, in powder form, were placed in gas-permeable paper packets and which were kept in sealed containers ready for use. A comprehensive account of a fumigation of bulk grain for insect control with the Delicia packet formulation is given by Mischon (1939). Complete decomposition of the formulation took about 8 days, and the control obtained was satisfactory.

A disadvantage of early phosphine formulations was the risk of spontaneous

combustion of high concentrations of the gas in the presence of oxygen. Phosphine is also highly toxic to man, and has a corrosive action on certain metals, particularly copper. Nevertheless, the fumigant penetrates well, and is highly toxic to insects. Harmful residues are unlikely (Heseltine and Thompson, 1957; Bruce et al., 1962), and the ability of grain to germinate is not impaired (van den Bruel and Bollaerts, 1956; Gunn, 1959). It is therefore surprising that phosphine was not used as a grain fumigant outside Germany until about twenty years ago when a new formulation was produced by another Germany company, Deutsche Gesellschaft für Schädlingsbekämpfung (Degesch) of Frankfurt am Main. This formulation consisted of finely powdered aluminium phosphide and ammonium carbamate compressed into 3 g tablets. The phosphide evolved 1 g of phosphine while the ammonium carbamate dissociated to give ammonia, the odour of which gave an early warning of the breakdown of the tablet, and carbon dioxide, which reduced the fire risk. In a series of toxicity experiments on the tablet formulation in bins containing sorghum, van den Bruel and Bollaerts (1956) obtained good control of S. granarius and T. granarium at a range of temperatures, especially when the bins were sealed. Heseltine and Thompson (1957), quoting results obtained in Germany, reported that all stages of grain beetles of the genera Cryptolestes and Oryzaephilus were killed by a CT product of 10 mg h/l. Younger pupae of S. granarius, however, proved exceedingly tolerant and a CT product of 300 mg h/l was required for complete control. For the best results the manufacturers advised minimum exposure periods of three to five days, depending on temperature.

Exposure time is a major consideration in phosphine fumigations, as different stages of the same species vary greatly in susceptibility to the fumigant, and development continues during the actual exposure (Reynolds et al., 1967; Barker, 1969; Burns Brown et al., 1969; Howe, 1973). Variable results are obtained with short exposures, even in tests on adults. Barker (1969) found that in 5-hour exposures at 24°C, a complete kill of adult Cryptolestes spp.

could not be obtained even with a phosphine concentration of 6.48mg per l, while concentrations between 0.03 and 0.076mg/l were sufficient for 50% kill.

An advantage of the tablet formulation of phosphine is that relatively unskilled workers can perform fumigations because little gas is evolved in the first hour of exposure, and a test area can be treated and vacated within this time (Heseltine and Thompson, 1957). The formulation has been further improved by the addition of a water-resistant coat comprised of hard wax or tristearin containing a disruptive agent to control the initial rate of evolution of gas (Rauscher et al., 1962; 1962a). Under normal conditions of humidity, very little phosphine is evolved during the first four hours after tablets were exposed to air, giving extra time to perform the fumigation.

Today, formulations of phosphine are produced by four separate companies (Prevett and Blatchford, 1972). The tablet formulation of Degesch described above is exported under the trade name Phostoxin. Pellets one-fifth the weight of tablets are also available. Excel Industries of Bombay market similar tablet and pellet formulations under the name of Celphos. The first company to market phosphine has now split into two, one in East Germany and one in West Germany. The eastern concern still markets under the name of Delicia and produces both bags and tablets. The western offshoot, Chemische Fabrik of Weinheim, produces bags and tablets under the names of Detia Gas-Ex-B and Gas-Ex-T. Standard recommendations for precautions in the use of aluminium phosphide preparations in the control of insects and mites have been prepared by the U.K. Ministry of Agriculture Fisheries and Food (1973). Dosages varying from 2-15 tablets per ton are generally applied for grain fumigation, depending on temperature, and on the extent to which the fumigation area can be rendered gas tight. Table 2 summarises the recommendations of manufacturers for treating grain for insect control in various situations, assuming reasonable conditions for the retention of gas.

TABLE 2. Dosages recommended by manufacturers for phosphine fumigations.

TRADE NAME :	PHOSTOXIN	DETIA GAS-EX-B	CELPHOS	DELICIA
Dosage	2-6 tablets (1) per ton	1 bag (2)/1-4 tons	1-2 tablets (1) per ton	1 bag/1-3 tons
Exposure times at various grain temperatures	12-15°C : 5 days 16-20°C : 4 days Over 20°C : 3 days	Silos :) 6-10 days at 20°C Bulk grain) Stacks : 3-6 days at 20°C	Below 15°C : 7 days 15-21°C : 6 days Above 21°C : 5 days	6-8 days depending on temperature

(1) Each tablet releases 1 g phosphine (2) Each bag releases 11 g phosphine

Literature on the toxicity of phosphine to moth pests of stored products is sparse. Much of the work done in Germany before the Second World War has fallen into obscurity. Mischon (1939) stated that many species including E. elutella could be effectively controlled by use of the Delicia formulation and Tomazewski (1941) reported effective control of E. kuchniella in empty storerooms. No detailed account of the relative toxicity of phosphine to insects appeared until after the marketing of the first tablet formulation, and it was only comparatively recently that such work included moths among the test insects. Successful control has however been reported from time to time in a number of field trials involving P. interpunctella (Ilic, 1965; Adesuyi and Cornes, 1967; Sinha et al., 1967), E. elutella (Todorovski, 1960), E. kuehniella (Horak and Strosova, 1963), and Ephestia cautella (Walker) (Tropical warehouse moth) (Riley and Simmons, 1968; Carmi and Calderon, 1969). Lindgren and Vincent (1966) included P. interpunctella larvae in toxicity tests at 26.7°C and obtained values of 0.01 and 0.078 mg/l respectively for the LD 50 and LD 99 in 24-hour exposures, giving CT products of 0.24 and 1.87 mg h/l. Brown et al. (1969), exposing all stages of development to phosphine, found that E. cautella was completely controlled by a CT product of less than 20, and P. interpunctella by a CT product of less than 40 mg h/l in 2-day exposures at 25°C. In 4 and 7-day exposures, both species were far more susceptible. In early work of the present project (Bell and Glanville, 1970), the egg stage of four Pyralid moths, E. elutella, E. kuehniella, E. cautella and P. interpunctella, was found to be very tolerant. All species survived a CT product of 38 mg h/l in a 2-day exposure, but the tolerant phase only spanned the first two days of development in the egg at 25°C, and in 4-day exposures, a CT product of 5 mg h/l killed all stages. Baskaran and Mookherjee (1971) fumigating E. cautella in 24-hour exposures at 29± 1°C found 0-1 day old eggs to be four or five times more tolerant than larvae. The LD 50 for eggs varied from about 0.12 to 0.13 mg/l.

In contrast, Muthu (1973), testing eggs of E. cautella in 24-hour exposures at 25°C, reported an LD 50 value of 1.2 mg per l, and an LD 99 value of 28.0 mg per l. Information is lacking, however, on the age or numbers of eggs treated. All stages of P. interpunctella have now been tested at 26.7°C (Vincent and Lindgren, 1972a) and the egg again proved to be the least susceptible stage. requiring 1.9 mg/l for 24 hours to achieve 95% kill. Larvae, pupae and adults required 0.060, 0.087 and 0.021 mg/l respectively, for a similar level of kill.

Phosphine has not been employed in vacuum fumigation as its toxic action is dependant on the presence of oxygen (Bond et al., 1967; Raüscher, 1972). As its method of application is different, phosphine is seldom used with other fumigants. However, in Germany mills have been fumigated with mixtures of hydrogen cyanide and phosphine, good results have been obtained. The effect of insecticide synergists on the toxic action of phosphine has been investigated, and synergism was observed against T. castaneum and houseflies using piperonyl butoxide (Rajak and Hewlett, 1971).

FACTORS AFFECTING FUMIGANT TOXICITY TO INSECTS

Chemicals injurious to life can kill only if they reach the sites of action in the target organism. In large scale fumigations, many factors can combine to divert the toxicant from the pest to be controlled. Much of the original dose applied can be absorbed by the commodity being treated, lowering the gas concentration in free spaces. A high power of penetration is a major requirement for an efficient fumigant. It ensures a rapid arrival at the site of action, and often enables a rapid evolution of gas during airing. The nature and state of the commodity influences penetration rate. In large food bulks, penetration may be slow or irregular due to such factors as sorption, variable temperatures within the bulk (perhaps due to 'hot spots' associated with an infestation), local air movements and draughts, leakage from the fumigation area, and chemical

reaction with the commodity itself or with certain substances in the fabric of the building. The provision of an air circulatory system greatly improves gas penetration and distribution within a food bulk (Turtle, 1950), and certain application methods, such as the distribution of aluminium phosphide tablets within bulk grain for phosphine fumigations, can effectively reduce the distances to be penetrated. In general, penetration is enhanced by increasing the length of the exposure period, as long as sufficient gas is available in free spaces. Long exposures also prove advantageous when pests continue to develop during fumigation out of short resistant phases in the life cycle into susceptible ones (Reynolds et al., 1967).

Fumigants enter insects by passing through the cuticle, but passage is assisted if the spiracles are open and the insect is actively respiring. Factors increasing the rates of metabolism and respiration will generally increase susceptibility to fumigation (Cotton, 1932). Enormous differences exist in the tolerance levels of different species to a particular fumigant. Within a single species, different stages commonly differ widely in susceptibility, greatest tolerance usually being shown by eggs or pupae (Howe and Hole, 1966; Howe, 1973). Different stocks of the same species may differ in susceptibility (Barker, 1967; 1969; 1970; Lindgren and Vincent, 1965) and differences in tolerance have been noted between the two sexes in both pupae and adults (Loschiavo, 1960; Girish, 1966).

The appearance of true resistance to fumigants among field populations of stored product insects has not yet become a problem in economic entomology. A resistant population may be regarded as one with an added ability to withstand a toxicant, acquired by breeding from survivors of exposures to the toxicant which kill

substantial numbers of the colony (Hoskins and Gordon, 1956). It is essentially a preadaptive phenomenon in the sense that the gene or genes which confer resistance through control of some biochemical process are present in the population before the introduction of a selection pressure. After Melander (1914) first raised the question of the ability of insects to develop resistance, Quayle (1916; 1922; 1938) found that certain scale insects infesting Californian citrus orchards had developed resistance to hydrocyanic acid gas in tent fumigations. The level of resistance was low, but the increased dose required for satisfactory control was too high for the safety of the trees.

In most circumstances fumigants do not favour the development of resistance because they are not persistant and are usually poisons with many different modes of action. The resistance of scale insects to hydrogen cyanide remains the only well documented case of insect resistance to fumigation in practice, although strains have been specially selected in the laboratory. Monro et al. (1961) increased tolerance to methyl bromide 5.5 times in S. granarius adults after 27 selections. After 13 more selections, tolerance to methyl bromide was increased from 5.5 times to 7 times (Monro, 1964). From these results, Monro concluded that resistance to methyl bromide was unlikely to appear rapidly in the field, and that the form of resistance obtained was best described as vigour tolerance because it was non-specific and of a low order. More recently Ellis (1972), working with the same strain after 67 selections, found a difference in the rates of uptake and metabolism of radiolabelled ethylene dibromide, and expressed a need to define vigour tolerance as a multifactorial, non-specific, low order difference in susceptibility.

In his work on selection with methyl bromide, Monro (1961) tested for cross tolerance to other fumigants. Under selection pressure by methyl bromide, the tolerance induced to phosphine in one strain was five times greater in 5-hour exposures than for methyl bromide itself.

S. granarius adults have also been subjected to selection with phosphine (Monro et al., 1972). After 28 selections, a few adults were able to survive 78-hour exposures to a concentration falling from about 13mg/l to 2-3mg/l at 25°C, whereas unselected insects succumbed after 18 hours. Cross tolerance to other fumigants was low. The possibility of cross resistance to fumigants arising as a result of selection pressure by chemicals of a more persistent nature has been little investigated. Bhatia and Bansode (1971) reported no significant cross resistance to a range of fumigants in a DDT-resistant strain of T. castaneum, but nevertheless resistance to fumigants in practical situations may be more likely to occur where strong selection by other toxicants has occurred.

Different fumigants act in a variety of ways. Some such as phosphine require the presence of oxygen (Bond et al., 1967; 1969), while others are most toxic in anoxic conditions. The absence of oxygen may prove advantageous against some species but not with others because of the wide variability in response to anoxia (Bond and Monro, 1967). However, very low oxygen concentrations kill insects in long exposures probably because the spiracles open in response to a build up of lactic acid and other metabolites of anaerobic respiration, giving rise to desiccation (Wigglesworth, 1935; Harein and Press, 1968; Jay et al., 1971). In storage bins where an effective seal can be maintained, infestations become self limiting due to the depletion of oxygen (Bailey, 1955; 1965).

With fumigants not requiring oxygen in order to be toxic, low pressure generally increases the level of kill, but in some cases a marked lowering of toxicity may occur at certain pressures, the exact level depending on the species tested (Monro, 1959). This effect is linked with vapour pressure, because in general fumigants with low boiling points and high vapour pressures are involved (Monro et al., 1966). Lowered pressure reduces the availability of oxygen with the result that carbon dioxide becomes more toxic to insects (Harein and Press, 1968; Marzke et al., 1970). Carbon dioxide has often been used with fumigants, notably with ethylene oxide under vacuum, usually in the dual capacity of fire depressant and secondary toxicant. It can also assist fumigant penetration and distribution (Peters and Ganter, 1935a; Calderon and Carmi, 1971; 1973). Also it stimulates insect spiracles to remain open (Hazelhoff, 1927; Mellanby, 1934; Wigglesworth, 1935), which facilitates the entry of fumigants and increases water loss. Adults of the bug Rhodnius prolixus Stål die within 3 days in a dry atmosphere containing 5-10% carbon dioxide (Wigglesworth and Gillett, 1936; Wigglesworth, 1972). In general, much higher levels of carbon dioxide are required for high mortality than can be administered in large scale fumigations.

Relative humidity (RH) at atmospheric pressure does not greatly affect the results of fumigations, unless insects are exposed to very high or very low levels for some time before the introduction of gas. Under reduced pressure, high RH generally increases susceptibility (Monro, 1959), probably because spiracular control is loosened (Miller, 1964). In very low oxygen or high carbon dioxide concentrations, high RH increases survival in the absence of fumigants (Pearman and Jay, 1970; Jay et al., 1971), as reduced saturation deficits retard rate of water loss through the spiracles. High RH can also increase the sorptive capacity of the treated commodity.

Perhaps the most important factor affecting fumigation results is temperature. Metabolic and developmental rates of all insects are temperature dependent. Increased temperature within the developmental range of a species generally reduces both the concentration and exposure period needed for a particular level of kill (Cotton, 1932; Peters and Ganter, 1935; 1935a; Sun, 1947; Muthu and Pingale, 1955; Kenaga, 1957; Lindgren and Vincent, 1960; Estes, 1965). However, temperatures below the lower limit for development may increase susceptibility by combining the effects of cold and fumigation (Peters and Ganter, 1935). Temperature also influences the extent and rate of sorption. Physical sorption is reduced at high temperatures, and as total sorption is usually lower, more fumigant is available for pest control (Lindgren and Vincent, 1960). On the other hand, chemical sorption increases with temperature rises (Heuser, 1961; Lindgren et al., 1962), and in certain cases an overall reduction in the amount of free fumigant can occur. Another effect of high temperature is the increase in rates of diffusion and penetration, which by enhancing fumigant efficiency again increases the level and rate of kill.

Random measurement of temperature within a bulk of stored grain is not a good enough guide to the temperature at which pests are actually developing. Insects produce metabolic heat which causes 'hot spots', areas of greatly increased temperature within the bulk (Back and Cotton, 1924). The amount of heating is proportional to the number of actively feeding larvae present (Flanders, 1933), and is limited by the heat tolerance of the insects themselves rather than by any differential between internal and external temperatures (Howe, 1962). Insect populations are usually distributed around the edge of expanding areas of hot grain because of the continual outward movement of individuals from areas of high population density and temperature.

Heating can be a problem in cultures prepared for fumigation experiments at controlled temperatures. Temperature rises of over 5°C have been reported in cultures of E. kühniella set up in 15cm square cardboard boxes with a 1-15cm layer of food (Wishart, 1942). Temperatures over 27°C are reported to cause a retardation of spermatogenesis in males of this species (Norris, 1933; Raichoudhury, 1936) and high-density cultures reared at 25°C therefore may produce infertility in males that emerge. There is also increased competition for food at high population densities, and populations tend to become self limiting. Flanders (1933) found that cultures of the Angoumois grain moth Sitotroga cerealella (Olivier) set up with 2 newly-hatched larvae per grain of ordinary white corn, produced more adults than cultures set up with 4 larvae per grain. Although high population pressure may lead to reduced biological efficiency in terms of fecundity and percentage survival from egg to adult, it can increase the tolerance to control measures firstly because of competition for food leading to starving and a reduction of metabolic rate (Sun, 1947), and secondly because resting stages appear in response to crowding or the contamination of food with faecal material. Two storage pests where diapause may be induced by such conditions are P. interpunctella (Tsuji, 1959) and T. granarium (Burges, 1956a).

It is well recognised that rearing food can influence susceptibility of insects to poisons (Sun, 1947; Lal and Singh, 1966; Lal and Attri, 1967; Urs and Mookherjee, 1967). Insects reared on foods of high nutritional value, or on the preferred food of the species, are generally more susceptible than those reared on other foods (Punj, 1970; 1971; Murthy and Srivastava, 1971). Different stages of the same insect, however, may differ in their order of susceptibility on a range of foods (Baskaran and Mookherjee, 1971). No results have yet appeared on the effect of rearing food on the susceptibility of

insects in diapause, but unless nutrition limits healthy development, its effect should not be so marked as in stages with an active metabolism. However, rearing food may be instrumental in producing diapause, as reported for E. elutella by Waloff (1948), and for P. interpunctella by Williams (1964).

In conclusion, some methods of interpreting toxicity results must be mentioned. The toxicity of a fumigant is usefully evaluated only as the minimum dosage required for a total kill, as all practical fumigations are aimed at complete control. The statistical uncertainty of the 100% mortality level has caused a marginal level, 99.9% kill, to be adopted instead for comparative purposes. In practical situations this dosage has appeared to give adequate results (Brown and Reynolds, 1965). The LD 99.9 is commonly obtained by extrapolation from a probit mortality against log CT product regression line. The probit transformation for dose-mortality responses is described by Bliss (1934a, 1934b, 1935). Because the transformation does not give linear results for very high mortalities, extrapolation of regression lines for the LD 99.9 yields inaccurate results. In tests on the immature stages of S. granarius, Howe and Hole (1967) showed that the slope of the regression line can change long before the 99.9% mortality level. Extrapolated values for the LD 99.9 consistently over-estimated the true result, although for very homogenous populations, the discrepancy was not so apparent.

Another factor affecting results is the definition of death in test insects, and the timing of the mortality assessment. Death after fumigation may be very delayed, and assessments conducted after a week or longer yield higher mortalities than those conducted soon after airing (Peters and Ganter, 1935a; Sun, 1947; Phillips et al., 1959; Sinha et al., 1967). Hence doses which fail to kill insects rapidly may still achieve adequate control. It is necessary to assess mortality long after fumigation in field trials so that the more tolerant stages of the life cycle, which are often difficult to isolate,

have a chance to progress to the adult stage. For a complete evaluation of fumigation efficiency, it is essential to examine the fertility of survivors. Loschiavo (1960), working with the flour beetle Tribolium confusum duVal, observed a reduced fecundity and fertility of adult survivors of fumigations with ethylene dibromide. Howe and Hole (1967) observed low fertility of survivors from tests with methyl bromide on larvae and pupae of S. granarius. Winks (1971) reported that doses of phosphine giving some kill of T. castaneum pupae lowered fertility in the subsequent adults, and when adults were fumigated with doses giving a high level of kill, the infertility of survivors was total. For practical purposes it is of little consequence how long individuals take to die if they have been sterilized, even if feeding continues for a while. The disadvantage of toxicants giving a delayed action is the disquieting effect of observing insects moving soon after a fumigation, which may give rise to an erroneous assumption about the effectiveness of the control procedure.

DIAPAUSE IN RELATION TO MOTHS INFESTING STORED PRODUCTS

Diapause has been developed by many arthropods that have become established in temperate zones. It has survival value as an overwintering mechanism, and is closely linked to the natural environment, typically being controlled by seasonal daylength, temperature, and nutrition. In the storage environment, changes in microclimate are greatly buffered, and changes tend to be sudden when hot spots are produced by an infestation, or when some of the food bulk is removed or disturbed. Here, diapause may have biological significance as a synchronising mechanism for emergence in favourable conditions, rather than as an overwintering device (Howe, 1962a).

Diapause is usually characterised by inactivity and a halt in growth, coupled with a lowered metabolic rate and an increased tolerance to adverse conditions such as heat, cold, drought, or stresses imposed by control procedures. It is known to occur in all stages of development, depending on the species, and in some cases it occurs more than once in the life cycle (Howe, 1962a). The term diapause originates from embryological studies on grasshopper eggs, where it was used to describe the halt in the movement of the embryo between anatrepsis and catatrepsis. Henneguy (1904) applied the term to any condition of arrested growth whether in the developing or the adult insect. Shelford (1929) suggested restricting the term to instances where an arrest occurred for no apparent reason, and not simply as a result of unfavourable conditions. Thus diapause was distinguished from the more general state of quiescence, in which development was stopped or started in immediate response to environmental changes. Shelford's definition is no longer accurate as, in an increasing number of cases, the stimulus for diapause can be traced back to the environment, and the condition is better considered as one in which development does not proceed, although the environment is favourable for growth (Lees, 1955). Recently, there have been attempts to classify diapause according to how many environmental factors play a part in its control. Agreement on this idea, however, has not yet been reached (Thiele, 1973).

Diapause is a physiological condition which, once produced, requires the completion of a number of processes before it is terminated. Andrewartha and Birch (1954) recognised that physiological change was necessary during diapause before resumption of active morphogenesis, and introduced the term "diapause development". Certain stimuli, such as prolonged exposure to cold, often help to bring about the termination of diapause, and may be said to favour diapause development.

Based on whether or not the environment plays a part in its formation, diapause is divided into two types. Diapause is defined as obligatory where the arrest occurs in every generation automatically, such as in northern races of many Lepidoptera, including the Silk moth Bombyx mori (L). Such races are restricted to one generation each year and are said to be univoltine. In other insects, diapause is facultative and environmental conditions decide whether or not an arrest appears in a particular generation. Such insects can be bivoltine or multivoltine as two or more generations can occur each year. Most species fit into this definition, although the range of environmental conditions favouring development without diapause may be limited. Many insects with a facultative diapause can be rendered univoltine by their environment. Many moths inhabiting northern Europe complete only one generation a year, including Ephestia elutella in Scotland, where slow development through the cool summer does not produce the sensitive stage until autumn, when conditions result in diapause. E. elutella enters diapause at 25°C when the daylength experienced by newly - moulted last instar larvae is 14 hours or less (Strümpel, 1964), or when temperature is lowered during development (Waloff, 1949). It is known as a long-day species, as long days favour active development.

The sensitive phase for diapause induction is usually quite separate from the diapause phase itself. In E. elutella diapause follows completion of feeding in the last larval instar while sensitivity occurs at the last larval moult (Strümpel, 1964). In the Large white butterfly Pieris brassicae (L), the conditions experienced by larvae between the third moult and the early days of the fifth instar initiate the pupal diapause (Claret, 1966; 1972). Races of Bombyx mori possessing a facultative diapause exhibit a short-day response, as diapause is induced when the daylength exceeds 14 hours (Kogure, 1933). Here, the sensitive phase occurs in older eggs and young larvae, but diapause does not occur until early in the development of the next generation of eggs laid. The

unusually wide separation of sensitive phase and diapause perhaps explains why long days are required to ensure that a developmental arrest occurs at the right time for overwintering.

The photoperiodic range at a given temperature over which diapause increases from 0 to 100% in a particular population is quite narrow. Strümpel (1964) found that whereas in a 15-hour photoperiod no larvae of E. elutella entered diapause at 25°C, virtually all did so in a 13-hour photoperiod. The term critical photoperiod is used to denote that photoperiod at which the rate of change in the incidence of diapause becomes most rapid (Lees, 1955). In this country, the critical photoperiod often lies between 14 and 15 hours, but elsewhere, different races of various species may have critical photoperiods varying from 14 to 19 hours, increasing with the latitude at which the insects were taken (Danilevskii, 1961; Maslennikova and Mustafayeva, 1971). With very long daylengths of 22 hours or more, in continual light, in total darkness, or with very short daylengths, results are often irregular, with some of the population entering diapause and others not. It is therefore apparent that in many species, both the light and dark phases need to be of a minimum length for the photoperiodic message to be clearly interpreted.

Critical photoperiod is influenced by the temperature experienced by the light sensitive stages. Firstly, a fall in temperature of 5°C generally lengthens the critical photoperiod by 1-1½ hours, but in Pieris brassicae, temperatures from 12 to 26°C give similar results (Danilevskii, 1961). Another species similar to P. brassicae in this way is the European corn borer Pyrausta nubilalis (Hübner) (Beck and Hanec, 1960). Secondly, a minimum temperature is required for the reception of light stimuli, but the photoperiodic response is largely determined by the temperature during the

dark phase (Lees, 1955; Danilevskii, 1961), high temperatures inhibiting diapause.

Recently, it has been found that at different times of year, the relation between temperature and the photoperiodic response can alter (Geyspits et al., 1972). In the Pine moth Dendrolimus pini L., the critical photoperiod for larvae obtained between March and June is independent of temperature within the range of 15-25°C, but from August onwards, there is marked variation in the critical photoperiod obtained at various temperatures.

In most species, diapause is induced by a minimum number of photoperiods shorter than the critical photoperiod. Danilevskii (1961) quotes values between 5 and 20 photoperiods for various species. The number required does not seem to be affected by temperature (Tyshchenko et al., 1972; Goryshin and Tyshchenko, 1973).

In some species, diapause is controlled by progressive changes in photoperiod, rather than by a particular number of short daylengths. This situation was first observed in the dragonfly Anax imperator Leach (Corbet, 1954). Recently, diapause in the Green lacewing Chrysopea carnea Stephens, which can be induced by short photoperiods, has also been shown to be affected by changing daylength (Tauber and Tauber, 1970). Diapause was induced by progressively shortening photoperiods even when these were longer than the critical photoperiod. Furthermore, photoperiods which were progressively lengthening, but were still shorter than the critical photoperiod, prevented the induction of diapause, and terminated diapause that had previously been induced.

Light intensity does not influence photoperiodic reaction in arthropods,

provided that a certain threshold is exceeded. Minimum intensities of 0.025 to 10 lux have been reported for various species (de Wilde, 1962), but information is lacking on the effect of temperature on threshold levels. All species tested have a higher sensitivity to light towards the blue end of the spectrum. In P. brassicae induction or inhibition of diapause is controlled by wavelengths between 420 and 520 mμ (Claret, 1972a).

Physiologically, hormonal control is only the last step in a series of processes leading up to diapause (Andrewartha and Birch, 1954). The organs and hormone-producing systems involved depend on the stage at which the insect is sensitive to stimuli, and the stage during which the arrest occurs, but the cerebral ganglion, the brain of the insect, always plays a major rôle. It has been shown to be the site of photoreception in P. brassicae, and in the silkworm Antheraea pernyi (Guer) (Claret, 1966; Norris, et al., 1969). In the Silkmoth Bombyx mori(L), diapause occurs in the eggs laid when a secretion of certain cells in the suboesophageal ganglion of the female is released (Yamashita and Hasegawa, 1966). In females destined to lay eggs that develop without diapause, the brain, acting through the oesophageal connectives, completely inhibits the release of the neurosecretory material into the blood.

Diapause occurring in the larval or pupal stages is characterised by a lack of the growth inducing hormones, notably the moulting hormone, ecdysone, produced by the prothoracic glands, or by a high titre of juvenile hormone (Chippendale and Yin, 1973). In diapausing pupae of the Giant silkworm Hyalophora cecropia (L), the prothoracic glands are inactive due to failure of the neurosecretory cells of the brain to produce an activating hormone (Williams 1946). Chilling the brain promotes release of the activating factor, and normal development is resumed. Histological studies of the larval diapause induced in Plodia interpunctella by rearing below 20°C, revealed that neurosecretory

cells located in the pars intercerebralis of the brain, which were actively secreting in non-diapausing larvae, were not functioning in diapausing larvae (Waku, 1960). Examination of the prothoracic gland cells revealed no activity until the cells of the pars intercerebralis resumed their secretory function at the end of diapause. It was also observed that during diapause the corpora allata showed enhanced activity. In many insects, certain hormones of the corpora allata stimulate metabolism and gonad maturation. These hormones are, however, absent in Lepidoptera. Waku (1960) suggested that the activity in P. interpunctella could indicate the production of juvenile hormone, which in high concentration would prolong the larval stage. Strümpel (1964) in examining the histology of endocrine organs in diapausing larvae of Ephestia elutella, found no comparable enhancement of corpora allata activity, but again found that the brain-prothoracic gland system was inactive.

Where diapause occurs in the adult stage, ovarian development is retarded by failure of the brain to activate the corpora allata.

Of the species regarded as pests of stored food products, about two dozen are thought to possess a diapause, including at least 8 species of Lepidoptera, all of which enter diapause as larvae. Diapause has been induced by lowering the rearing temperature in P. interpunctella (Tsuji, 1958; Tzanakakis 1959; Waku, 1960), the Carob moth Ephestia calidella Guenée (Franquiera, 1955), E. figulilella (Donahoe et al., 1949; Sardesai, 1968), E. elutella (Waloff, 1948) and the Stored nut moth Paralipsa gularis (Zeller) (Smith, 1965). Temperature is also a controlling factor in the diapause of the Brown house moth Hofmannophila pseudospretella (Stainton), as diapause can be avoided by rearing at temperatures gradually increasing from about 8°C (Woodroffe, 1951). Diapause in Sitotroga cerealella, and in the European grain moth Nemapogon granella (L), is most likely temperature dependent (Howe, 1962a).

Photoperiod has been found to induce diapause in P. interpunctella (Danilevskii, 1956; 1961; Tsuji, 1963; Sardesai, 1968), E. elutella (Strümpel, 1964), E. figulilella and E. calidella (Cox, 1973). Nutrition has been shown to be important for diapause in P. interpunctella (Williams, 1964; Tsuji, 1966), E. elutella (Waloff, 1948), and E. calidella (Cox, 1973). Little work has been done on the diapause of storage moths in relation to nutrition and daylength, however, and these factors may prove of importance in other species. Population density is another little investigated factor in relation to diapause. It is of importance in P. interpunctella (Tsuji, 1959), and probably in Paralipsa gularis where disturbance of cocoons causesdelay in development (Joseph and Oomen, 1960). No diapause has yet been demonstrated in Ephestia kuehniella, E. cautella, the Rice moth Corcyra cephalonica Stainton, the Meal moth Pyralis farinalis (L) or the White-shouldered house moth Endrosis sarcitrella (L).

Ephestia elutella was the first pest of stored products in which a diapause was noted (Richards and Waloff, 1946). Observations were made on a London warehouse population of this species feeding on Manitoba wheat. Each year, emergence of adults commenced during May and reached a peak in late June or early July. Very few moths were evident by the end of August when fully grown larvae were observed wandering from the grain. By mid-September, the number of wandering larvae reached a peak, and about this time a small second period of adult emergence commenced. Most larvae, after wandering for about 2 days, spun cocoons in the fabric of the building, particularly in crevices in the ceiling, and few were active after the end of October. Winter was passed in the larval stage and development proceeded in the spring when permitted by temperature. Further observations (Waloff and Richards, 1946) showed that early larval instars were spent actually within grain embryos, but, after the second instar, the larva left the grain and carried on feeding

from the outside. About 48 grain embryos were consumed to complete development. Most larvae were found in the top 30 cm of grain, with the highest number in the top 5 or 10 cm.

Diapause can be induced in E. elutella by rearing on foods containing a high proportion of starch, such as white flour or soft wheat. On these foods the diapause-initiating factor may be lack of glucose, linoleic acid or vitamins of the B complex, rather than starch itself (Waloff, 1948). Normally, diapause is induced by lowered temperature or shortening daylength in the autumn. Waloff (1948; 1949) described a stock isolated by Basden which entered diapause at 21 or 17°C, but not at 25°C.

Strümpel (1964) found that the percentage of insects entering diapause at 25°C changed from 0 to 100% as daylength was reduced from 15 to 13 hours. The sensitive stage was found to be the first few days of the last larval instar. At this time larvae would presumably still be feeding under the surface of the food bulk where change in light density is small. Of larvae exposed to continual darkness at 20°C, all entered diapause, while 96% entered diapause in darkness at 25°C. Only by increasing the temperature to 30°C was the occurrence of diapause reduced to a low level. Thus it is likely that in many situations a proportion of every generation of E. elutella developing in food bulks will enter diapause, regardless of the season. If photoperiodic stimuli are important in development, the sensitivity of E. elutella to light must be high.

Diapausing larvae are heavier than fully grown non-diapausing larvae, and have less free water. They show high cold tolerance and are able to survive 40 days at -2°C (Waloff, 1949). The duration of diapause in English unheated stores is about 8 months, although the latter part of this period may simply be quiescence because post-diapause development is prevented only by low

temperatures. Waloff (1949) found that the range of times required for pupation was reduced from 20-240 days in samples moved from a warehouse to the laboratory at 25°C in November, to 10-60 days for January samples and 10-40 days for February samples. The most rapid change occurred between early and mid December when times before pupation in samples moved to 25°C changed from 10-180 days to 20-70 days. In laboratory experiments, a 2-week exposure to -2°C slightly advanced pupation at 25°C when administered to samples removed from the warehouse before mid December, and delayed pupation in samples moved after mid December, indicating some stimulation of diapause development, and interference with early post-diapause development.

In samples held at constant temperatures, diapause may last a year or more (Waloff, 1948). Pupation occurred after a reduction in larval weight of about 35%. Strümpel (1964) observed weight losses of 40-60% before pupation and suggested weight loss as the diapause terminating agent in darkness or short daylength. He found light important in the termination as well as in the induction of diapause, and a 16-hour daylength stimulated pupation within three months, especially when larvae had previously been held in diapause for about a month. Neither weight-loss nor daylength, however, can explain the change in the times required for pupation in samples moved from the warehouse to 25°C in early December, and those moved in mid December.

Plodia interpunctella prefers high temperatures for development, and in this country is near the northern limit of its range. It was first recognised to enter diapause as a fully grown larva by Zacher (1950). However, larvae have long been recognised to overwinter in this country. Potter (1935) described a population hibernating in a London warehouse in association with E. elutella. The duration of diapause at fixed temperatures is less than in E. elutella, seldom approaching 300 days at 20°C (Tzanakakis, 1959; Tsuji, 1963; Williams, 1964). Photoperiod was first related to diapause in this species in 1956

(Danilevskii, 1961). Tsuji (1958) found that the onset of diapause could be controlled by temperature. Larvae reared at 30°C and dropped to 20°C during the egg stage, or any of the first three larval instars, entered diapause. Few or no larvae entered diapause when temperature was lowered during the 4th or 5th larval instars. When over 100 larvae were reared on 30 g of rice bran at 30°C, some entered diapause, indicating that high population density was another factor inducing diapause (Tsuji, 1959). By continued selection, Tsuji (1960) isolated stocks that would either develop without diapause at 20°C, or give high proportions of diapausing insects in response to high population density at 30°C.

Tzanakakis (1959) confirmed the presence of a temperature dependent diapause in P. interpunctella. He found that a temperature drop in early development from 25°C or above, to 20°C or below, induced diapause, and that more insects entered diapause in the absence of light. Temperature drops from 30°C to 25°C did not produce diapause. Two strains were tested, one with a markedly greater tendency to enter diapause. The duration was not shortened by exposures of up to 1 hour at -18°C. Similarly, no effect was observed after exposing to -5°C for several days, or to 10°C for 14 weeks. Diapause breaking was however hastened by short exposures to 50°C, by repeated destruction of the cocoon, or by wounding of larvae with a red-hot needle.

Tsuji (1963), continuing his studies on P. interpunctella, found that no diapause occurred at 20°C in a 16-hour photoperiod, while all insects entered diapause in darkness or under 8-hour photoperiods. The principal light sensitive phase occurred in the 3rd and 4th larval instars. The previously established 'density-dependent' diapause was found to be stimulated by young larvae being crowded together, rather than by food shortage or faecal contamination. Less density-dependent diapause was induced in a 16-hour daylength at 30°C than in darkness. All diapausing larvae were

characterised by being heavier, and by having more fat and less free water than fully grown non-diapausing larvae. In contrast with the work of Tzanakakis (1959) at 25°C, diapause termination at 20°C was enhanced by chilling at 8 -10°C for 30 or 60 days. Nutrition was demonstrated to play a rôle in terminating diapause. Placing diapausing larvae on filter papers impregnated with rice bran extracts stimulated pupation. Later work (Tsuji, 1966) showed that the neutral fraction of the n-hexane extract was the most potent in this respect. Williams (1964) showed that nutrition also played a part in the induction of diapause in P. interpunctella. A sizeable proportion of larvae reared on maize entered diapause, while larvae reared in parallel experiments on sultanas did not.

Sardesai (1968) confirmed that diapause in P. interpunctella was encouraged by lowered temperature, high population density, darkness, or short photoperiod. The mean value for the duration of diapause at 20°C was 22 weeks, and diapause termination was preceded by an increase in respiration. Diapausing larvae were found to be slightly more tolerant to methyl bromide than non-diapausing larvae, but, surprisingly, no difference in tolerance was observed with hydrogen cyanide.

There are many similarities in the initiation and maintenance of diapause in E. elutella and P. interpunctella, although the emphasis of various factors may be different. Both species have a diapause that is presumably suited to the storage environment, but which is still comparable with the diapause of species which live in natural habitats.

METHODS

1. HISTORY OF STOCKS

A. Laboratory Stocks of Ephestia spp. and Plodia interpunctella

The four laboratory stocks have been bred at Slough for many years at 25°C. Until late 1968, E. elutella was reared in darkness, while the other species were reared in continual light. From 1968, all were reared under a 16-hour daylength at 25°C.

B. Field Stocks

The four field stocks were maintained at the laboratory in an outbuilding where they experienced daily and seasonal changes in humidity, temperature and photoperiod. Ephestia elutella was collected from the Central Granary at Millwall, London, in July 1969, and Ephestia kuehniella from a mill near Southampton in May 1970. Plodia interpunctella obtained from an infested parcel of dandelion roots which arrived via England and Belgium, was received in January 1971 from the Department of Agriculture for Scotland. At the laboratory, the stock was bred at 25°C for three generations before transfer to the outbuilding. Four adults of Ephestia cautella, 3 males and one female, were obtained from a chocolate factory at Slough in May 1971, and were moved to the outbuilding after two generations at 25°C.

C. Pieris brassicae and Bombyx mori

Eggs of P. brassicae were supplied by the Insect Virus Group, Glasshouse Crops Research Institute based at Cambridge.

Eggs and cocoons containing pupae of the univoltine race of B.mori were supplied by the Insect Pathology Research Institute, Department of Fisheries and Forestry, Canada.

2. INSECT REARING

A. Preparation of Food

The principal food used for rearing both laboratory and field stocks of Pyralid species was wheatfeed, which consists of fine bran with a little endosperm. As a precaution against spores of pathogenic protozoa and of Bacillus thuringiensis, a disease organism of many moth species, wheatfeed was sterilised in covered stainless steel trays for at least 2 hours at over 105°C.

To replace vitamins and sterols that may have been destroyed by the heat, dried, debittered brewer's yeast was added to the oven-treated wheatfeed. Glycerol was included as an additional nutrient and also as a humidity stabilizer and mould depressant. The rearing food comprised 10 parts by weight (p.b.w.) wheatfeed, 1 p.b.w. dried yeast, and 2 p.b.w. glycerol, thoroughly mixed.

B. Culturing of Pyralid Laboratory Stocks

Cultures were set up fortnightly by transferring 50-100 adults anaesthetised with carbon dioxide to 325 g wheatfeed mix in a 3-1 culture jar (fig. 1). A drinking fountain was provided in each culture because adults with access to water were more consistent in producing viable eggs. A disc of glasspaper filter material with a pore size fine enough to exclude bacterial spores, was secured to the rim of each culture jar with molten wax. To prevent the escape of larvae chewing through the spore-proof cover, a piece of cotton cambric was attached across the neck of each culture jar with an elastic band. Cultures were kept on stands on oil filled trays in a room maintained at 25 ± 0.2°C and 70 ± 3% RH, and provided with a 16-hour daylength by a "warm white" or "daylight" 40-Watt fluorescent tube. All glassware used for culturing was sterilized in a 5% solution of a sodium hypochlorite preparation (Chloros) to which potassium permanganate was added as an indicator.

C. Culturing of Pyralid Field Stocks

Field stocks were reared in glass tanks measuring 28 by 22 cm, and standing 41 cm high (fig. 2). Each culture contained 390 g of wheatfeed mix spread into a layer 2 or 3 cm deep. Before use in experiments, the first cultures of each stock were checked for the presence of disease by allowing a dence second generation to develop.

New cultures were set up with 50-100 adults, which were sucked from the parent tank into a glass tube. Tanks were covered by cotton cambric secured with adhesive tape. No drinking water was provided. Tanks were kept in a windowed outbuilding in which variations of temperature and humidity were not controlled. During winter, some heating was provided for cultures of E. cautella by placing a hot plate on the floor under the rack containing cultures. To supply insects for tests, extra cultures were set up in 3-1 jars at 25°C, as described for laboratory stocks. Not more than two generations were allowed to pass at 25°C before starting a new line from tank cultures.

D. Pieris brassicae

P. brassicae was reared from the egg stage on cut Brussels sprout plants in tanks similar to those used for the field stocks of Pyralid moths. No more than 100 larvae were reared in each tank. Food was added as required, and faecal pellets and stale leaves were removed from cultures to minimise risk of disease.

Following the data of David and Gardiner (1962), diapausing pupae were obtained from larvae reared under an 8-hour daylength at 20°C, and non-diapausing pupae from larvae under a 16-hour daylength at 25°C. After the emergence of adults (fig. 3) fresh leaves were added to encourage egg laying.

Fig. 1. Pyralid moth laboratory stock culture: 3-1 jar showing drinking fountain, emerging moths, and in the upper layer of food, larval feeding tubes and silken cocoons.

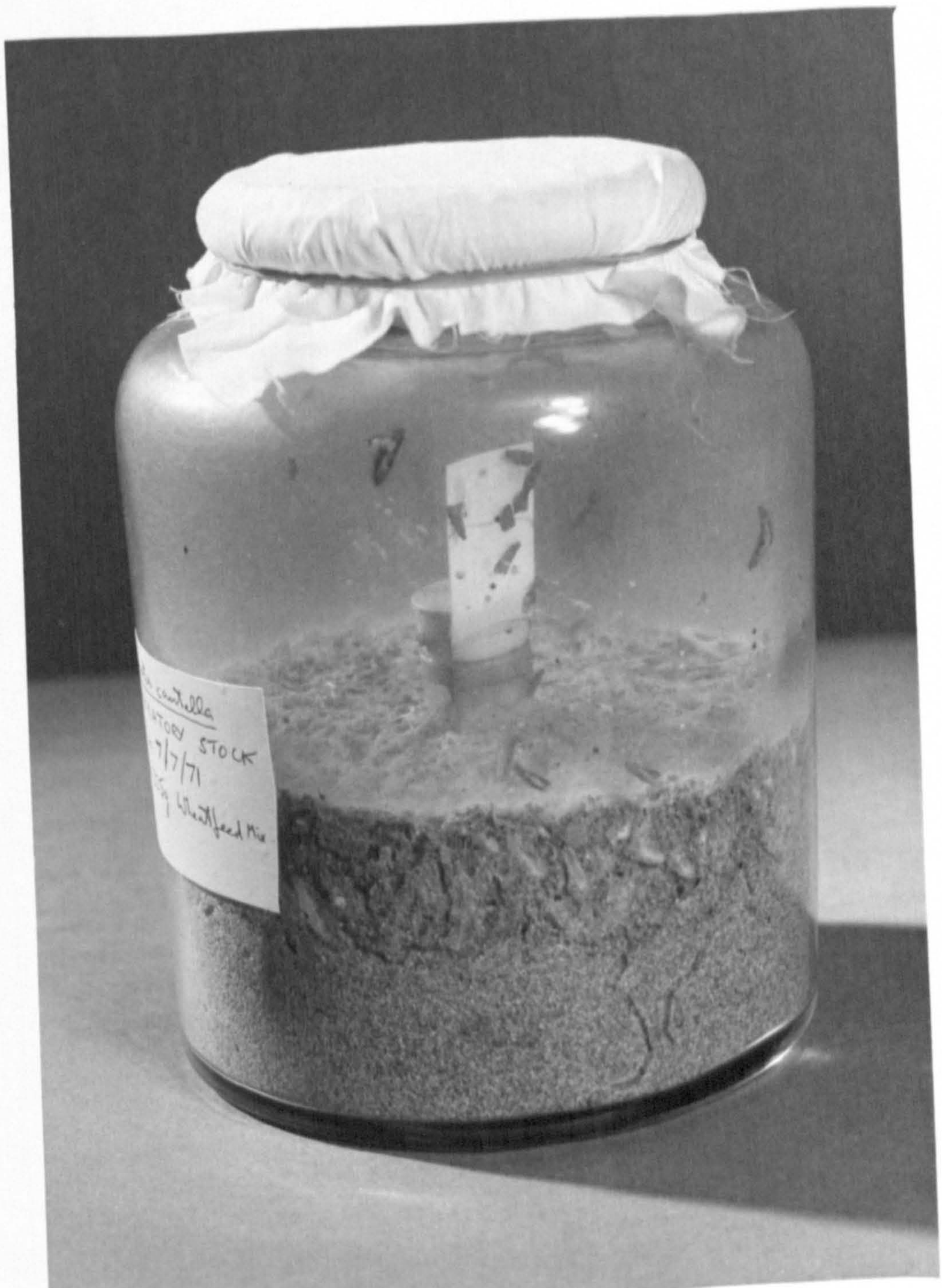


Fig. 2. Glass tank used for rearing field stocks of Pyralid moths, showing shallow food layer and some newly-emerged moths clinging to silk laid down by wandering larvae on interior surfaces.

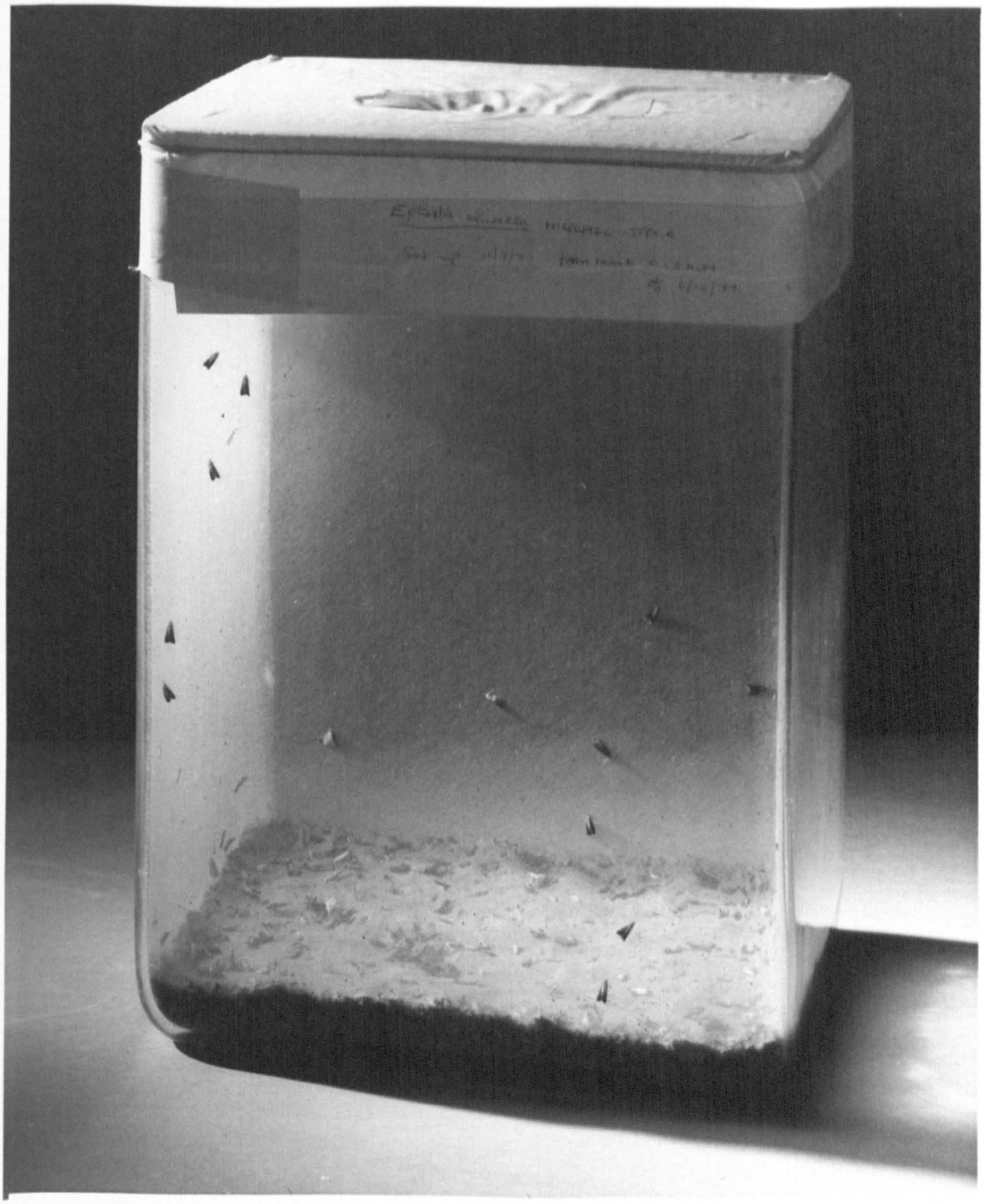


Fig. 3. Tank containing pupae, pupal skins, and adults of Pieris brassicae

E. Bombyx mori

To obtain young diapausing or non-diapausing eggs, groups of 6 cocoons were confined at 25°C, 70% RH, on waxed filter paper under an inverted plastic funnel 15 cm in diameter. After emergence, cocoons were removed and adults were sexed. The sex ratio was adjusted so that, as far as was possible, three males and three females were present under each funnel. Filter papers were replaced daily after laying commenced. To avoid diapause, eggs less than 24 hours old were suspended in 6 N hydrochloric acid for 5-6 minutes at 30°C, washed in water, dried on filter papers, and then returned to 25°C (Nayar and Fraenkel, 1963). The method proved 100% successful in preventing diapause, and caused little mortality.

3. BIOLOGICAL EXPERIMENTS

A. Temperature and Development

i) Isolating eggs

Most experiments were started with eggs which were isolated in the following manner. A piece of damp cotton wool was taped inside a 10 cm crystallizing dish. After anaesthetising with CO_2, 40- 60 moths were placed in a hemispherical nylon sieve 11.5 cm in diameter. The 10 cm dish with drinking pad attached, was placed over the sieve to confine the insects. The unit was then placed over another crystallizing dish 12.5 cm in diameter containing a black filter paper (fig. 4) so that eggs laid were clearly visible.

ii) Developmental limits

For experiments on developmental limits, 1 g of wheatfeed mix was compressed into a layer about 7 mm thick in 5 x 2.5 cm glass tubes. These were laid sideways against a dark background on a tray (fig. 5). An egg newly-laid at 25°C was

placed in each tube using a fine bruch about 2 cm from the food. The tubes were checked daily for hatch, and, after an interval, for adult emergence. Some adults from each rearing condition were paired to check fertility, which was assessed on the basis of adults developing from eggs laid.

For experiments at low humidity, trays containing tubes were placed in glass tanks containing a dish of saturated potassium acetate solution (fig. 6), which was expected to give 21 - 22% RH (Winston and Bates, 1960). Tanks were sealed by a thick piece of polythene sheeting secured with Canada balsam. A small dish of 10% potassium hydroxide solution was included to absorb carbon dioxide. The atmosphere in each tank was changed three times a week with air dried by passing through a column of calcium chloride. Cobalt thiocyanate (Co (CNS)2) papers were used to read humidity inside tanks, with the aid of a Lovibond comparator.

iii) Investigations on pupae

The length of the pupal stage at various temperatures was found by adding fully grown larvae in pairs to 1 g food in 2.5 x 7.5 cm glass tubes, and checking daily for pupation and then adult emergence. Some batches of pupae were exposed to temperatures below the developmental range. After return to 25°C, some deformed adults were seen. These were boiled for 10 minutes in 10% potassium hydroxide solution, cleared in glacial acetic acid, washed, and mounted in glycerol. They were then examined under the microscope for deformities of the mouthparts or genitalia, using a few control specimens and the keys of Richards and Thomson (1932) for guidance.

iv) Heat production

Temperature variations in stock cultures were measured with thermometers graduated in divisions of 0.1°C. One was pushed down one side to the bottom

Fig. 4. Apparatus set up to isolate eggs of Pyralid moths.

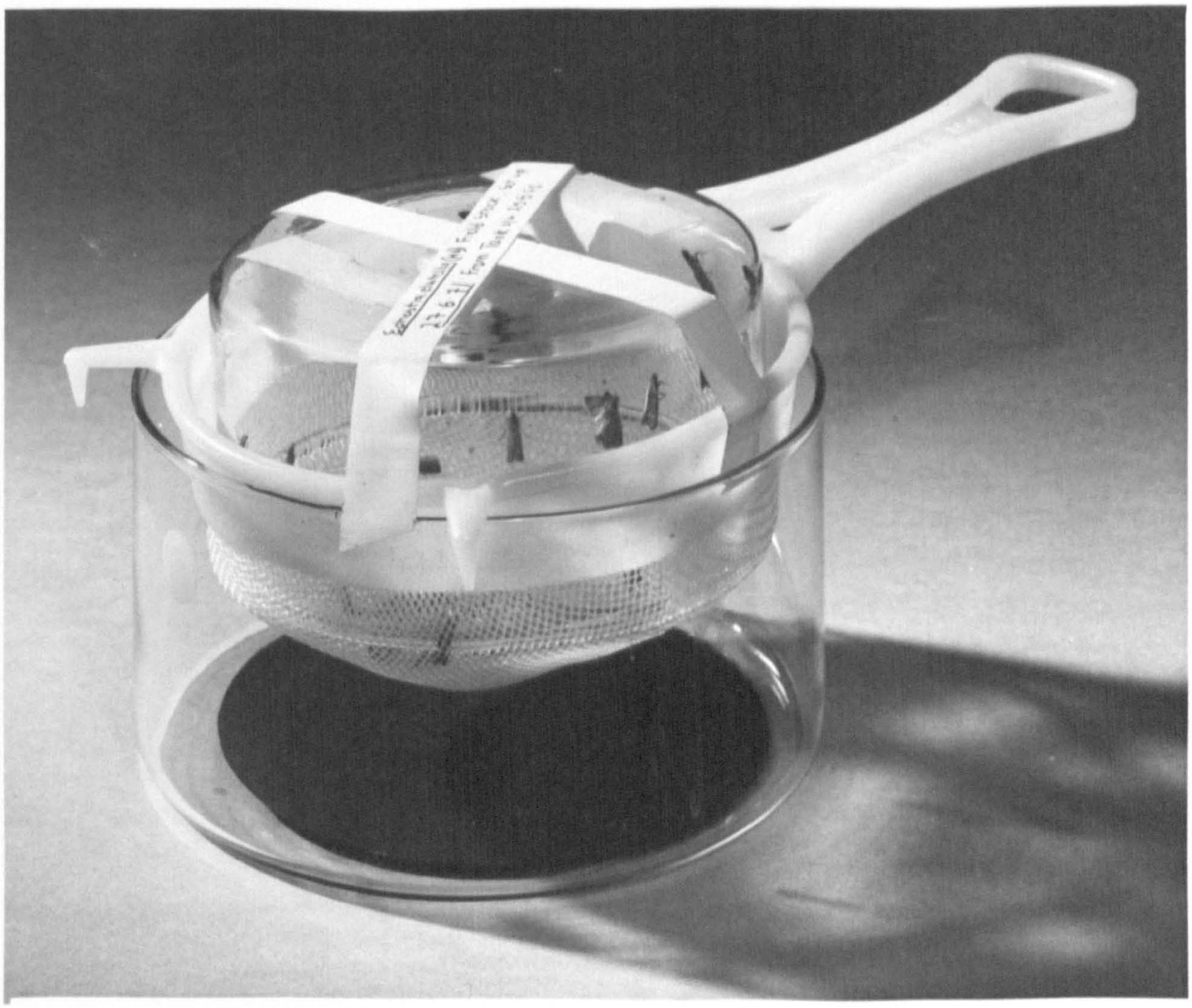


Fig. 5. Arrangement of tubes against a dark background for individual rearing of moths from the egg stage in development time studies.

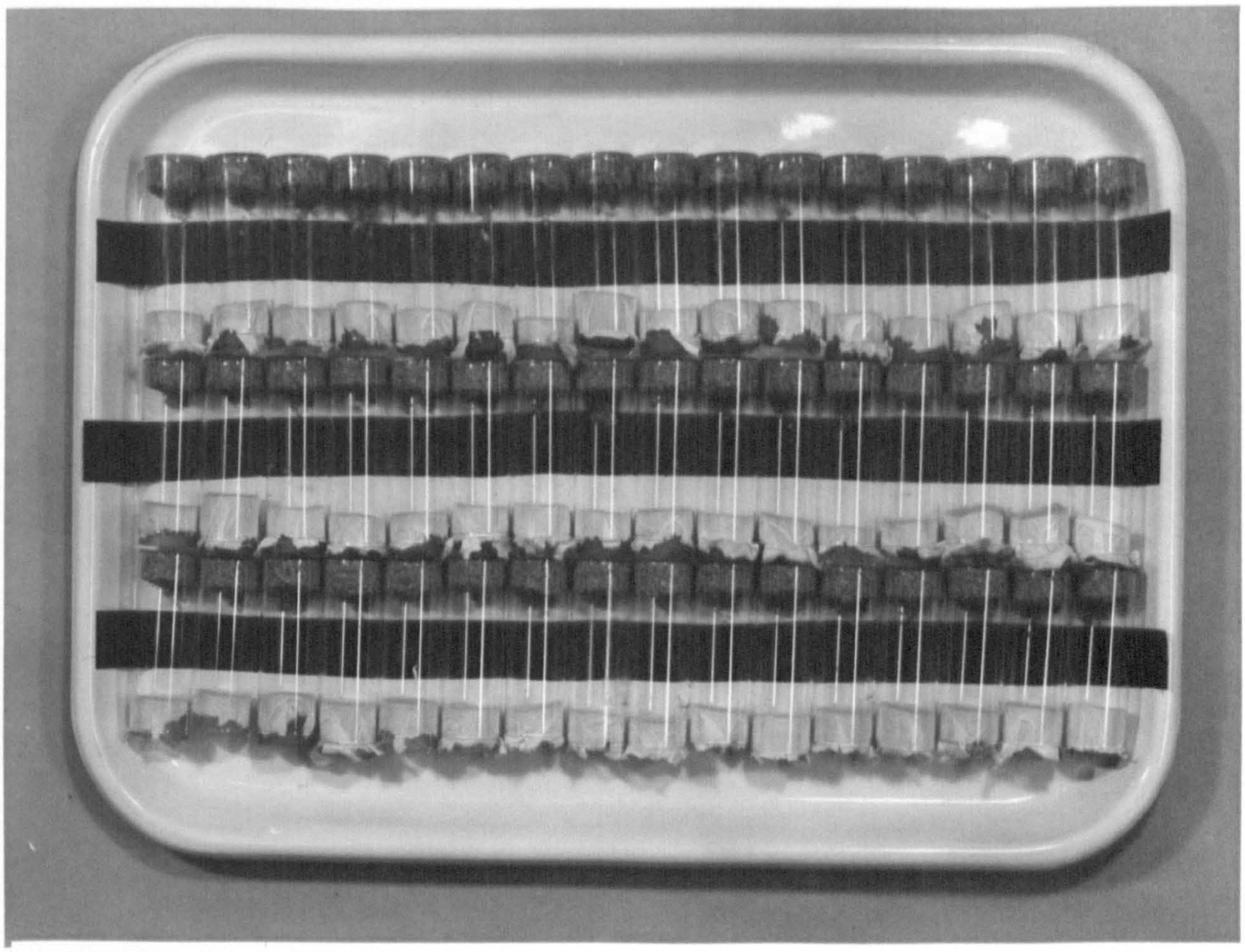
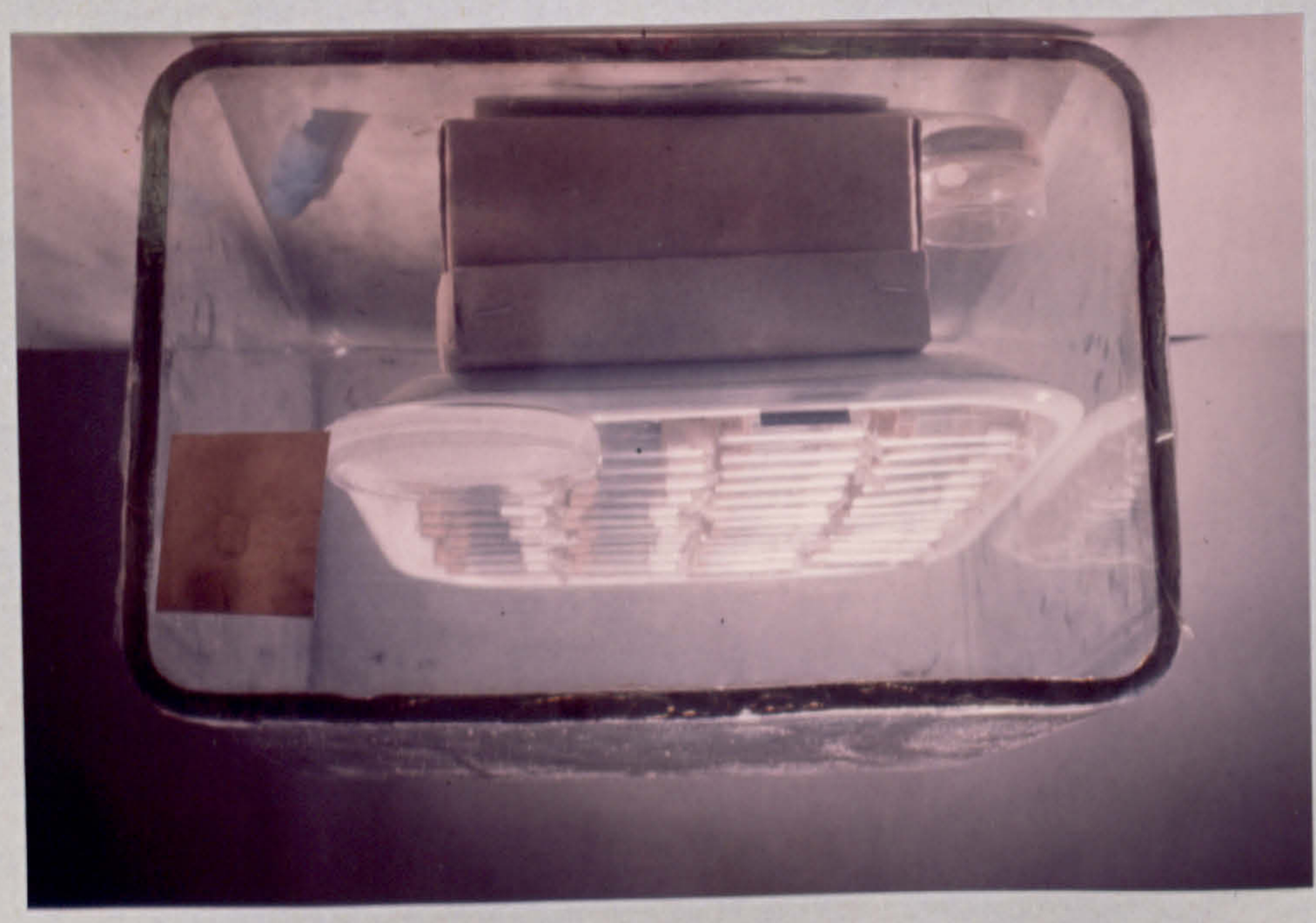


Fig. 6. Tank sealed with polythene used for experiments at low humidity. Dish containing saturated potassium acetate solution mounted on tray, with cobalt thiocyanate paper below in bottom right hand corner of tank.



of the culture, while the others were pushed into the food until the bulbs were just covered, one at the centre, and one at the outside edge of the food surface. Temperatures were read at least three times a week. Humidity at the food surface was estimated from a piece of cobalt thiocyanate paper in a small gauze tray near the middle of the culture. A control paper was placed just outside the culture.

B. Larval Interaction

Development times and percentage survivals were recorded of various numbers of insects developing in cultures of different cross sectional area, and of different volume. When small numbers of insects (up to about 40) were required, eggs layed through sieves into dishes were left to hatch and first instar larvae were carefully transferred with a fine paint brush to the prepared food bulk. Mortality of larvae handled in this way was very low. When larger numbers of insects were required (up to 400), batches of eggs were counted in marked watch glasses which were placed in the test cultures. Hatch was assessed by removing the watch glass after one week and counting the dead eggs remaining.

When still larger numbers of insects were required, such as for experiments on density and temperature rise, 1000 eggs of each species were counted out and weighed. Results were as follows: E. elutella, 36 mg; E. cautella, 27 mg; E. kuehniella, 24 mg; P. interpunctella, 18 mg. The required numbers of eggs were then estimated by weight, and control batches of 100 were set up in watch glasses to estimate the percentage hatch.

C. Diapause

i) Lighting systems

Light in constant temperature rooms was provided by 40-W warm white or daylight fluorescent tubes mounted over benches and regulated by time switches. Also in the rooms were two cabinets. Each cabinet (fig. 7) measured 1.09 x 0.51 m, and stood 0.61 m high. Each was partly divided into 2 compartments by a transparent perspex sheet. The lower compartment contained the experimental material, while the upper compartment was reserved for a warm white 13-W fluorescent tube mounted on the ceiling and, at one end, a Philips HR 3404 extractor fan to draw air out of the cabinet. A 7.5 cm gap in the partition at the opposite end of the cabinet to the fan, and 2 rows of air holes mounted below the fan in the lower compartment, ensured that air was first drawn across the insect material, and then across the lighting unit on its journey through the cabinet. Readings of temperature were taken at frequent intervals during the experimental period. Light intensities in the cabinet as measured with a Gossen Lunasix light meter were 800 - 1000 lux.

In one cabinet, daylength was controlled by a Venner solar time clock which gave progressively lengthening or shortening daylengths according to the date setting, while the other was fitted with a standard clock so that any constant photoperiod could be selected. In both, the extractor fan was automatically switched on with the light to remove heat from this source. The cabinets were covered with black polythene sheeting to minimise light diffusing in or out.

As test insects were to be reared on the wheatfeed mix, the ability of light to penetrate this medium was examined by standing crystallising dishes containing wheatfeed layers of known depth on a light source adjusted to an intensity of 1000 lux. A 7 mm layer of wheatfeed was found necessary for complete extinction of the light source measured on a Gossen Lunasix 3 light meter.

ii) Handling of insects

For experiments on diapause, a layer of food less than 1/4 mm deep to allow penetration of light was set up in 7.5 x 2.5 cm glass tubes (c. 1¼ - 1½ food). Sieves were set up as described for experiments on development, and the eggs laid were allowed to hatch. As it was desired to expose as many insects as possible in a given space without incurring a second generation before the end of the experiment, 2 first instar larvae were added to each of the prepared tubes. Higher numbers of insects were added for an experiment on diapause at high population density. Each tube was covered with a numbered muslin square by a piece of polythene tubing, and exposure to the experimental conditions was started. Upon entering diapause, larvae commonly moved up the tube to spin hibernacula (fig. 8).

iii) Experiments with light intensity

At the surface of glass tubes stood in various positions on benches or in cabinets, the intensity reading on a Gossen Lunasix 3 light meter varied between 200 and 1000 lux. For a light intensity of no more than 1 lux, tubes were placed in open cardboard boxes (fig. 9) and covered with a thin sheet of black polythene.

iv) Assessing the duration of diapause

Diapause in larvae of E. elutella and P. interpunctella begins at the cessation of feeding, but because of the difficulty in assessing this date, the start of diapause in each batch was taken as the first day adults developing from non-diapausing larvae were seen at the experimental temperature, and, if possible, at the experimental daylength. This date represented a fair average for the cessation of feeding in each sample, although, of course, some individuals would have stopped feeding well before this time while others would continue for some time afterwards. In the case of the field stock of E. elutella at 15°C, when

no individuals developed without diapause, the starting date for diapause was taken as the first day an adult of the laboratory stock was seen. In all batches, the termination of diapause was taken as the day of pupation, although in fact, diapause must end before the start of the prepupal phase.

In assessing the mean and standard deviation for the duration of diapause in each batch, it was assumed that the distribution of pupation times was normal. Usually, however, a positive skew was apparent, and if the minimum period for 80% of the batch to pupate was less than the range of the S.D. about the mean, which should include about 68% of the population in a normal distribution, then mean and S.D. were recalculated omitting stragglers requiring over twice the first calculated mean time for pupation.

4. FUMIGATION EXPERIMENTS

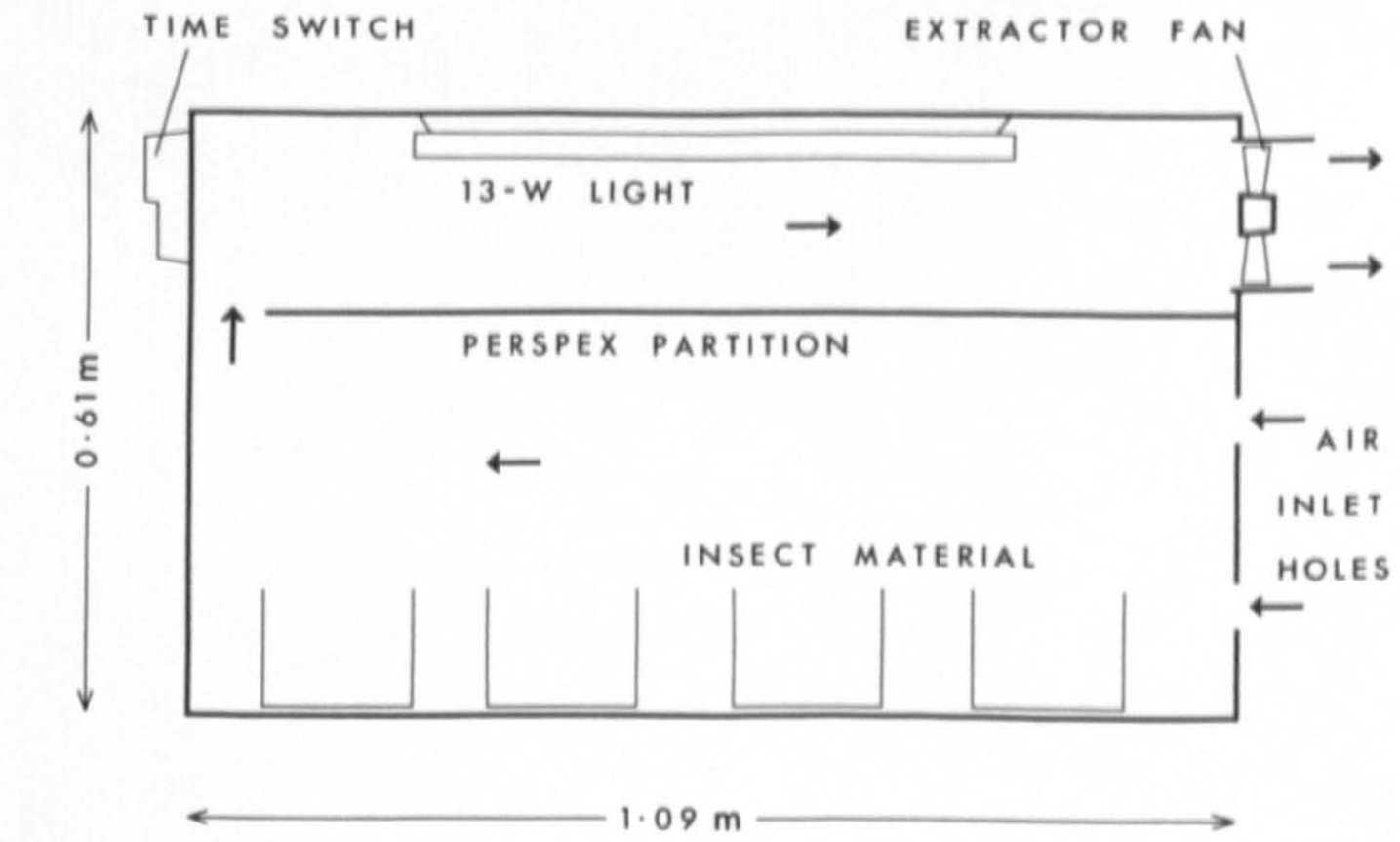
A. Fumigation Chambers

Early in the programme, a few tests were carried out in a 14000-l brick-built chamber. Subsequently, fumigations were conducted in 1700-l chambers fitted with a door at the front, and additional access points of various size (fig. 10). Each chamber was situated in a room maintained at constant temperature and humidity, and was provided with a light, a circulatory fan, a mercury-in-steel thermometer, and a vacuum pump by means of which fumigant could be expelled through piping opening outside the building. Inside the chamber, experimental material was accommodated on sheets of plate glass. Bulky material could be loaded into the chamber before dosing through the main door, or alternatively could be loaded after dosing through an arrangement of polythene sleeves fitted to a 15 cm port hole in the side of the chamber (fig. 11) which was opened by removing a steel plate.

Fig. 7. Cabinets fitted with lighting and an air circulatory system for use in experiments on photoperiod:-

Upper: Diagram of cabinet showing layout and direction of air movement.

Lower: View of loaded cabinet with front hatch raised, showing insect material, air holes, fan and light.


TIME SWITCH
EXTRACTOR FAN
13-W LIGHT
PERSPEX PARTITION
AIR
INLET
HOLES
INSECT MATERIAL
0·61 m
1·09 m

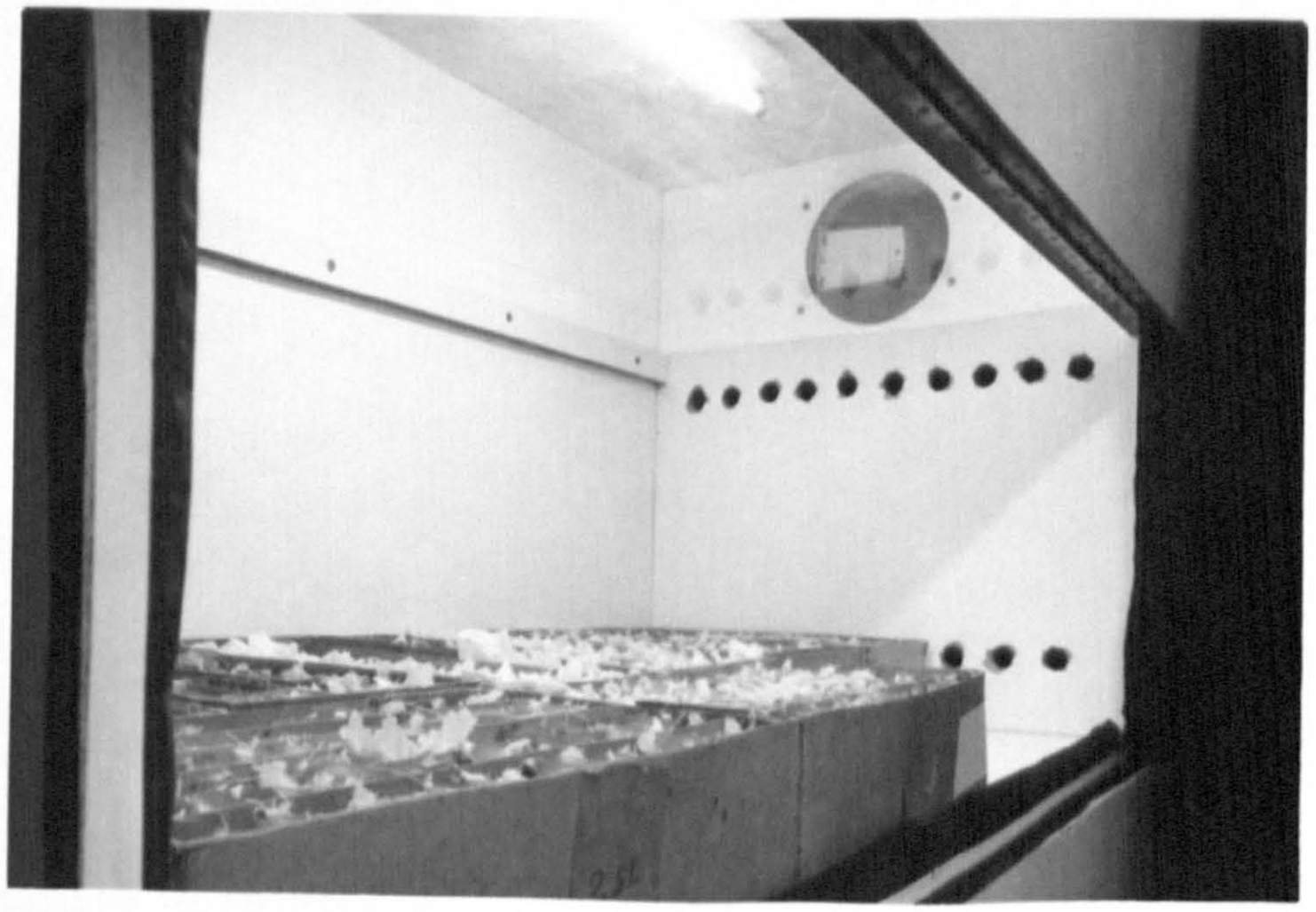


Fig. 8. Diapausing larvae and hibernacula of (upper) Ephestia elutella and (lower) Plodia interpunctella in 7.5 x 2.5 cm glass tubes with muslin stoppers. One of the P. interpunctella larvae has not yet spun a hibernaculum.

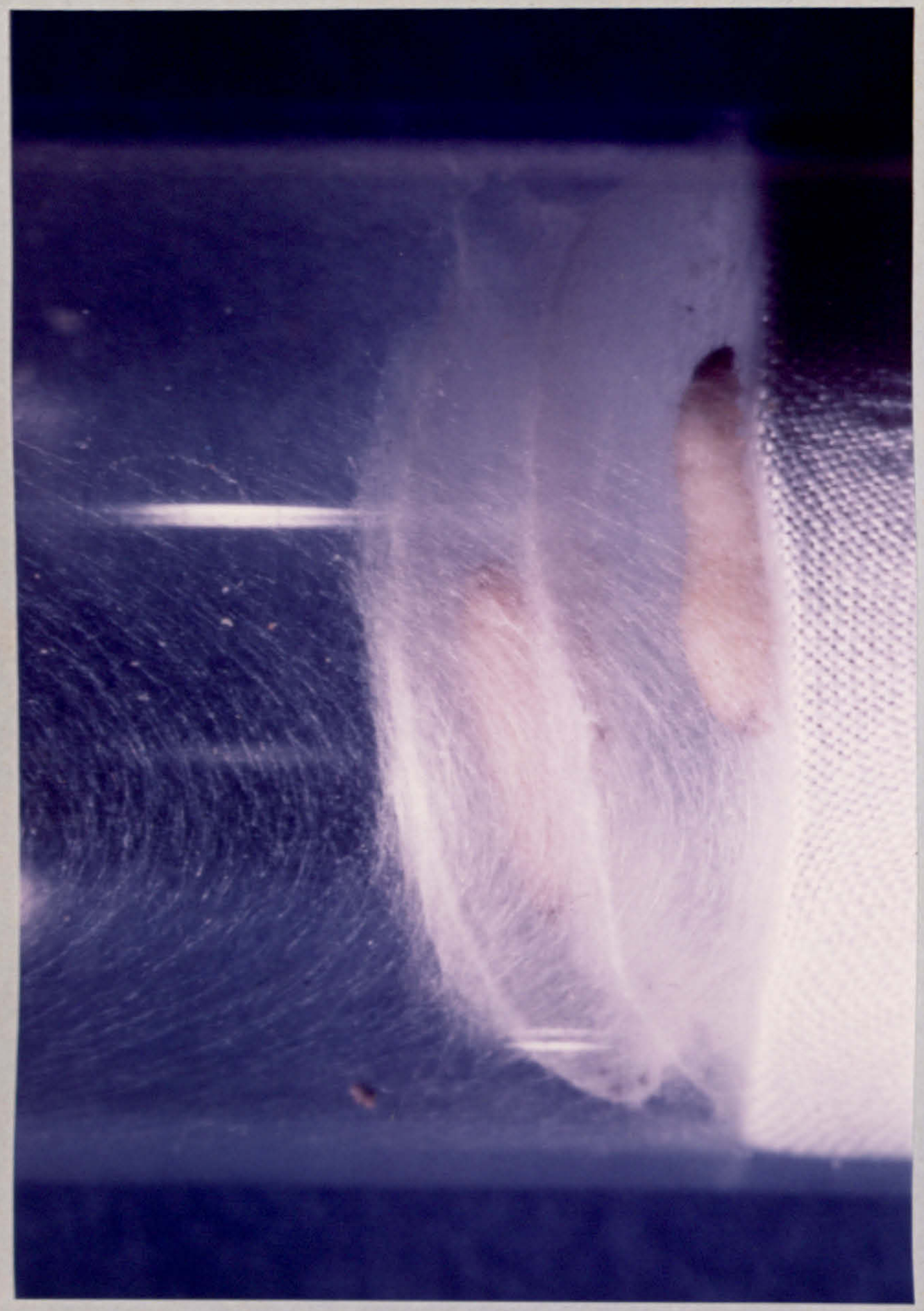


Fig. 9. Cardboard box used to contain tubes set up with young larvae in experiments on diapause induction.

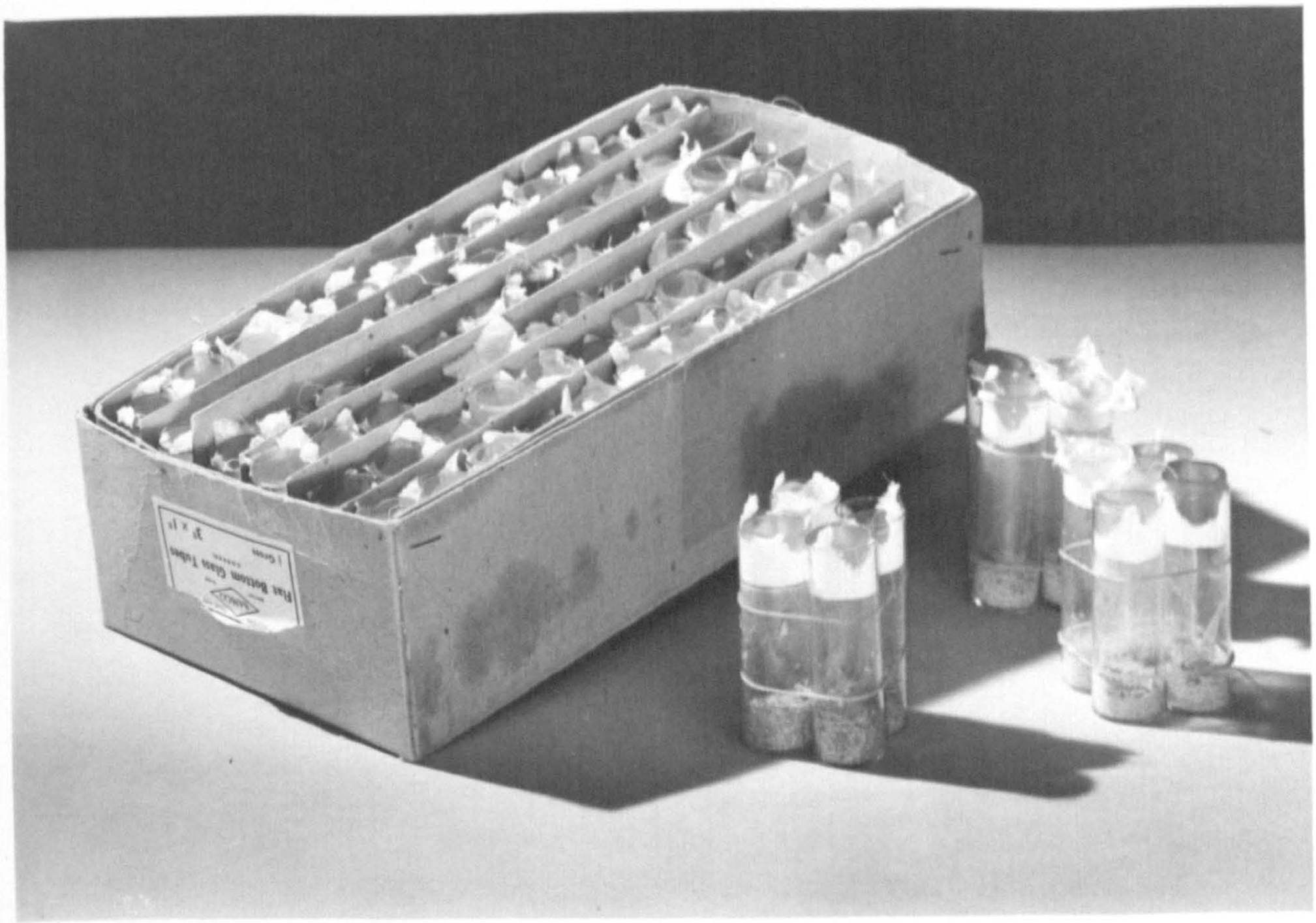

Flat Bottom Glass Tubes
1 Gross
3" x 1"

Fig. 10. 1700-l stainless steel chamber used in fumigation tests showing (upper) the chamber fully loaded with cultures containing all stages of various stored product species, and (lower) the same chamber fitted with the polythene sleeves and bags used to minimise gas escapes during removal of bulky test material.

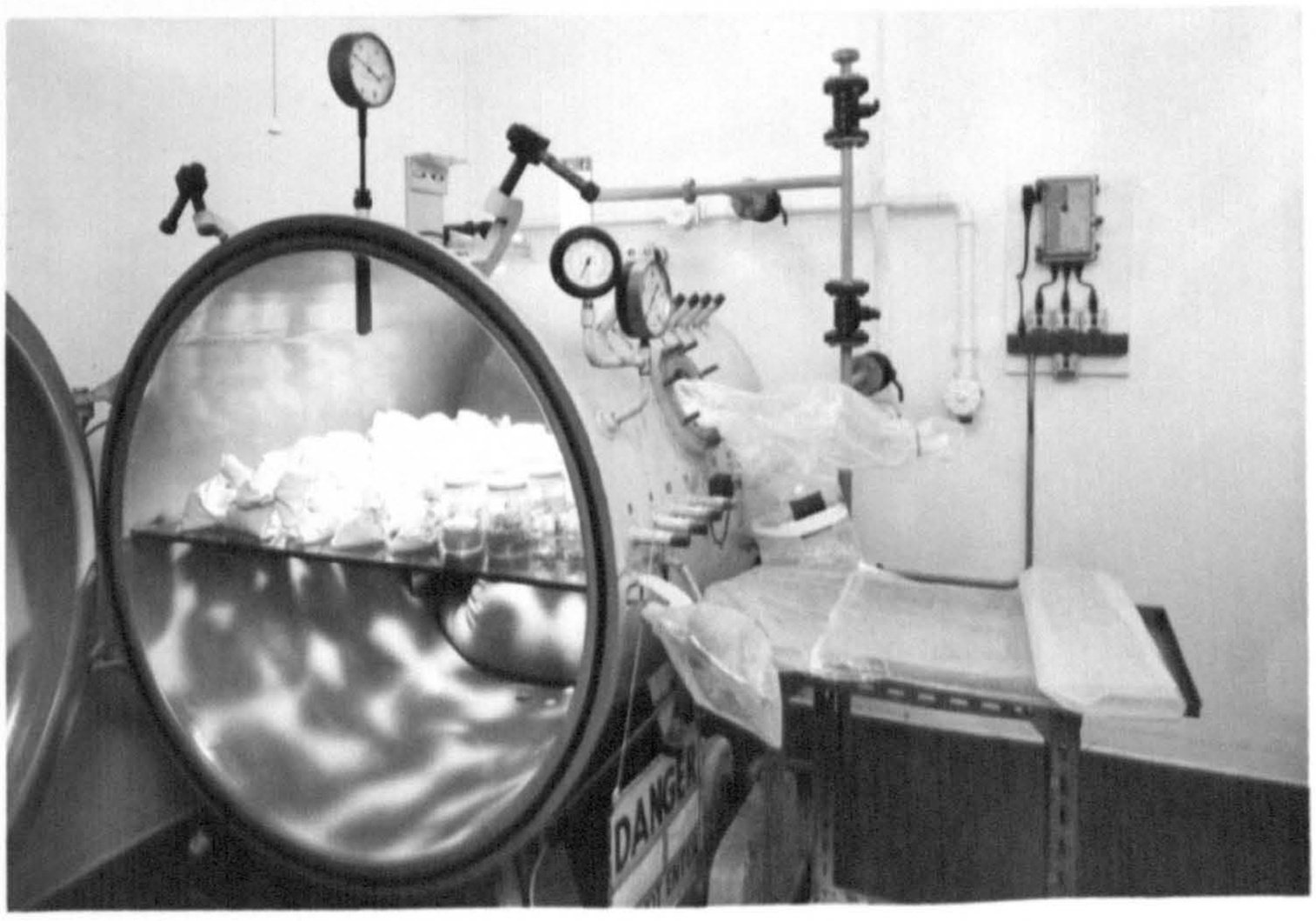

DANGER

Fig. 11. Loading a box containing diapausing larvae in glass tubes into the chamber through the 15 cm diameter port via a polythene sleeve.

B. Dosing and Sampling of Fumigants

Pure liquid methyl bromide (CH_3 Br) supplied in cylinders by May and Baker Ltd was weighed out for the concentration required. Phosphine (PH_3) was generated from Phostoxin, a proprietary formulation containing aluminium phosphide and ammonium carbamate. In the presence of moisture, the formulation decomposes to yield phosphine, carbon dioxide and ammonia. Tablets, which released about 1 g of phosphine, or pellets, which released about 0.2 g, were counted out according to the dose required, and, after weighing, were placed on trays in the chamber together with a dish containing about 1 ml of water for each pellet added. The amount of sorption in tests with phosphine is much less than in tests with methyl bromide, and so it was possible to include a sack of grain in the chamber as an atmospheric buffer. The exposure of insect material was commonly started with the exposure of the formulation, but in tests on stable concentrations, 2 days were allowed at 25°C, or longer at lower temperatures, for the complete decomposition of the formulation. With both fumigants, the gas concentration in the chamber could be lowered by partial evacuation.

Within half an hour of loading test insects into the chamber, gas samples were taken in triplicate to estimate fumigant concentration. Further samples were taken before the removal of insects for each exposure, except in tests lasting 8 hours or less, when sampling was confined to the beginning and end of the test. During sampling, methyl bromide was absorbed into cyclohexylamine in evacuated glass flasks, and was estimated chemically by the method of Lewis (1945). Occasionally, concentrations of methyl bromide were measured using a calibrated thermal conductivity meter (Heseltine, 1961). Phosphine was sampled by absorption into acid potassium permanganate solution in an evacuated glass flask, and was estimated colorimetrically by the method of Bruce et al. (1962).

C. Preparation and Exposure of Insects

i) All stages together

A 7 cm layer of wheatfeed mix (130g) was placed in a glass jar of 7.5 cm diameter (a 2 lb jam jar). Each species was represented in the tests by 2 series of cultures, one with the older, and the other the younger developmental stages. Eggs laid over 3 or 5 days were counted into watch glasses, and were added at weekly intervals firstly to the culture destined to provide the older stages, and then to the younger culture. Immediately before the addition of each batch of eggs, the watch glass from the previous week was removed to assess the number of larvae which had hatched and entered the culture. To avoid temperature increases within the culture and severe competition for food, cultures were limited to about 200 insects. A day or so before the start of the test, older cultures were checked to ensure that adults were present, and if numbers were low, up to 50 extra adults were added. Rearing for tests on all stages was conducted at 25°C, 70% RH. For tests at 15°C, a three-day acclimatisation period at the test temperature was allowed for all insects other than the last batch of eggs, which were counted out 2 - 3 hours before fumigation. After exposure, cultures were returned to 25°C for incubation after an airing-off period of at least 2 days.

ii) Eggs

For all tests, eggs were collected daily from sieves, counted into small foil trays, and stood at 25°C, 70% RH, in 7.5 x 2.5 cm glass tubes containing about 2 g of food. Tubes were grouped in fours with elastic bands, or were packed upright in beakers, to facilitate transfer in and out of the fumigation chamber. A period of at least one hour acclimatisation was allowed for all age groups at the test temperature before exposure to gas. After fumigation, eggs were aired for at least 24 hours before returning to 25°C. A hatch count was performed

after a week at 25°C, and, in the event of more than 4 eggs hatching, the contents of tubes were tipped into jars containing extra food.

iii) Non-diapausing larvae and pupae

For tests on non-diapausing larvae, feeding last instar larvae were removed from a 3-1 stock culture of suitable age three days before fumigation, and were added in pairs to 7.5 x 2.5 cm tubes containing about 1 g of food. Before fumigation batches were checked for pupation and were acclimatised for at least 4 hours at the test temperature.

For tests on pupae, a similar procedure was adopted, but two age groups were fumigated. The older pupae were placed on wheatfeed in tubes or jars three days before fumigation. Care was taken to remove all pupae from the stock culture. On the day of the test, the culture was again searched to obtain young pupae. All samples were acclimatised for at least three hours at the test temperature.

Pupae for tests with methyl bromide at 15 and 20°C, were obtained by allowing larvae set up in glass tubes to pupate before fumigation. Larvae in tests with phosphine at 20°C were reared from the first instar in glass tubes under a 16-hour daylength at 25°C.

Batches of tubes for fumigation were packed into cardboard boxes for transfer in and out of the chamber. An airing-off period of at least 2 days was allowed after the withdrawal of fumigated samples from the chamber before returning to the rearing room at 25°C.

iv) Diapausing larvae

For toxicity tests, diapausing larvae of E. elutella and P. interpunctella

were reared under an 8-hour daylength at 20°C. A few batches were reared under different conditions for comparative purposes. Larvae were set up in glass tubes as described for experiments in diapause. Before loading into the chamber, tubes were packed in cardboard boxes. Samples were acclimatised for at least 3 days at test temperatures below 20°C, while for tests above 20°C, 1 day only was allowed to avoid the possibility of terminating diapause. After fumigation, samples were aired for at least 2 days before moving to a 16-hour daylength at 25°C for the termination of diapause. In certain experiments the effects of other post-fumigation conditions on susceptibility were investigated.

v) Pupae of Pieris brassicae

Pupae required for tests were removed from tanks used for rearing by severing the silk threads supporting the thorax, and loosening the grip of the pupal claspers at the posterior and of the abdomen. For fumigation, 40 pupae were placed on a thin layer of wheatfeed mix in a glass jar 7.5 cm in diameter (fig. 12). Samples were allowed at least 24 hours acclimatisation at the test temperature, and, after fumigation, were left to air for at least 2 days. Diapausing pupae were then chilled for 11 or 16 weeks at 7.5 - 10°C before returning to a 16-hour daylength at 25°C.

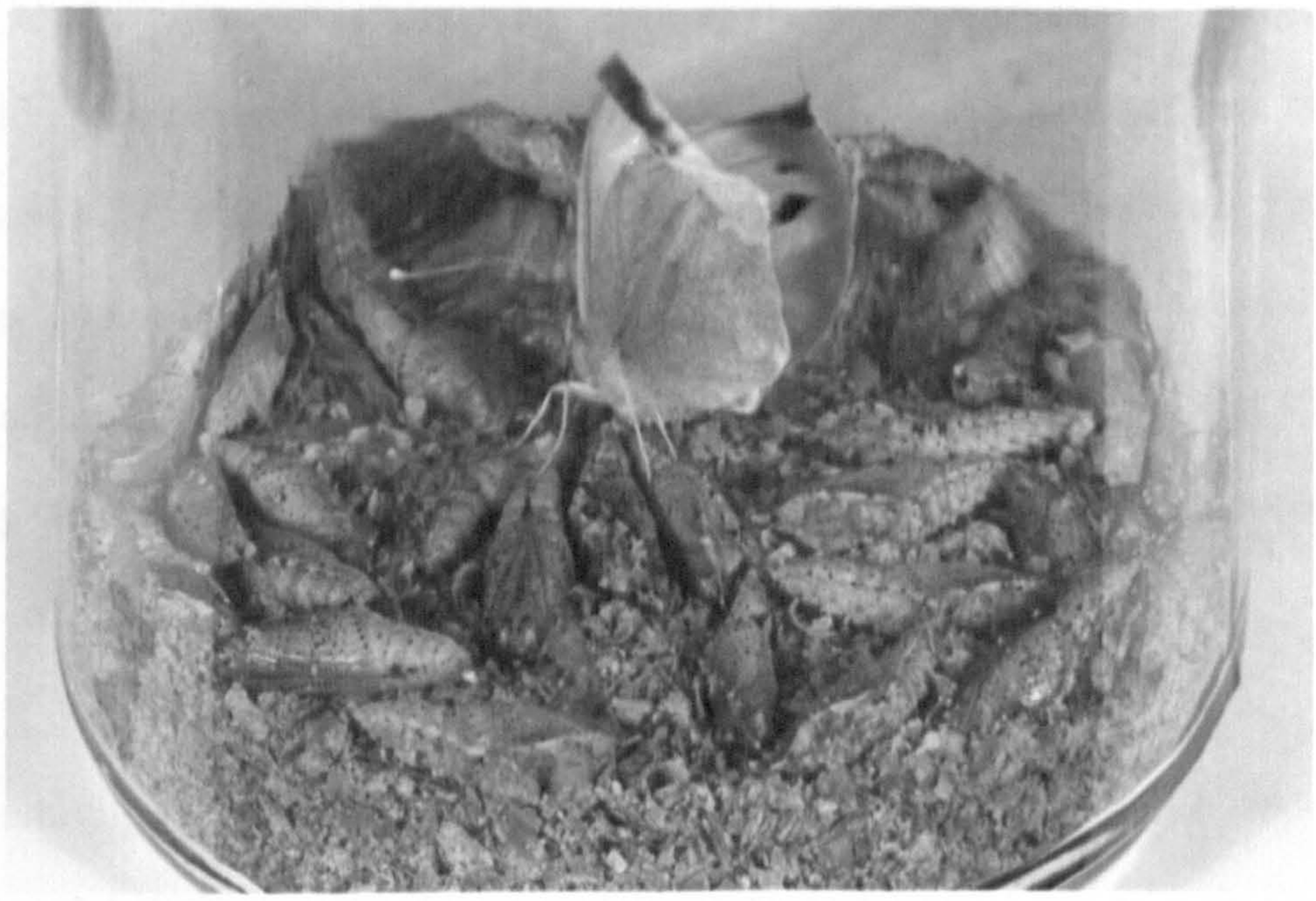
vi) Eggs of Bombyx mori

Waxed papers bearing eggs were cut into short strips which were placed in 7.5 x 2.5 cm glass tubes for fumigation. 200 - 500 eggs were set up per exposure. At least 24 hours acclimatisation was allowed at the test temperature, and, following fumigation, samples were left to air for at least 48 hours. Diapausing eggs fumigated at 10°C were allowed a total of 25 weeks at this temperature before returning to 25°C, while those fumigated at other temperatures were chilled for 16 weeks at 7.5°C. Eggs treated for the avoidance of diapause, or the breaking of diapause, were returned to 25°C immediately after airing.

Fig. 12. Pupae of Pieris brassicae set up for fumigation experiments on wheatfeed in jars 7.5 cm in diameter:-

Upper: Emergence of a butterfly in a control jar.

Lower: 5 fumigated jars showing dead pupae which have darkened in colour, and adults which have emerged and died.



D. Evaluation of results

Mortality of all stages other than eggs of B. mori was assessed on the basis of adult emergence. Adults failing to completely free themselves from the pupal case were judged not to have emerged. Survival was not accredited to adults surviving less than a day, or showing marked deformity. In cultures containing many insects, adults emerging were removed daily to prevent the rapid build-up of a second generation.

Genitalia and mouthparts of deformed adults were investigated as described in experiments on cold tolerance, ~~for deformities of genitalia and mouthparts.~~ Additional tests were performed on the fertility and weight of adult survivors. Adults were weighed individually within 1 day of emerging. In tests on pupae, the weight loss at emergence was assessed in control batches and in batches which had been fumigated.

The mortality of eggs of B.mori was assessed on the basis of hatch alone.

In all tests, percentage mortalities were corrected for control mortality by the method of Abbott (1925). For eggs, the control hatch was used to estimate the number of live eggs treated, and results were corrected for the control mortality between hatching and adult emergence. In tests with methyl bromide, percentage mortalities for eggs and other susceptible stages were transformed to probits (Bliss, 1934a; 1934b; 1935), and LD 50 and 99.9 values were read from eye-fitted lines. The probit transformation was not employed in tests with phosphine on eggs, because large changes in susceptibility occurred rapidly during development, and mortality was controlled chiefly by the length of the exposure.

Results for diapausing larvae with either phosphine or methyl bromide were subjected to a mathematical probit analysis for calculation of LD 50, 90, and 99 values, together with fiducial limits (Finney, 1971).

B I O L O G I C A L R E S U L T S

1. TEMPERATURE AND DEVELOPMENT

A. Developmental Times and Limits of Four Storage Moths

i) Span of complete life cycle on wheatfeed mix

Laboratory and field stocks were set up with newly-laid eggs in 3-1 jar cultures incubated at 70% RH at a range of temperatures. A 16-hour daylength was provided by a 40-W fluorescent tube controlled by a time switch. The first emergence of adults in each culture was noted, and the time of peak emergence was estimated from daily observations (table 3). At 20-30°C, P. interpunctella and E. cautella completed development faster than E. kuehniella and E. elutella. At 25 and 30°C the developmental period was shortest for P. interpunctella, followed in order by E. cautella, E. kuehniella, and E. elutella. In all species variation between developmental periods for laboratory and field stocks was small at higher temperatures. Diapause, which greatly extended development time, occurred in all larvae of the field stock of E. elutella at 15°C, and in most at 20°C. Results at 20°C for the field stock, and 15°C for the laboratory stock, were compiled from those insects developing without diapause. P. interpunctella and E. cautella did not complete development at 15°C.

ii) Span of egg and pupal stages

All investigations were carried out at 70% RH and under long daylength. At each temperature, differences between stocks of the same species were small, but were often significant (tables 4 and 5). In field stocks development was usually a little slower than in laboratory stocks. P. interpunctella and E. cautella completed the egg and pupal stages faster than the other 2 species at 20°C or above. At all temperatures tested, E. elutella took longer than other species to complete development in either stage. Assessments of hatch, pupation and adult emergence were performed daily.

TABLE 3. Days for first emergence and peak emergence in moth cultures set up with 500 eggs at 70% R H, and under a 16-hour daylength, at 15-30°C.

E. e. E. elutella

E. k. E. kuehniella

E. c. E. cautella

P. i. P. interpunctella

STOCK	TEMPERATURE °C							
	15		20		25		30	
	1st	Peak	1st	Peak	1st	Peak	1st	Peak
E.e. LAB	146(2)	167(2)	60	70	39	45	30	35
FIELD	271(1)	306(1)	66(2)	77(2)	41	47	30	35
E.k. LAB	132	147	59	68	38	43	29	34
FIELD	124	138	58	66	38	43	30	34
E.c. LAB	-	-	53	59	32	36	25	29
FIELD	-	-	55	63	33	38	27	31
P.i. LAB	-	-	55	60	29	34	22	26
FIELD	-	-	52	57	30	34	23	27

(1) All enter diapause

(2) Many enter diapause

TABLE 4. Duration of the egg stage of four stored product moths at a range of temperatures at 70% RH and under long daylength.

SPECIES	TEMP °C	LABORATORY STOCK			FIELD STOCK			p, SIGNIFICANCE OF DIFFERENCE BETWEEN STOCKS
		RANGE	MEAN AND S.D.*	NO. IN SAMPLE	RANGE	MEAN AND S.D.*	NO. IN SAMPLE	
Plodia interpunctella	20	6-9	7.2	92	7-10	8.0 ± 0.7	83	
	25	3-5	4.1	88	4-6	5.3	91	
	30	2-4	3.0	86	3-5	4.0	90	
Ephestia cautella	15	16-20	17.5 ± 1.0	71	17-22	18.9 ± 1.1	62	<0.001
	20	6-8	7.4	78	6-9	7.8	58	
	25	4-5	4.7	65	4-5	4.7	70	
	30	3-4	3.4	76	3-5	3.6	63	
Ephestia kuehniella	15	15-18	16.6 ± 0.7	92	14-18	16.1 ± 1.0	82	<0.001
	20	7-9	7.8	94	7-10	8.2 ± 0.8	83	
	25	4-6	5.2	89	4-6	5.3	86	
	30	3-5	4.0	91	3-5	4.0	83	
Ephestia elutella	15	18-22	19.4 ± 0.9	64	18-24	20.0 ± 1.6	81	NOT SIG. AT 5%
	20	8-11	9.9 ± 0.8	72	9-12	10.3 ± 0.8	74	<0.001
	25	5-7	6.2	65	5-7	6.5	77	
	30	4-5	4.5	76	4-5	4.6	78	

*Standard deviations (S.D.) have not been calculated for means less than 8 days because the interval between counts (1 day) is too large.

TABLE 5. Duration of the pupal stage of four stored product moths at a range of temperatures at 70% RH, and under long daylength.

SPECIES	TEMP °C	LABORATORY STOCK			FIELD STOCK			p, SIGNIFICANCE OF DIFFERENCE BETWEEN STOCKS
		RANGE	MEAN AND S.D.*	NO. IN SAMPLE	RANGE	MEAN AND S.D.*	NO. IN SAMPLE	
Plodia interpunctella	20	16-20	17.7 ± 0.8	48	15-19	17.3 ± 0.9	45	< 0.05
	25	8-11	9.4 ± 0.6	76	8-12	9.7 ± 0.9	87	< 0.02
	30	6-8	7.2	36	6-9	7.6	37	
Ephestia cautella	20	15-19	17.5 ± 0.9	44	15-20	18.1 ± 1.0	40	< 0.01
	25	8-10	8.9 ± 0.7	43	8-11	9.3 ± 0.6	44	< 0.01
	30	6-8	7.0	45	6-8	7.1	38	
Ephestia kuehniella	15	36-43	39.8 ± 1.5	30	35-44	40.4 ± 1.9	34	NOT SIG. AT 5%
	20	18-22	20.1 ± 0.8	36	17-21	19.7 ± 0.8	33	< 0.05 ⊙
	25	10-13	11.7 ± 0.7	44	10-14	11.9 ± 0.6	41	NOT SIG. AT 5%
	30	8-10	9.0 ± 0.5	31	7-10	9.1 ± 0.7	33	NOT SIG. AT 5%
Ephestia elutella	15	42-48	44.0 ± 1.4	39	40-51	45.2 ± 2.6	56	NOT SIG. AT 5%
	20	18-22	20.8 ± 0.9	106	19-23	21.3 ± 0.8	78	< 0.001
	25	11-15	13.2 ± 0.7	125	12-15	13.6 ± 0.7	90	< 0.001
	30	9-12	10.5 ± 0.7	37	9-12	10.3 ± 0.8	34	NOT SIG. AT 5%

* Standard deviations (S.D.) have not been calculated for means less than 8 days because the interval between counts (1 day) is too large.

⊙ Only case where duration of the pupal stage in a field stock was significantly shorter than in the laboratory stock.

iii) Limits for development

At 70% RH, neither E. elutella and E. kuehniella at 10°C, nor E. cautella and P. interpunctella at 15°C, completed development (table 6). In three species development under these conditions halted during the egg stage, but the eggs of E. cautella hatched at 15°C, and, as no large larvae were seen, mortality occurred during early larval development. E. elutella and E. kuehniella completed development at 15°C even with an RH of 25%, although larval mortality was about 80% in the laboratory stocks. The field stocks were tolerant to the low RH. At 30°C, 70% RH, eggs of these two species laid at 25°C developed readily to the adult stage. Attempts to produce a second generation from the emergences at 30°C were however unsuccessful in both laboratory and field stocks of E. kuehniella, and in the field stock of E. elutella. In the laboratory stock of E. elutella, 12 out of a sample of 81 eggs hatched, but only 3 adults were produced. 44 eggs of each stock of E. elutella, E. cautella, and P. interpunctella were exposed at 30°C, 25% RH. The mean development time to the adult stage was 10-14 days longer than at 70% RH. Mortality in pre-adult stages at 25% RH was greater in E. cautella than in other species. In all species, the field stocks were again more tolerant to the low RH. No hatch was recorded in eggs laid by the emerging adults of either stock of E. elutella at 30°C, 25% RH. E. cautella and P. interpunctella adults reared under these conditions and set up over food were able to produce a second generation.

B. Cold Tolerance

i) Eggs

Eggs laid at 25°C were exposed to temperatures below the range for complete development for various periods, and were then incubated at 25°C. At 10°C, eggs of E. cautella and P. interpunctella failed to hatch after a 2-week exposure (tables 7 and 8). No adults were produced from eggs of the laboratory stock of P. interpunctella exposed for just 7 days at 10°C. At 15°C eggs of

E. cautella survived well, hatch occurring within about three weeks. Eggs of P. interpunctella aged about 2 days at 25°C before transfer to 15°C also hatched at the lower temperature, and developed to the adult stage after returning to 25°C. After a 14-day exposure to 15°C, younger eggs failed to produce adults at 25°C. Fig. 13 illustrates the decline of adult emergence with length of exposure of eggs to 15°C for both E. cautella and P. interpunctella.

Eggs of E. elutella and E. kuehniella were exposed to 7.5 and 10°C (table 9). In both stocks of E. elutella, some eggs of each age group gave rise to adults after a 14-day exposure at 10°C, and some of the youngest and oldest age groups survived a similar period at 7.5°C. 21 days at either temperature resulted in 100% mortality of all eggs. Eggs of the two stocks of E. kuehniella showed a wide difference in cold tolerance. Results for the laboratory stock resembled those for E. elutella except that no 0-1 day-old eggs gave rise to adults after a 14-day exposure at 7.5°C. In the field stock, all age groups gave rise to adults after 21 days at 10°C or 14 days at 7.5°C. Eggs over 1 day old showed a small survival after 28 days at 10°C, or 21 days at 7.5°C.

In all species other than E. elutella, eggs became more tolerant to cold when aged over 1 day at 25°C.

ii) Pupae

After a 3-week exposure at 15°C, 48 pupae of E. cautella produced normal adults at 25°C. When another 48 pupae were maintained at 15°C indefinitely, 30 completed development, but 17 of the adults produced remained partly trapped in the old pupal case, and wings were expanded only partly in 7 of the remainder. The adults obtained did not produce offspring, but examination of mouthparts and genitalia revealed no structural deformities. Larvae maintained in cultures at 15°C after hatching at 25°C failed to pupate and perished within three months.

TABLE 6. Development of 4 species of stored product moths at temperatures and humidities near the limits for survival.

SPECIES	STOCK	TEMP °C	% RH	NO. IN BATCH	% HATCH	% SURVIVAL EGG TO ADULT	OVIPOSITION TO ADULTS (DAYS)	
							RANGE	MEAN ± S.E.
Ephestia elutella	LAB	10	70	46	0	0		
		15	25	44	77.3	11.4	178-383*	300 ± 103
			70	46	82.6	43.5	137-403(2)	279 ± 113
		30	25	44	68.6	54.5(3)	38-58	44.8 ± 3.9
			70	46	87.0	76.1	30-39	33.4 ± 2.0
	FIELD	10	70	46	0	0		
		15	25	44	90.9	59.1	344-668(1)	408 ± 65
			70	46	91.3	76.1	302-462(1)	377 ± 44
		30	25	44	90.9	80.4(3)	40- 57	44.6 ± 4.0
			70	46	82.6	76.1	29- 38	33.3 ± 2.2
Ephestia kuehniella	LAB	10	70	46	0	0		
		15	25	44	56.7	22.7	167-211	186 ± 14
			70	46	93.5	89.1	132-169	143 ± 10
		30	70	46	93.5	84.8(3)	31- 37	33.6 ± 1.8
	FIELD	10	70	46	0	0		
		15	25	44	79.6	61.3	162-197	178 ± 12
			70	46	91.3	87.0	125-158	134 ± 8
		30	70	46	89.1	82.6(3)	31- 38	33.4 ± 2.0
Ephestia cautella	LAB	15	70	100	83.0	0		
		30	25	44	77.3	22.7	31- 51	42.6 ± 4.0
			70	100	93.0	79.0	25- 33	28.4 ± 1.6
	FIELD	15	70	100	75.0	0		
		30	25	44	84.1	45.5	38- 54	43.5 ± 4.4
			70	100	88.0	80.0	27- 34	29.9 ± 1.5
Plodia inter-punctella	LAB	15	70	100	0	0		
		30	25	44	91.3	50.0	34- 43	37.4 ± 2.5
			70	100	94.0	89.0	24- 31	26.2 ± 1.7
	FIELD	15	70	100	0	0		
		30	25	44	88.6	70.5	35- 46	39.2 ± 3.2
			70	100	92.0	88.0	24- 32	27.0 ± 1.9

* Only 5 adults, three of which enter diapause.

(1) All enter diapause

(2) Many enter diapause

(3) Adults infertile

TABLE 7. Ephestia cautella: percentage survivals to the adult stage, calculated from hatch in control batches, of batches of 100 eggs exposed for various periods at 10° and 15°C (% hatch indicated in parenthesis).

TEMP °C	DAYS EXPOSED	AGE OF EGGS AT 25°C WHEN TRANSFERRED TO LOW TEMPERATURE (DAYS)					
		LABORATORY STOCK			FIELD STOCK		
		0-1	1-2	2-3	0-1	1-2	2-3
10	0	94 (88)	89 (73)	87 (80)	88 (78)	90 (89)	93 (84)
	7	10 (74)	8 (40)	5 (24)	9 (24)	33 (68)	25 (49)
	10	0 (26)	2 (21)	1 (17)	0 (15)	5 (33)	2 (30)
	14	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
15	0	88 (83)	88 (90)	87 (82)	88 (83)	84 (81)	86 (79)
	4	86 (78)	88 (87)	96 (86)	-	-	-
	8	92 (85)	80 (88)	71 (74)	-	-	-
	11	80 (70)	66 (73)	72 (68)	70 (78)	63 (69)	73 (71)
	15	52 (70)	63 (75)	62 (73)	-	-	-
	21	64 (66)	81 (81)	87 (77)	61 (65)	77 (72)	75 (67)
	28	50 (63)	64 (80)	74 (71)	-	-	-
	35	43 (58)	65 (74)	69 (70)	49 (65)	58 (60)	66 (75)

TABLE 8. Plodia interpunctella: percentage survivals to the adult stage, calculated from hatch in control batches of batches of 100 eggs exposed for various periods at 10°C and 15°C (% hatch indicated in parenthesis).

TEMP °C	DAYS EXPOSED	AGE OF EGGS AT 25°C WHEN TRANSFERRED TO LOW TEMPERATURE (DAYS)					
		LABORATORY STOCK			FIELD STOCK		
		0-1	1-2	2-3	0-1	1-2	2-3
10	0	87 (93)	93 (86)	91 (89)	88 (91)	92 (87)	95 (82)
	5	15 (20)	64 (63)	71 (80)	11 (24)	32 (54)	38 (67)
	7	0 (0)	0 (37)	0 (10)	5 (25)	32 (48)	28 (50)
	10	0 (0)	0 (0)	0 (0)	0 (0)	0 (2)	0 (0)
	14	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
15	0	96 (82)	97 (84)	95 (80)	94 (83)	89 (81)	95 (80)
	2	96 (81)	95 (86)	-	-	-	-
	4	85 (78)	81 (78)	96 (83)	-	-	-
	8	37 (67)	35 (42)	89 (82)	-	-	-
	11	1 (27)	5 (23)	72 (70)	13 (46)	22 (52)	75 (71)
	14	0 (2)	2 (15)	72 (78)	0 (12)	10 (30)	69 (75)
	19	0 (0)	2 (14)	64 (77)	-	-	-
	25	0 (0)	2 (14)	66 (79)	-	-	-
	32	-	1 (11)	64 (74)	-	-	-

Fig. 13. Effect of periods spent at 15°C on the subsequent survival at 25°C of eggs of the laboratory stocks of Plodia interpunctella and Ephestia cautella laid and held for different times at 25°C.

KEY: ○ Plodia, aged 0–1 days
□ " , " 1–2 "
△ " , " 2–3 "
● Ephestia , " 0–1 "
■ " , " 1–3 "

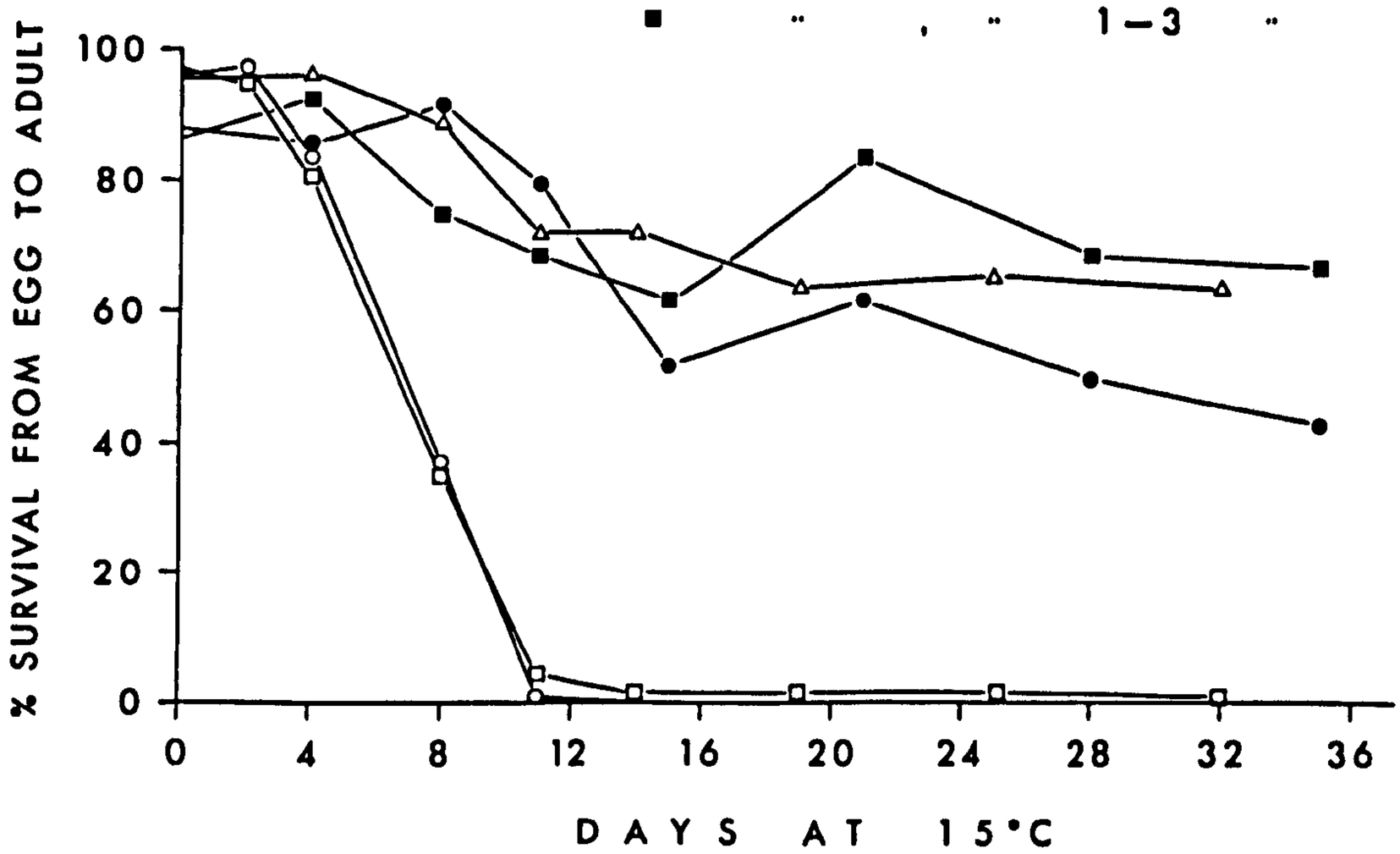


TABLE 9. Percentage survivals to the adult stage, based on hatch in controls, of batches of 100 Ephestia elutella and Ephestia kuehniella eggs exposed for various periods at 7.5° and 10°C (% hatch indicated in parenthesis).

STOCK	TEMP (°C)	DAYS EXPOSED	AGE OF EGGS AT 25°C WHEN TRANSFERRED TO LOW TEMPERATURE (DAYS)					
			E. elutella			E. kuehniella		
			0-1	1-2	2-3	0-1	1-2	2-3
LAB	7.5	0	98 (79)	91 (85)	93 (80)	97 (91)	98 (87)	95 (94)
		7	20 (43)	46 (54)	45 (61)	43 (75)	39 (79)	53 (86)
		14	4 (26)	0 (26)	5 (10)	0 (22)	0 (11)	4 (36)
		21	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	10	7	39 (61)	33 (48)	64 (77)	87 (83)	89 (85)	93 (93)
		14	11 (40)	2 (17)	11 (34)	15 (30)	6 (13)	22 (47)
		21	0 (0)	0 (0)	0 (0)	0 (10)	0 (4)	0 (18)
		28	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
FIELD	7.5	0	84 (78)	87 (71)	90 (78)	89 (91)	96 (89)	84 (88)
		7	47 (52)	51 (59)	67 (62)	60 (81)	38 (90)	85 (94)
		14	6 (33)	0 (15)	10 (29)	51 (64)	70 (85)	52 (61)
		21	0 (0)	0 (0)	0 (0)	0 (40)	3 (30)	3 (17)
		28	-	-	-	0 (0)	0 (0)	0 (0)
	10	7	54 (66)	62 (67)	74 (72)	55 (71)	92 (87)	80 (92)
		14	6 (20)	3 (25)	13 (16)	49 (72)	81 (87)	76 (82)
		21	0 (0)	0 (0)	0 (0)	16 (56)	24 (62)	27 (40)
		28	0 (0)	0 (0)	0 (0)	0 (14)	2 (7)	3 (22)
		35	-	-	-	0 (0)	0 (1)	0 (12)

With P. interpunctella, no adults emerged from 36 pupae held at 15°C, and another 36 pupae exposed 21 days at this temperature gave rise to infertile adults at 25°C. A second generation was produced when 29 adults of E. kuehniella were placed over food after emerging from pupae exposed 21 days at 10°C. The proportions of normal and abnormal adults of E. elutella appearing after exposing pupae for 21 days at 2, 7.5 or 10°C are shown in table 10. Several of the emergences from the 7.5 and 2°C batches had wings entirely or partly devoid of scales. Deformities of the genitalia or mouthparts were nevertheless rare and survivors from each test temperature when placed over food produced a second generation.

iii) Larvae in diapause

Diapausing larvae were very tolerant of cold. Cultures of E. elutella overwintered in an unheated outbuilding where temperatures fell on occasions to -4°C. Cultures of P. interpunctella tolerated minimum temperatures of about 2°C in a slightly heated room. In the laboratory, several hundred diapausing larvae of E. elutella were exposed to 5°C for up to 10 weeks and to 2.5°C for up to 4 weeks. The adults subsequently produced were normal in appearance and fertility. Diapausing larvae of P. interpunctella exposed for 6 weeks at 2.5 or 7.5°C, or for 10 weeks at 10°C, showed little mortality, and normal fertility in the adults produced.

C. Heat Movements in Stock Cultures

i) Results from 3 points inside cultures 14 cm in diameter

Temperature rises of several degrees above ambient were recorded in cultures of all species (figs. 14 - 17). The hottest region occurred in the middle of the culture within the upper 2 cm of the food layer. Graphs of temperature in this region against time showed an accelerating rise to a sharp peak 17 - 24 days

TABLE 10. Effect of 21-day exposures to selected low temperatures on pupae of the laboratory stock of Ephestia elutella

TEMPERATURE °C	NO. OF PUPAE EXPOSED	NORMAL ADULTS	DEFORMED* ADULTS	SCALELESS ADULTS	DEAD PUPAE
10	46	26	16	0	4
7.5	44	15	22	2	7
2	46	11	27	6	8

* Including scaleless adults

(depending on the species) after introduction of adults, followed by a somewhat slower decline. At the base of the culture 7 - 8 cm below the surface, temperature increased irregularly to a smaller peak roughly a week after the maximum rise at the centre. In cultures of E. kuehniella, a second peak was observed near the food surface at this time.

ii) Variation of humidity at the food surface with culture temperature

Glycerol lowered the relative humidity at the food surface in freshly-set-up cultures. Temperature changes within cultures produced inversely related changes in the humidity level (figs. 18 - 21). The discrepancy between humidity at the food surface and outside the culture decreased in cultures of E. cautella, and disappeared altogether in cultures of the other three species shortly before the emergence of adults. Significantly, only the top 2 or 3 cm of food had been utilised in E. cautella cultures, and the number of adults produced was low in comparison with other species.

2. CULTURAL FACTORS

A. Surface Area of Food

The area of the food surface controlled the yield of adults at high population densities (tables 11 - 14). In jars of cross sectional area 4.9 cm^2, the yield of adults of P. interpunctella, E. kuehniella, and E. elutella, was markedly depressed at a population density of 8 larvae / cm^2, while in cultures of E. cautella, yield was depressed at 4 larvae / cm^2. Although results were variable, development was a little faster in cultures of greater area or higher population density, provided that yield was not severely depressed. The difference was significant in all 4 species when development times of 40 insects in cultures of surface area 4.9 and 50.3 cm^2 were compared ($p = < 0.02$).

Fig. 14. Plodia interpunctella: Heat movements at 3 points within a 3-1 jar culture.

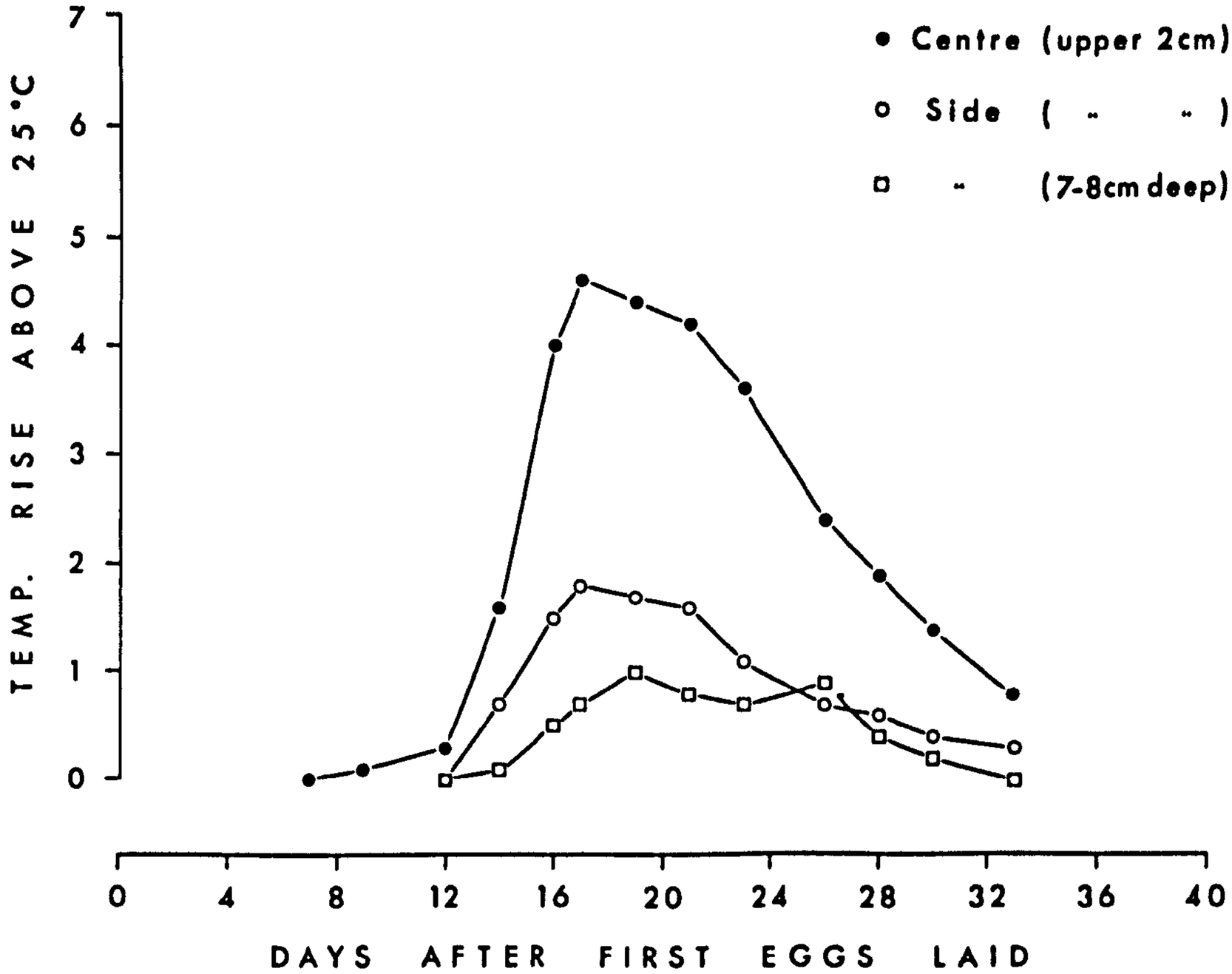

TEMP. RISE ABOVE 25°C
0
1
2
3
4
5
6
7
0
4
8
12
16
20
24
28
32
36
40
DAYS AFTER FIRST EGGS LAID
• Centre (upper 2cm)
o Side (" ")
□ " (7-8cm deep)

Fig. 15. Ephestia kuehniella: Heat movements at 3 points within a 3-l jar culture.

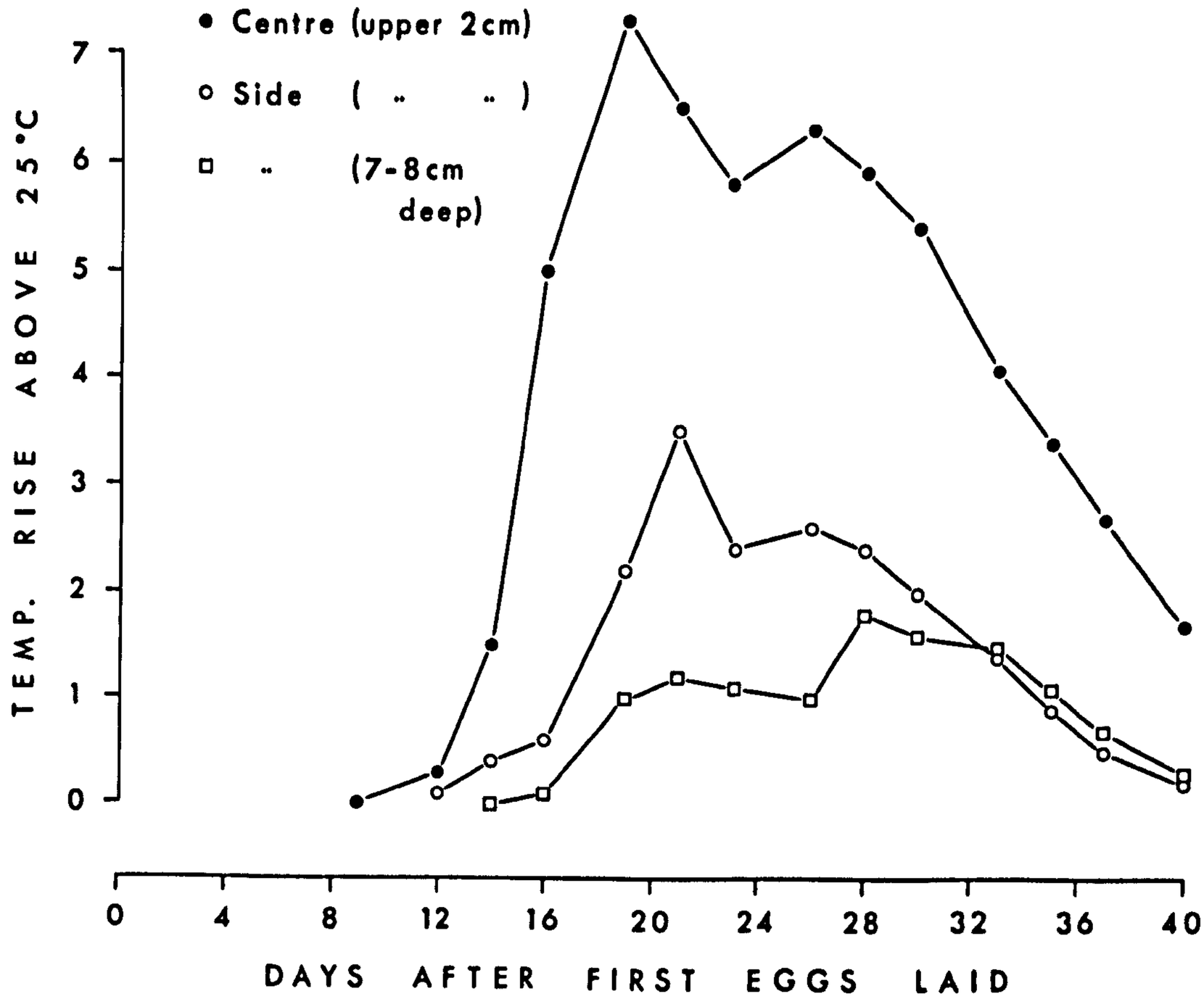

• Centre (upper 2cm)
○ Side (" ")
□ " (7-8cm deep)
TEMP. RISE ABOVE 25°C
0
1
2
3
4
5
6
7
0
4
8
12
16
20
24
28
32
36
40
DAYS AFTER FIRST EGGS LAID

Fig. 16. Ephestia cautella: Heat movements at 3 points within a 3-1 jar culture.

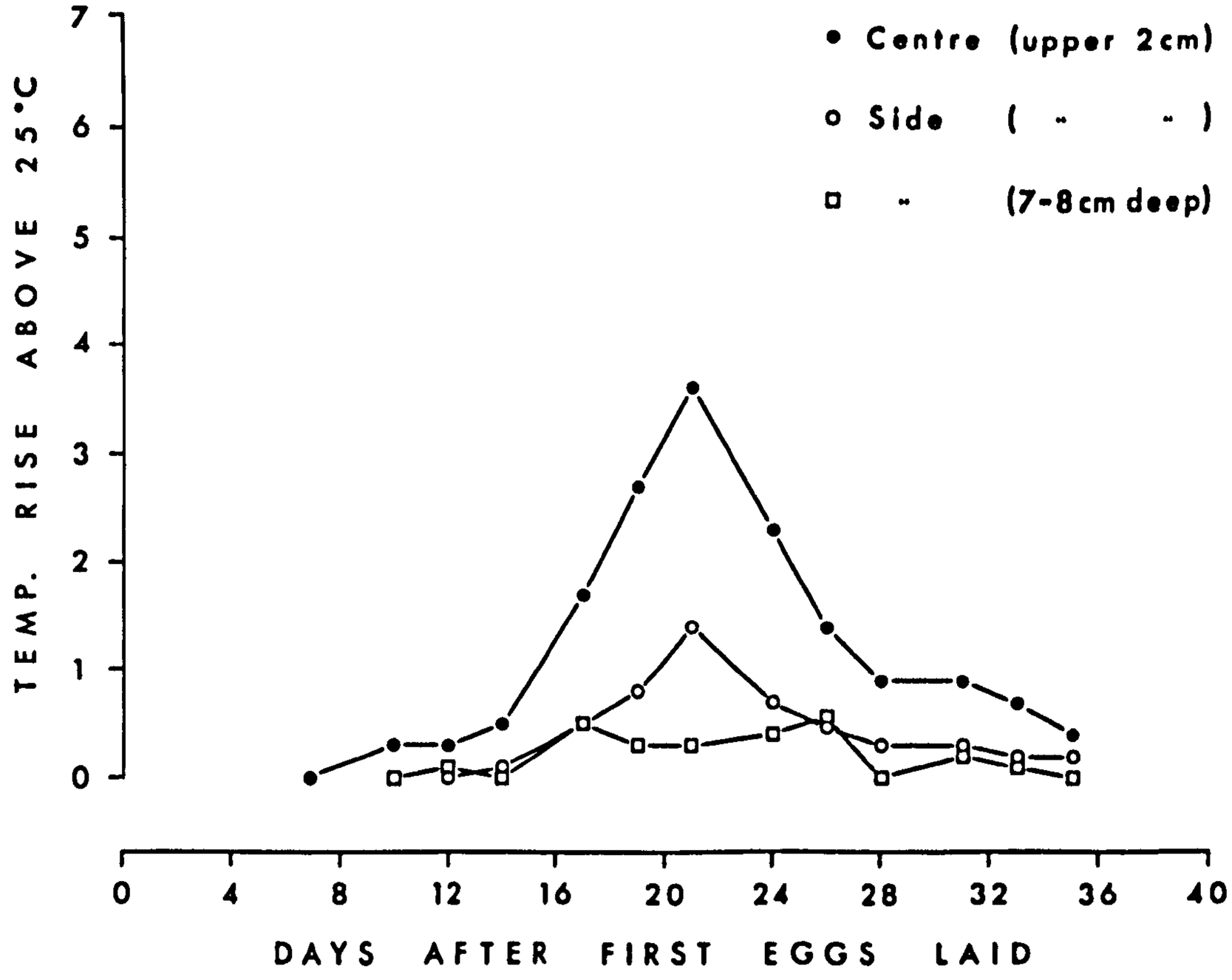

• Centre (upper 2cm)
o Side (" ")
□ " (7-8cm deep)
TEMP. RISE ABOVE 25°C
0
1
2
3
4
5
6
7
0
4
8
12
16
20
24
28
32
36
40
DAYS AFTER FIRST EGGS LAID

Fig. 17. Ephestia elutella: Heat movements at 3 points within a 3-l jar culture.

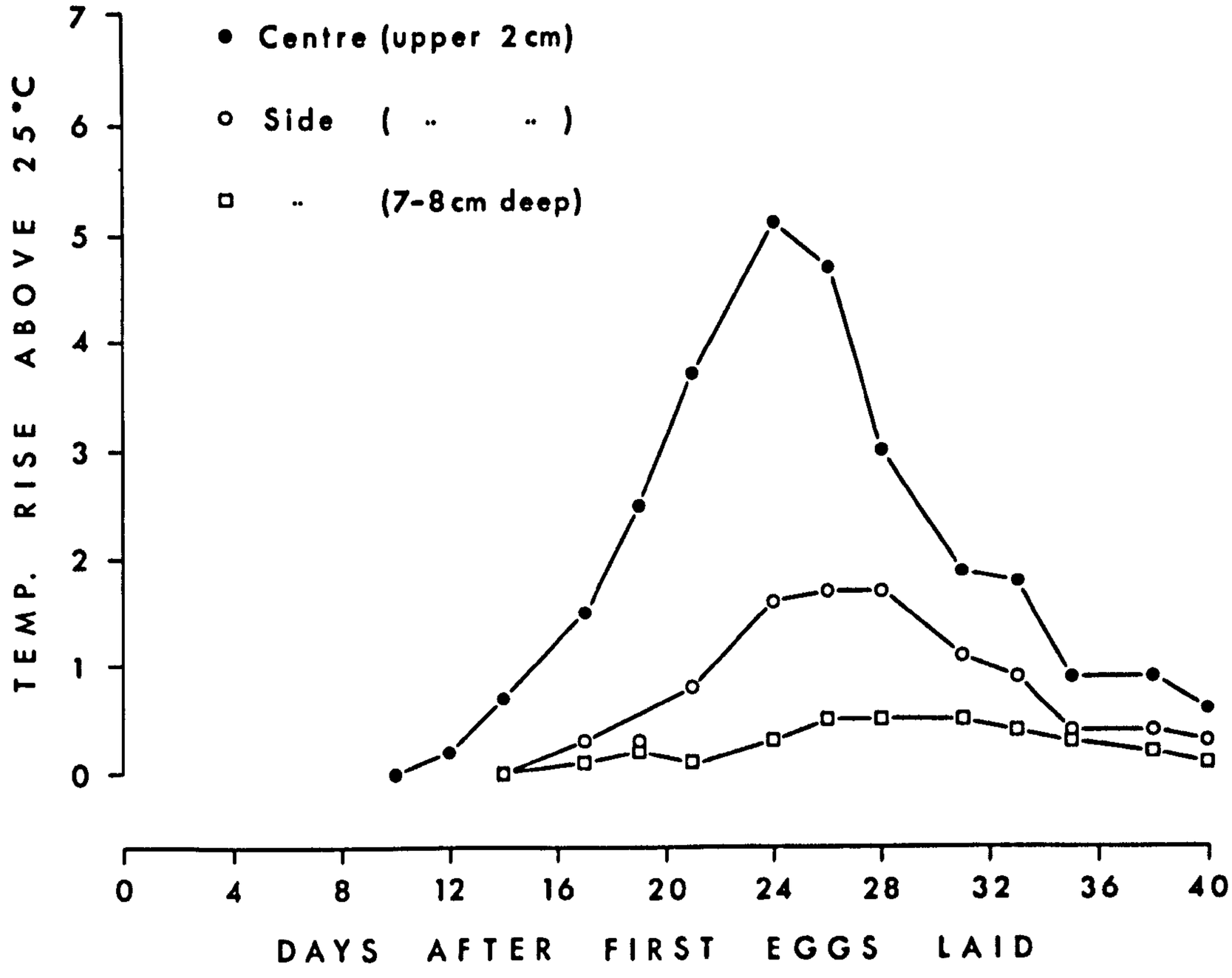

Centre (upper 2 cm)
Side (.. ..)
.. (7-8 cm deep)
TEMP. RISE ABOVE 25°C
0
1
2
3
4
5
6
7
0
4
8
12
16
20
24
28
32
36
40
DAYS AFTER FIRST EGGS LAID

Fig. 18. Plodia interpunctella: Heat movement and RH at the food surface of a 3-l jar culture

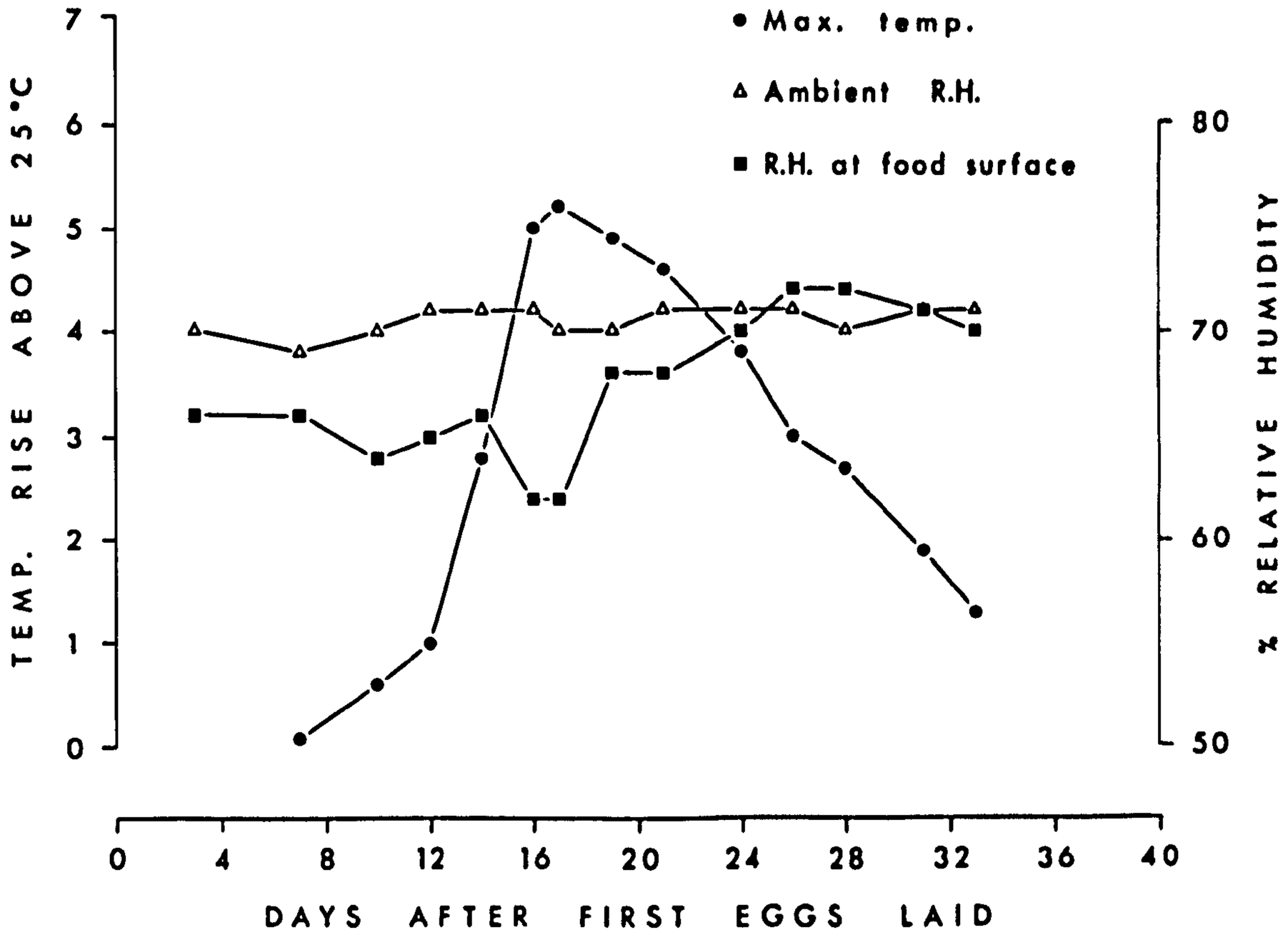

Max. temp.
Ambient R.H.
R.H. at food surface
TEMP. RISE ABOVE 25°C
% RELATIVE HUMIDITY
0
1
2
3
4
5
6
7
50
60
70
80
0
4
8
12
16
20
24
28
32
36
40
DAYS AFTER FIRST EGGS LAID

Fig. 19. Ephestia kuehniella: Heat movement and RH at the food surface of a 3-l jar culture.

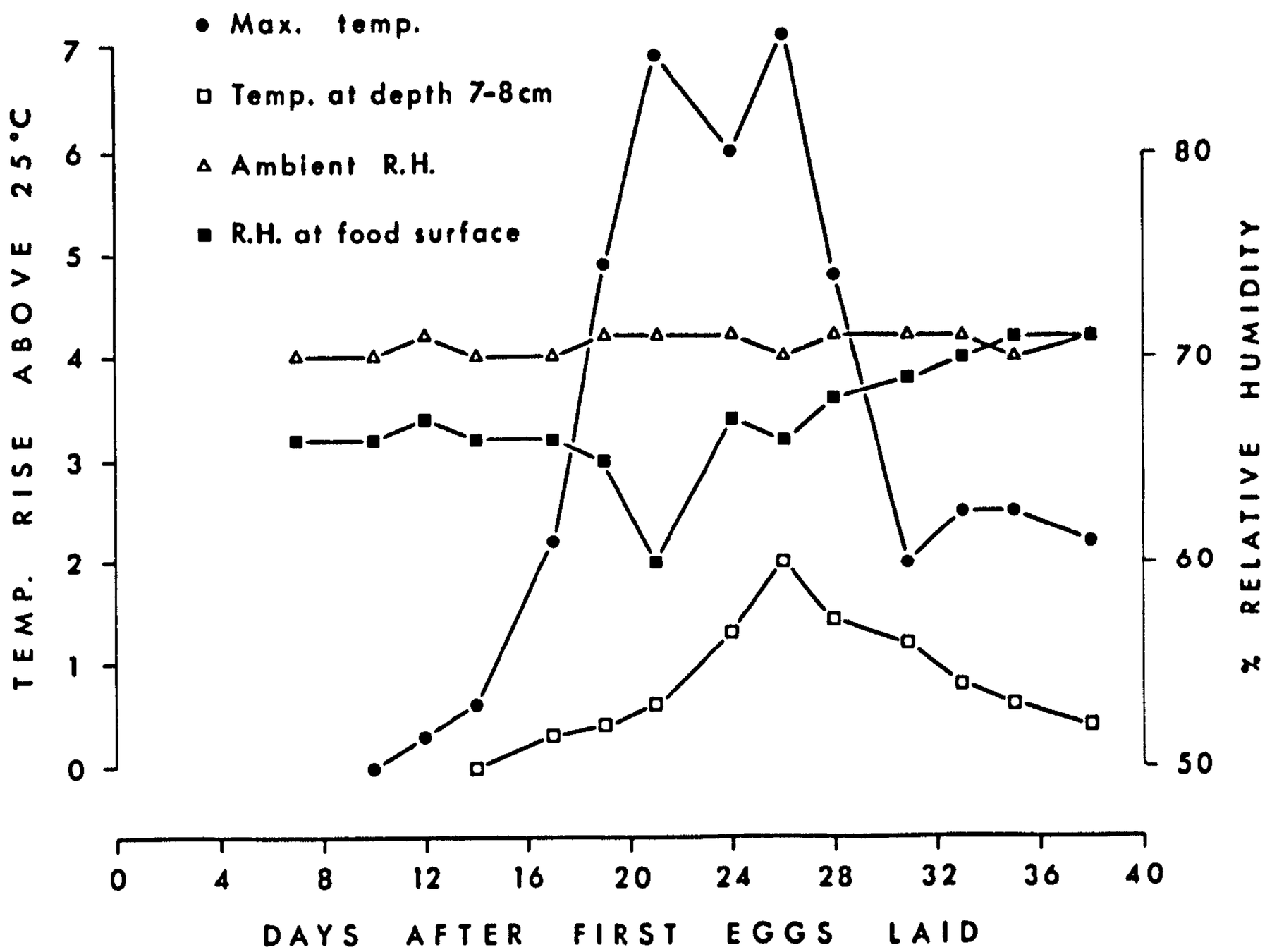

● Max. temp.
□ Temp. at depth 7-8cm
△ Ambient R.H.
■ R.H. at food surface
TEMP. RISE ABOVE 25°C
% RELATIVE HUMIDITY
0
1
2
3
4
5
6
7
50
60
70
80
0
4
8
12
16
20
24
28
32
36
40
DAYS AFTER FIRST EGGS LAID

Fig. 20. Ephestia cautella: Heat movement and RH at the food surface of a 3-l jar culture.

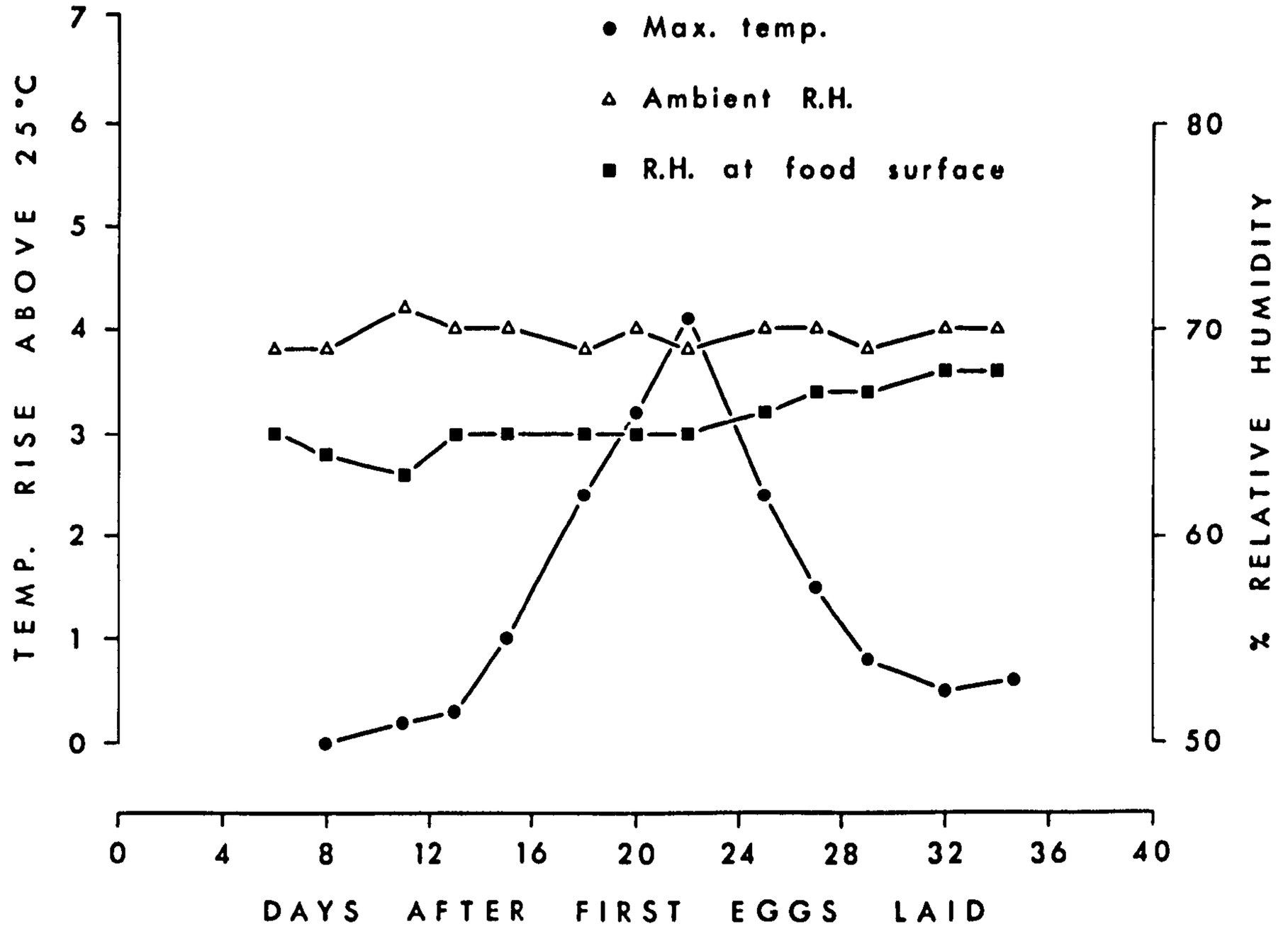

• Max. temp.
△ Ambient R.H.
■ R.H. at food surface
TEMP. RISE ABOVE 25°C
% RELATIVE HUMIDITY
0
1
2
3
4
5
6
7
50
60
70
80
0
4
8
12
16
20
24
28
32
36
40
DAYS AFTER FIRST EGGS LAID

Fig. 21. Ephestia elutella: Heat movement and RH at the food surface of a 3-l jar culture.

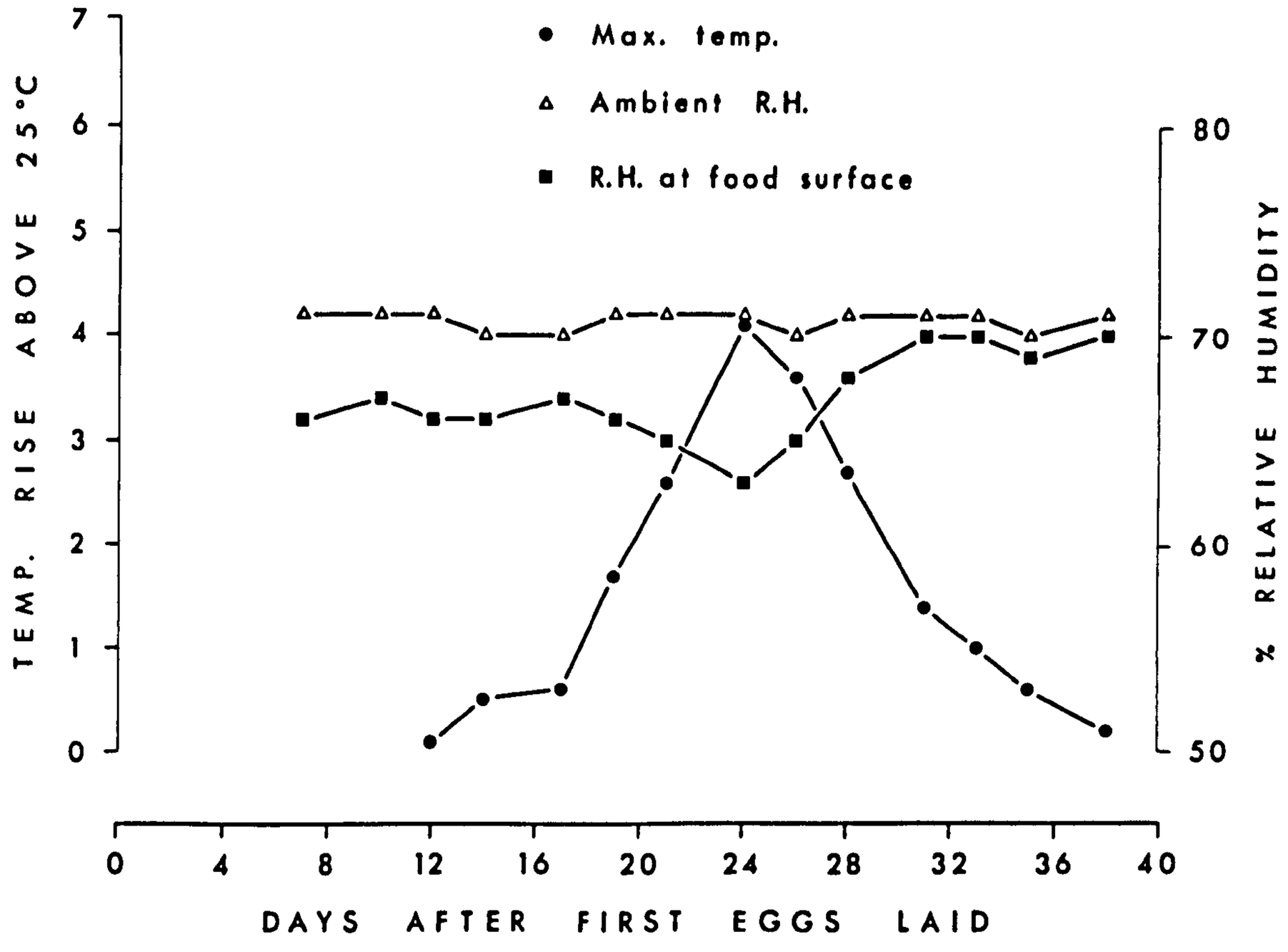

Max. temp.
Ambient R.H.
R.H. at food surface
TEMP. RISE ABOVE 25°C
% RELATIVE HUMIDITY
0
1
2
3
4
5
6
7
50
60
70
80
0
4
8
12
16
20
24
28
32
36
40
DAYS AFTER FIRST EGGS LAID

TABLE 11. Development of Plodia interpunctella on 5 g food bulks in containers giving different areas at the food surface

AREA OF FOOD SURFACE cm^2	NO. OF LARVAE ADDED	NO. OF ADULTS EMERGING IN 2 OR 3 REPLICATES	MEAN % SURVIVAL TO ADULT	RANGE OF DEVELOP-MENT TIMES (DAYS)	MEAN & S.D. FOR DEVELOP-MENT TIME (DAYS)*
4.9	10	8, 9, 9	87	31 - 41	36.3 ± 1.6
	20	18, 17, 15	83	31 - 42	35.9 ± 2.1
	40	33, 20	66	33 - 42	36.8 ± 1.8
22.8	10	10, 9, 10	97	32 - 44	36.2 ± 1.9
	20	14, 18, 19	85	32 - 42	36.1 ± 2.2
	40	34, 35	86	31 - 43	35.7 ± 2.0
50.3	10	9, 9, 10	93	31 - 41	36.0 ± 1.7
	20	18, 19, 18	92	31 - 47	35.9 ± 1.9
	40	37, 32	86	31 - 45	35.6 ± 2.1

* In this and the following three tables, counts of adult emergence were performed at least 3 times a week. For the estimation of means, the adults emerging in any 2 or 3-day period were taken to have appeared at the half-way point between counts.

TABLE 12. Development of Ephestia kuehniella on 5 g food bulks in containers giving different areas at the food surface

AREA OF FOOD SURFACE cm^2	NO. OF LARVAE ADDED	NO. OF ADULTS EMERGING IN 2 OR 3 REPLICATES	MEAN % SURVIVAL TO ADULT	RANGE OF DEVELOPMENT TIMES (DAYS)	MEAN & S.D. FOR DEVELOPMENT TIME (DAYS)
4.9	10	10, 9, 10	97	40 - 52	44.9 ± 2.6
	20	19, 17, 18	90	39 - 54	44.6 ± 3.4
	40	24, 29 -	64	40 - 54	44.8 ± 2.7
22.8	10	9, 10, 9	93	40 - 52	45.0 ± 2.8
	20	20, 18, 20	97	39 - 48	44.2 ± 2.6
	40	32, 38 -	88	39 - 55	44.0 ± 3.1
50.3	10	10, 9, 10	97	40 - 51	44.7 ± 3.2
	20	17, 20, 19	93	39 - 50	43.9 ± 3.0
	40	36, 36 -	90	39 - 53	43.6 ± 2.9

TABLE 13. Development of Ephestia elutella on 5 g food bulks in containers giving different areas at the food surface

AREA OF FOOD SURFACE cm^2	NO. OF LARVAE ADDED	NO. OF ADULTS EMERGING IN 2 OR 3 REPLICATES	MEAN % SURVIVAL TO ADULT	RANGE OF DEVELOP-MENT TIMES (DAYS)	MEAN & S.D. FOR DEVELOP-MENT TIME (DAYS)
4.9	10	9, 9, 10	93	42 - 59	46.1 ± 3.1
	20	16, 12, 15	73	41 - 51	46.7 ± 2.8
	40	18, 19 -	46	42 - 64	47.8 ± 3.5
22.8	10	9, 10, 9	93	42 - 52	46.4 ± 2.7
	20	17, 16, 19	87	41 - 50	45.9 ± 2.4
	40	35, 36 -	89	41 - 57	45.7 ± 2.8
50.3	10	8, 10, 9	90	41 - 50	45.8 ± 2.5
	20	18, 17, 16	85	40 - 58	46.3 ± 3.0
	40	35, 31 -	83	40 - 61	45.5 ± 2.9

TABLE 14. Development of Ephestia cautella on 5 g food bulks in containers giving different areas at the food surface

AREA OF FOOD SURFACE cm^2	NO. OF LARVAE ADDED	NO. OF ADULTS EMERGING IN 2 OR 3 REPLICATES	MEAN % SURVIVAL TO ADULT	RANGE OF DEVELOPMENT TIMES (DAYS)	MEAN & S.D. FOR DEVELOPMENT TIME (DAYS)
4.9	10	7, 9, 8	80	32 - 43	36.8 ± 1.9
	20	13, 14, 11	63	32 - 52	37.0 ± 2.5
	40	16, 11 -	34	32 - 49	37.4 ± 2.1
22.8	10	9, 9, 10	93	32 - 46	36.6 ± 2.1
	20	19, 20, 18	95	31 - 41	35.7 ± 1.8
	40	32, 26 -	73	31 - 43	36.2 ± 2.0
50.3	10	8, 10, 10	93	31 - 49	36.6 ± 2.3
	20	17, 16, 17	83	32 - 55	35.9 ± 2.7
	40	29, 34 -	79	31 - 47	35.4 ± 2.2

B. Depth of Food

In all species, only depths less than 3 cm limited the yield of adults (table 15). Percentage survival to the adult stage was generally greater in cultures set up with fewer insects. Development was usually a little faster when more insects were present, but in cultures of E. cautella set up with 320 larvae, a small resurgence of emergence occurred about two weeks after the main period, lengthening the mean development time. This resurgence has been divided from the main emergence at the 44th day for the calculation of mean development time. In table 15, the mean development times for both samples are presented with the range for the complete sample.

C. Population Density

Increasing population density decreased percentage survival at first gradually, and then more sharply (table 16). Fewer adults of E. cautella were obtained with 800 larvae per culture than with 400 larvae per culture. A second generation appeared in test cultures even when survival in the first generation was lowered. Development was usually delayed in cultures where percentage survival was less than 75%. In heavily populated cultures of E. cautella, many fully grown larvae were observed wandering when the first adults appeared, and, as in the experiments on different weights of food (table 15), a second period of emergence was observed 2 - 4 weeks later. The later adults comprised about 15 - 20% of the total emergence.

D. Heat Generation

Heat generated in cultures of developing larvae caused temperature rises of several ^{o}C above ambient. The maximum temperature rise in cultures 14 cm in diameter and containing 325 g of food, was proportional over a limited range to the logarithm of the number of insects added (see fig. 22). E.cautella

generated more heat than the other species with about 1000 or more insects, while E. kuehniella generated more heat with about 500 or less insects. Traces of temperature against time for each species (figs. 23 - 26) showed that temperature peaks tended to occur earlier in cultures of higher population density. Cultures of E. kuehniella set up with over 1000 insects showed two distinct temperature peaks, but only the first peak, which was the greater of the two at high population densities, was clearly related to the number of insects present (see figs. 24 and 22).

With the results of experiments on culture conditions and microclimate in mind, smaller cultures of diameter 7.5 cm and containing 130 g of food, were chosen for use in toxicity tests on all stages (Chemical Results, section 1). These cultures did not show temperature rises of more than 1°C when set up with a total of 200 insects added in 3 or 4 batches at weekly intervals.

3. INDUCTION OF DIAPAUSE

Diapause was induced in Ephestia elutella and Plodia interpunctella by lowered temperature within the developmental range, by short photoperiods or darkness, or by continual light. High population density was an additional factor inducing diapause in P. interpunctella. In both species, diapause was induced more readily, and over a greater range of temperatures, in the field stock than in the laboratory stock.

A. Induction by Temperature and Light in Ephestia elutella

i) The laboratory stock

Very few larvae entered diapause at 25°C or 30°C (table 17), probably because the stock had been bred continuously for many generations at 25°C. At 20°C, a high percentage of larvae entered diapause when daylength was between 8 and 12

TABLE 15. Development of storage moths (laboratory stocks) on different quantities of food in jars 8 cm in diameter

SPECIES	FOOD DEPTH (cm)	320 INSECTS/CULTURE			80 INSECTS/CULTURE			p VALUE FOR 80 AND 320 MEANS
		SURVIVAL TO ADULT STAGE(%)	TIME FOR DEVELOP-MENT (DAYS)		SURVIVAL TO ADULT STAGE(%)	TIME FOR DEVELOP-MENT (DAYS)		
			RANGE	MEAN & S.D.		RANGE	MEAN & S.D.	
Plodia interpunctella	7	88	29-48	35.3 ± 2.6	93	32-43	36.3 ± 1.8	0.1%
	5	87	30-47	35.2 ± 2.4	85	32-42	36.0 ± 2.3	5 %
	3	87	30-50	36.4 ± 2.7	96	31-44	36.6 ± 2.2	-
	2	76	30-48	37.8 ± 2.4	91	32-45	37.2 ± 2.3	-
Ephestia cautella	7	61	30-58 (26*)	35.6 ± 2.3 50.4 ± 3.2	79	32-56 (2*)	36.7 ± 2.1	0.1%
	5	66	32-55 (22*)	35.7 ± 2.4 50.0 ± 3.1	80	33-43 (0*)	36.8 ± 2.1	0.1%
	3	68	31-57 (24*)	36.3 ± 2.2 51.6 ± 2.9	84	32-53 (2*)	36.5 ± 2.0	-
	2	47	32-58 (19*)	36.0 ± 2.5 50.3 ± 3.4	81	32-47 (3*)	36.9 ± 2.0	1 %
Ephestia kuehniella	7	83	38-59	43.6 ± 3.3	93	39-55	44.4 ± 3.4	-
	5	89	38-62	43.2 ± 3.7	88	39-58	44.1 ± 3.5	-
	3	87	38-66	43.5 ± 2.9	91	41-60	44.9 ± 3.1	1 %
	2	81	39-63	43.9 ± 3.2	88	40-52	44.9 ± 2.9	2 %
Ephestia elutella	7	78	40-71	45.3 ± 3.0	90	41-63	45.9 ± 2.8	-
	5	82	40-60	45.1 ± 2.8	86	42-56	46.2 ± 2.7	2 %
	3	79	40-67	45.7 ± 3.0	83	41-67	45.9 ± 3.1	-
	2	62	40-69	46.1 ± 3.4	91	43-59	46.7 ± 2.7	-

* Number of adults emerging after 44th day. Mean development times are quoted for individuals emerging by 44th day (1st line) and, for 320-insect cultures, for individuals emerging later (2nd line).

TABLE 16. Percentage survival of storage moths to adult stage in jars 8 cm in diameter set up with 50 g of food and different numbers of newly hatched larvae.

NO. OF LARVAE ADDED	Plodia interpunctella	Ephestia kuehniella	Ephestia elutella	Ephestia cautella
100	93	92	85	82
100	96	83	90	86
200	93	84	78	76
200	92	83	84	85
400	85	78	75	66
400	85	80	72	62
800	65	62	51	23

Fig. 22. Population density in Pyralid moth cultures and maximum temperature rise.

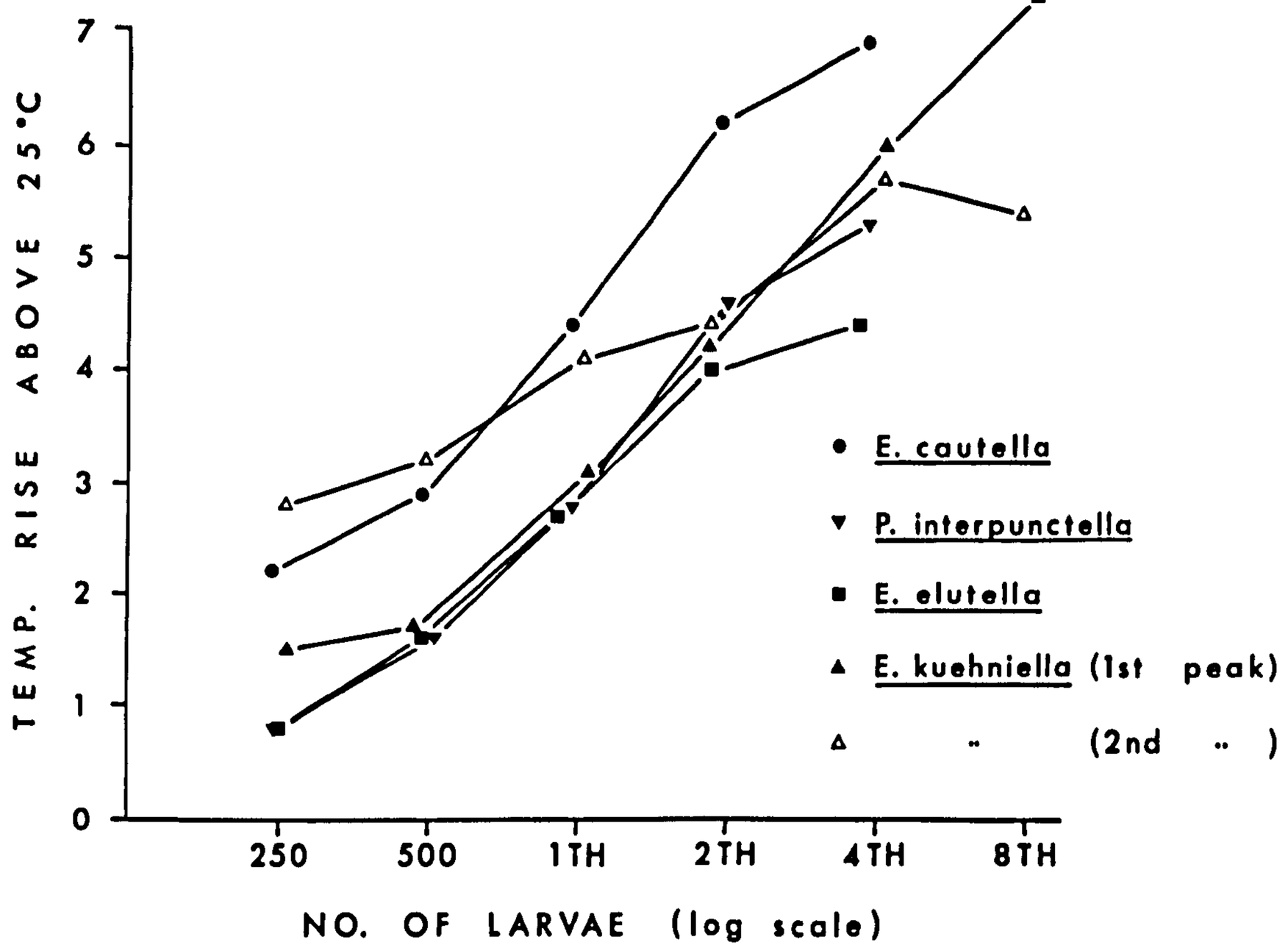

TEMP. RISE ABOVE 25°C
0
1
2
3
4
5
6
7
250
500
1TH
2TH
4TH
8TH
NO. OF LARVAE (log scale)
E. cautella
P. interpunctella
E. elutella
E. kuehniella (1st peak)
(2nd ..)

Fig. 23. Plodia interpunctella: Population density and heat movements in 3-1 jar cultures.

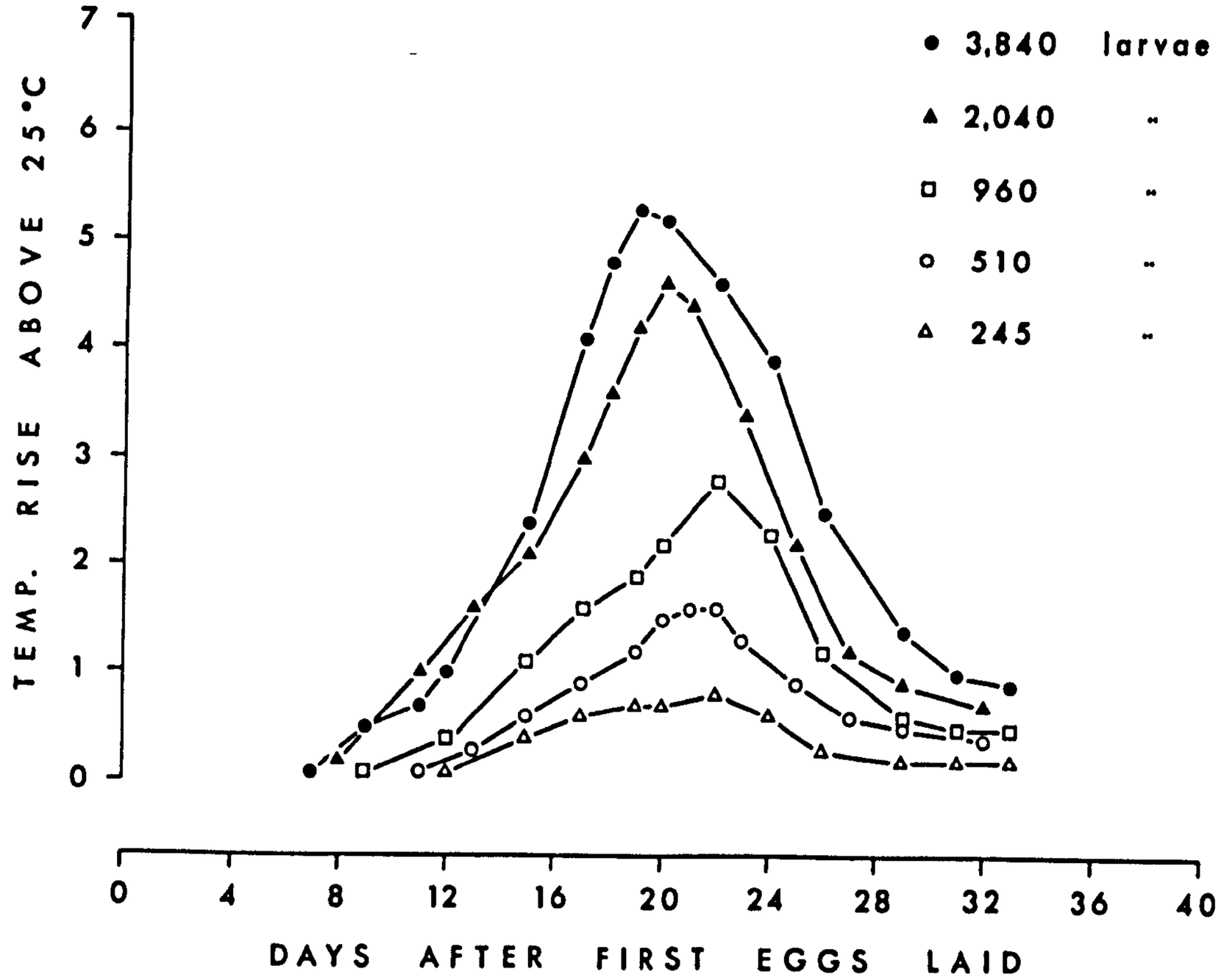

TEMP. RISE ABOVE 25°C
0
1
2
3
4
5
6
7
0
4
8
12
16
20
24
28
32
36
40
DAYS AFTER FIRST EGGS LAID
● 3,840 larvae
▲ 2,040 "
□ 960 "
○ 510 "
△ 245 "

Fig. 24. Ephestia kuehniella: Population density and heat movements in 3-1 jar cultures.

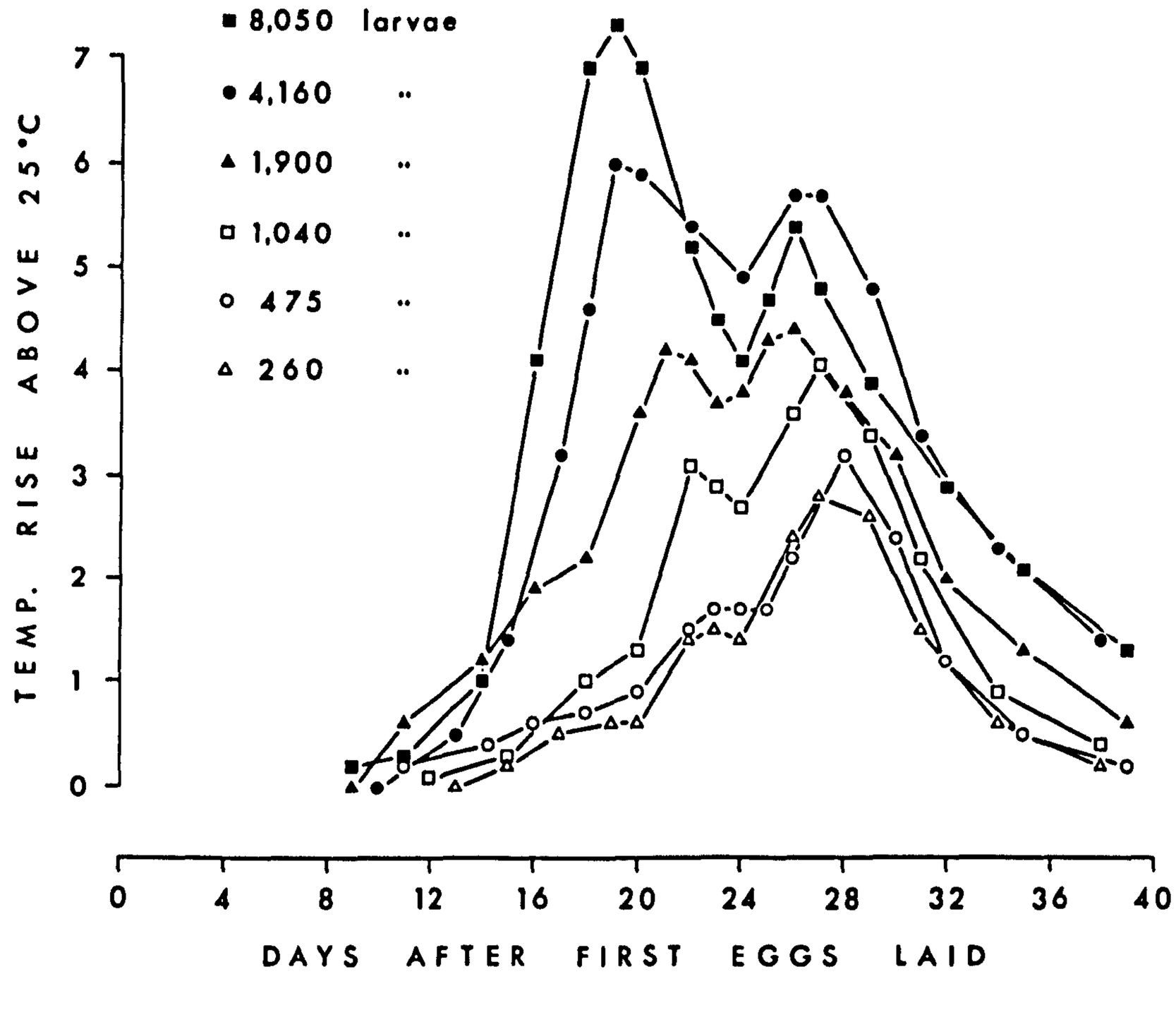

■ 8,050 larvae
● 4,160 ..
▲ 1,900 ..
□ 1,040 ..
○ 475 ..
△ 260 ..
TEMP. RISE ABOVE 25°C
0
1
2
3
4
5
6
7
0
4
8
12
16
20
24
28
32
36
40
DAYS AFTER FIRST EGGS LAID

Fig. 25. Ephestia cautella: Population density and heat movements in 3-1 jar cultures.

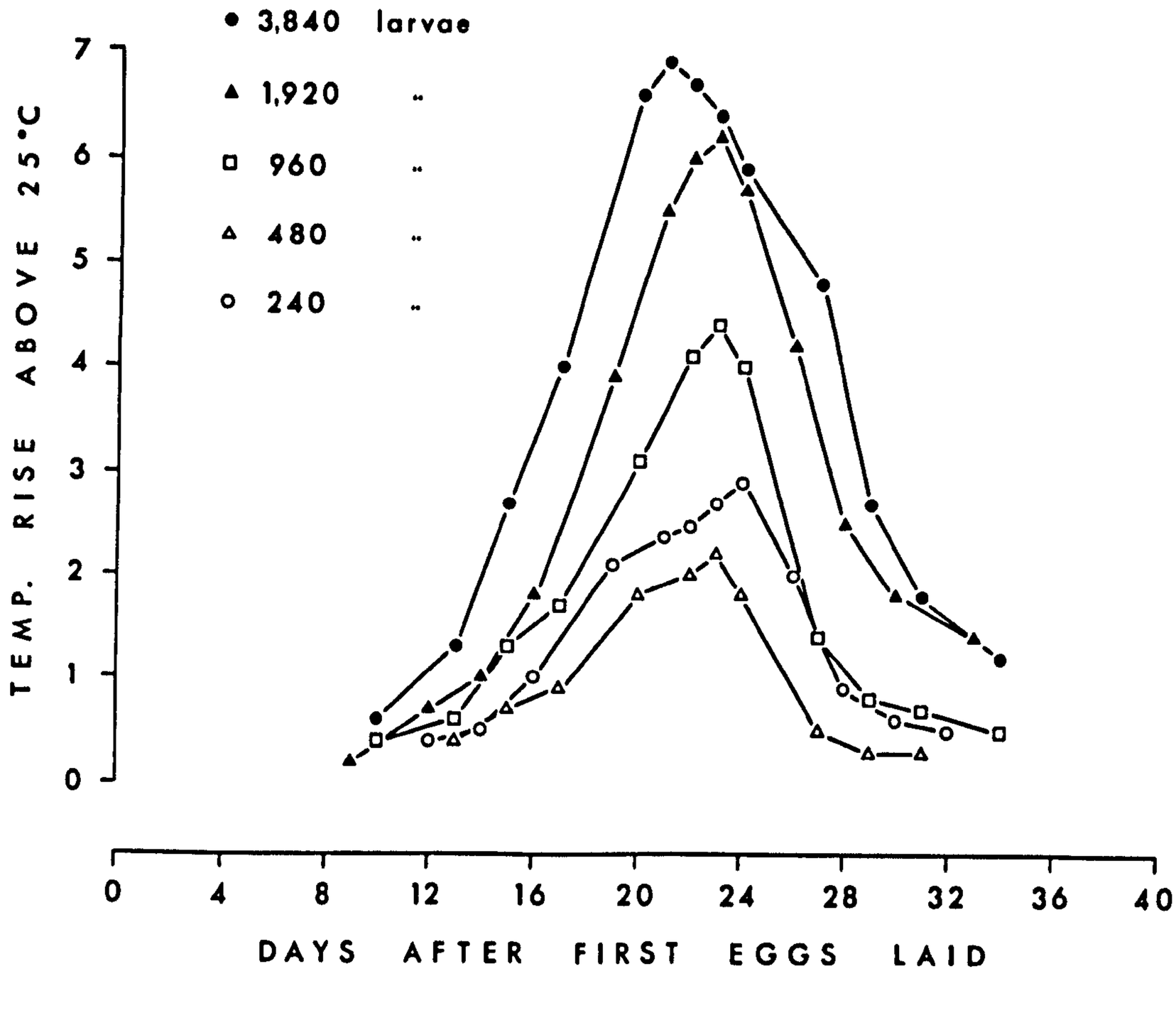

● 3,840 larvae
▲ 1,920 "
□ 960 "
△ 480 "
○ 240 "
TEMP. RISE ABOVE 25°C
0
1
2
3
4
5
6
7
0
4
8
12
16
20
24
28
32
36
40
DAYS AFTER FIRST EGGS LAID

Fig. 26. Ephestia elutella: Population density and heat movements in 3-1 jar cultures.

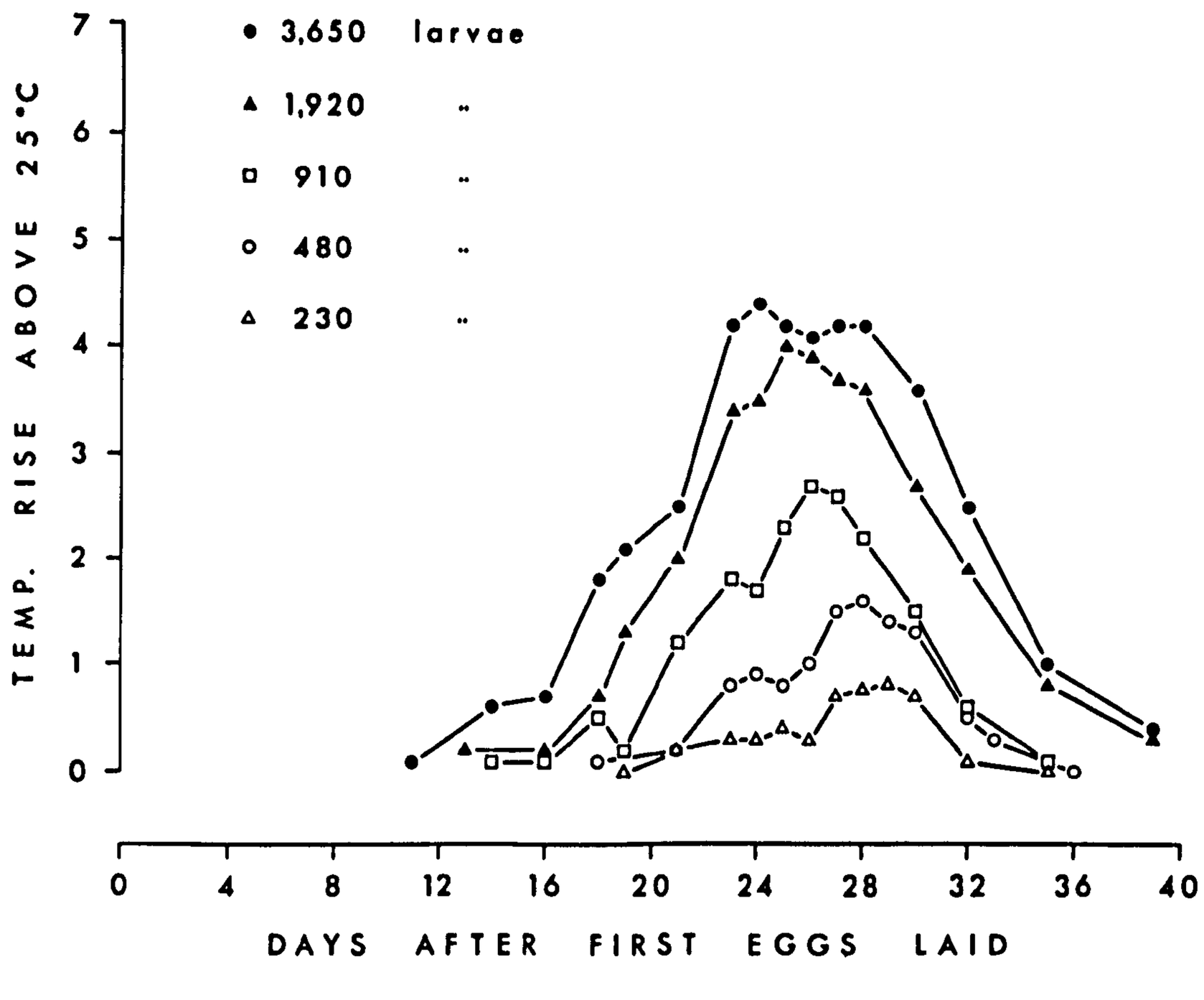

• 3,650 larvae
▲ 1,920 "
□ 910 "
○ 480 "
△ 230 "
TEMP. RISE ABOVE 25°C
0
1
2
3
4
5
6
7
0
4
8
12
16
20
24
28
32
36
40
DAYS AFTER FIRST EGGS LAID

TABLE 17. The induction of diapause in the laboratory stock of Ephestia clutella at various temperatures, and under various lighting conditions (all experiments at 70% RH)

TEMPERATURE °C	HOURS LIGHT PER DAY (AT 200-1000 LUX)		NO. IN BATCH	% MORTALITY	% OF BATCH ENTERING DIAPAUSE
30	0		69	7.2	0
25	0		66	4.5	1.5
	8		645*	1.7	4.1
	16		140	2.1	0
20	0		297	8.8	30.6
	0	(For 5wk, then 8Hr)	139	3.6	43.2
	8		778/	1.6	84.4
	8	(At < 1 lux)	47	2.1	51.1
	12		82	1.2	90.2
	14		67	3.0	34.3
	16		88	0	5.7
	16	(At < 1 lux)	86	1.2	2.3
	16	(At < 1 lux incl. 8 at 400 lux)	202	0.5	5.5
	24		43	0	2.3
	24	(At < 1 lux)	45	0	2.2
	12	(Shortening in 6 wks to 9.5)	274	1.5	88.7
	15.5	(Shortening in 6 wks to 13)	43	0	7.0
15	0		61	1.6	96.7
	24		58	1.7	81.0
2) Weeks at 3) 25°C before 4) dropping to 20°C	0 at 25°C (8 at 20°C (		133	1.5	83.5
			138	1.4	71.0
			274	1.5	4.0

* Results of 4 batches combined. In all four, diapause occurred in less than 7% of larvae present.

/ Results of 6 batches combined. Samples contained 79-267 larvae, and the percentage entering diapause varied from 79.4 - 91.1%.

hours. In continual darkness, or with a daylength of 14 hours, the proportion of insects entering diapause was considerably reduced, and with a daylength of 16 hours, or in continual light, only 2 - 6% of larvae exposed entered diapause. Progressively shortening daylengths at 20°C from 15.5 hours light at hatching to about 13 hours six weeks later, at which time pupation was proceeding, produced a similarly low percentage of diapausing individuals.

Continual light or 16-hour photoperiods of approximate intensity 1 lux were as effective as intensities of over 200 lux in encouraging continuous development, while an 8-hour photoperiod of intensity 1 lux was less effective in stimulating diapause than similar photoperiods at higher light intensities. The presence of an 8-hour photoperiod at 400 lux during a 16-hour photoperiod at 1 lux, did not, however, increase the proportion of larvae entering diapause.

Retaining insects for up to three weeks from egg laying in darkness at 25°C had little effect on the subsequent induction of diapause at 20°C. Of insects retained 4 weeks at 25°C, very few entered diapause at 20°C. Similarly, holding in darkness at 20°C for five weeks from egg laying, and then exposing to an 8-hour daylength, produced a much smaller proportion of diapausing larvae than when short daylength was experienced earlier in development.

At 15°C, high percentages of larvae entered diapause under continual light or continual darkness.

ii) <u>The field stock</u>

Results are summarised in table 18. At 30°C, some larvae entered diapause in darkness, but no diapause occurred under a 16-hour photoperiod. At 25°C, diapause did not occur in unchanging daylengths of 15-20 hours, or in daylengths progressively shortening from 16 hours at egg laying to 14 hours at pupation, but

a few individuals entered diapause in continual light. About 50% of insects reared in continual darkness, and over 95% of insects reared at daylengths of 8-13 hours, entered diapause.

At 20°C, a high proportion of larvae entered diapause under all light conditions tested. Apart from a small mortality, the response was total in continual darkness, or under daylengths of 12 hours or less. Light systems giving intensities of over 200 lux, and those giving intensities of no more than 1 lux, gave comparable results.

At 15°C, all insects that survived entered diapause under continual light or continual darkness.

Experiments were performed to locate the sensitive phase. When insects were maintained at 25°C under a 16-hour daylength for up to 18 days from egg laying, all entered diapause under an 8-hour daylength at 20°C. Samples kept for 21-28 days at 25°C before exposure to an 8-hour photoperiod at 20°C, showed a progressive reduction in the proportion of insects entering diapause, and after 31 days under long daylength at 25°C, by which time some pupation would have occurred, no insects entered diapause at all. In another experiment, larvae were reared from egg laying under a 13-hour daylength at 25°C and were transferred at intervals to a 16-hour daylength. The subsequent patterns of emergence were similar in samples remaining up to 28 days under the 13-hour daylength, but in samples remaining under this daylength 31 days or longer, many larvae delayed pupating, and the emergence was more protracted (fig. 27).

B. Humidity and the Induction of Diapause in Ephestia elutella

At 30°C, no diapause occurred in the laboratory stock regardless of light or humidity (table 19). A total of 159 insects were tested. In the field stock,

TABLE 18. The induction of diapause in the field stock of Ephestia elutella under various conditions of temperature and light. (All experiments at 70% RH)

TEMPERATURE (°C)	HOURS LIGHT/DAY (AT 200-1000 LUX)		NO. IN BATCH	% MORTALITY	% ENTERING DIAPAUSE
30	0		402	3.5	23.1
	16		135	1.5	0
25	0		178	5.1	48.9
	8		129	2.3	97.0
	12		132	1.5	96.2
	13		126	1.6	96.8
	14		70	2.9	57.1
	15		60	1.7	0
	16		66	0	0
	18		67	3.0	0
	20		66	1.5	0
	24		60	5.0	5.0
	16	(shortening in 5 wks to 14)	65	1.5	0
20	0		342*	1.5	98.5
	8		676*	0.9	99.1
	12		134	1.5	98.5
	14		62	3.2	91.9
	16		66	1.5	87.9
	16	(at < 1 lux)	68	2.9	76.5
	24		69	1.4	94.2
	24	(at < 1 lux)	67	0	95.5
15	0		42	4.8	95.2
	24		62	1.6	98.4
15)			) 135	1.5	98.5
18) Days	16 at 25°C		) 130	0.8	99.2
21) at 25°C			) 138	1.4	84.2
23) before	8 at 20°C		) 66	0	81.8
25) dropping			) 65	1.5	70.8
28) to 20°C			) 133	2.3	42.9
31)			) 62	1.6	0

* Results for several batches combined. All surviving insects entered diapause.

Fig. 27. Pupation of Ephestia elutella (field stock) larvae under a 16-hour daylength at 25°C, after holding from oviposition under a 13-hour daylength for 28 and 31 days.

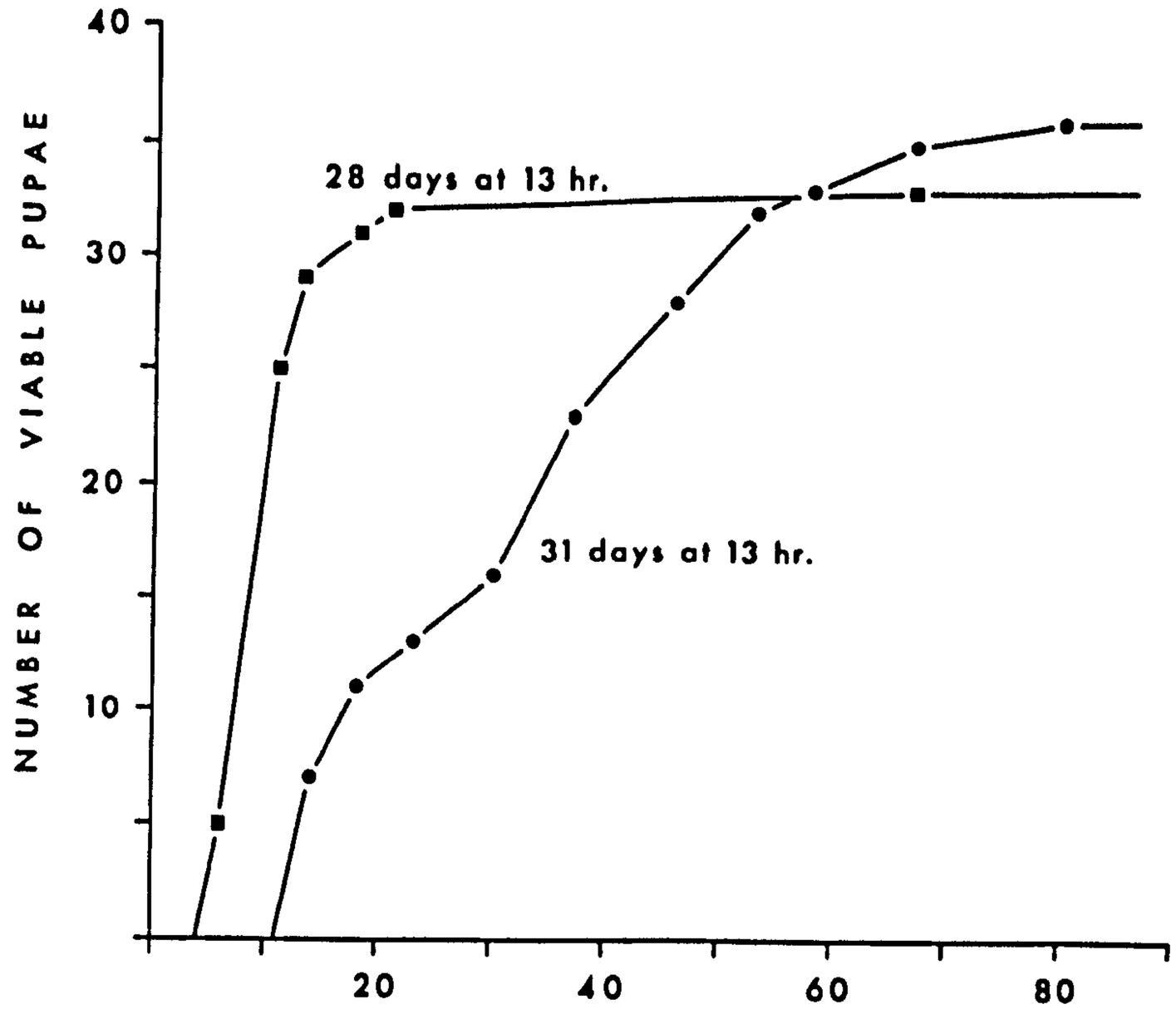

40
30
20
10
NUMBER OF VIABLE PUPAE
28 days at 13 hr.
31 days at 13 hr.
20
40
60
80
DAYS AT 25°C

TABLE 19. Mortality and the induction of diapause in Ephestia elutella at two humidities

	FIELD STOCK AT 30°C DARK		LABORATORY STOCK AT 15°C CONTINUAL LIGHT	
HUMIDITY (% RH)	25	70	25	70
NUMBER IN BATCH	44	46	44	46
% MORTALITY (INCLUDING DEATHS OF 1st INSTAR LARVAE)	15.9	13.0	84.1	41.2
% OF SURVIVORS ENTERING DIAPAUSE	45.9	22.5	71.4	74.1

low humidity increased the proportion of insects entering diapause in continual darkness. At 15°C, all surviving larvae of the 250 tested entered diapause regardless of light or humidity, while in the laboratory stock little difference occurred in the proportions of survivors entering diapause at 70% or 25% RH, although mortality and development time were increased at the lower humidity (c.f. Section 1.A.iii).

C. Induction by Temperature and Light in Plodia interpunctella

i) The laboratory stock

Diapause was induced by rearing at 20°C, or lowering the temperature from 25°C to 20°C during the first 17 days after oviposition, provided that the daylength was 12 hours or less (table 20). With a 14 or 16-hour daylength at 20°C, very few or no insects entered diapause. Even at an intensity of 1 lux at the surface of tubes containing insects, a 16-hour photoperiod was effective in encouraging continuous development. Under continual light at 20°C, diapause occurred in a small percentage of the insects exposed. No larvae kept at 25°C for 20 days or longer, regardless of daylength, entered diapause. Like the laboratory stock of E.elutella this stock had been bred continuously at 25°C for many years.

ii) The field stock

Diapause was induced in batches transferred from a 16-hour daylength at 25°C to 20°C up to 18 days from egg laying (table 21). The response was nearly total in batches moved to an 8-hour daylength at 20°C after 12 or 15 days at 25°C, but was much reduced in batches moved after 18 days. No insects entered diapause in a batch moved after 21 days. Dropping larvae to darkness, continual light, or a 12-hour photoperiod at 20°C after 12 days at 25°C, proved as effective in inducing diapause as dropping to the 8-hour photoperiod. In contrast, a photoperiod shortening by $\frac{1}{4}$ minutes a day from 14.5 hours, or constant 14 or 16-hour photoperiods, were effective in preventing diapause. Fewer than 12% of

larvae dropped from 25°C to these photoperiods at 20°C entered diapause. Similar results were obtained with a 16-hour photoperiod of light intensity no more than 1 lux to one at 400 lux.

When insects reared for 10 days at 30°C were dropped 10 days after egg laying to a 16-hour daylength at 20°C, a much greater proportion entered diapause than in batches dropped from 25°C (table 21).

At 25°C, some larvae reared in continual light or darkness, or under photoperiods of 13 hours or less, entered diapause. No diapause occurred in batches exposed to darkness or continual light at 30°C, or to a 14 or 16-hour photoperiod at 25°C.

D. Population Pressure

i) Ephestia elutella and Plodia interpunctella

Batches of the field stock of E. elutella reared at densities of 2, 4, 8 and 12 larvae per gram of food, gave similar percentages of larvae entering diapause in darkness at 25°C (table 22). Mortality, however, increased with increasing density. In the laboratory stock, which was set up in darkness at 20°C, results were variable. Higher proportions of insects entered diapause in samples with 12 larvae/g of food than in samples of lower densities, but this result could have arisen by chance. In contrast with the field stock, mortality at all densities was low.

The proportion of larvae the field stock of P. interpunctella entering diapause at 25°C in batches with 10 larvae/g of food was much greater than in batches with 2 or 4 larvae/g. Mortality was low in all batches.

ii) Attempts to induce diapause in Ephestia cautella

Attempts to induce diapause were unsuccessful when samples reared at high larval

TABLE 20. The induction of diapause in the laboratory stock of Plodia interpunctella by temperature drops from 25°C to 20°C under various lighting conditions (all experiments at 70%RH)

DAYS AT 25°C	HOURS LIGHT PER DAY AT 20°C (AT 200-1000 LUX)	NO. IN BATCH	% MORTALITY	% OF BATCH ENTERING DIAPAUSE
0	0	50	8.0	90.0
	8	76	0	100
	16	48	0	0
6	8	48	2.1	97.9
	12	47	0	100
	14	48	2.1	2.1
	16	46	2.2	0
	16 (at < 1 lux)	46	2.2	8.7
	24	43	2.3	7.0
12	0	406	1.2	98.3
	8	46	0	100
15	0	71	1.4	84.5
	8	412	1.2	97.3
	12	48	4.2	95.8
	16	47	0	2.1
17	0	69	2.9	91.3
	8	135	3.7	94.1
20	8	71	1.4	0

TABLE 21. The induction of diapause in the field stock of Plodia interpunctella under various conditions of temperature and light (all experiments at 70% RH)

TEMPERATURE (FROM EGG LAYING)	HOURS LIGHT/DAY AT 200-1000 LUX)	NUMBER IN BATCH	% MORTALITY	% OF BATCH ENTERING DIAPAUSE
25°C for 12 days then 20°C	0	280	0.7	97.1
	8	551	1.3	97.3
	12	71	1.4	98.6
	14	68	0	5.9
	16	70	1.4	11.4
	16 (at < 1 lux)	68	1.5	7.4
	24	64	3.1	89.0
	24 (at < 1 lux)	67	0	94.0
	14.5 (shortening by 4 min/day)	69	0	8.7
30°C for 10 days then 20°C	16	66	1.5	36.4
25°C for 15 days then 20°C	8	274	1.5	95.2
25°C for 18 days then 20°C	8	141	0.7	29.1
25°C for 21 days then 20°C	8	45	0	0
25°C throughout development	0	163	6.7	29.5
	8	548	1.5	19.0
	12	62	1.6	37.1
	13	66	4.5	10.7
	14	71	2.8	0
	16	47	0	0
	24	140	0	24.3
30°C throughout development	0	47	6.4	0
	24	45	2.2	0

TABLE 22. Diapause in batches of Ephestia elutella and Plodia interpunctella larvae set up in darkness at different densities.

SPECIES AND STOCK	TEMP. °C.	LARVAE/g OF FOOD	TOTAL NO. OF INSECTS	% MORTALITY*	% OF BATCH ENTERING DIAPAUSE
E.elutella (LAB.STOCK)	20	2	70	10.0	21.4
		4	100	10.0	15.0
		8	96	11.5	16.7
		12	144	13.2	30.0
E.elutella (FIELD STOCK)	25	2	96	5.2	45.8
		4	96	12.5	55.2
		8	96	22.9	40.6
		12	144	31.9	43.8
P. interpunctella (FIELD STOCK)	25	2	168	9.5	28.5
		4	288	10.4	29.2
		10	600	13.7	51.5

* Mortalities include deaths of 1st instar larvae

density, or on food contaminated with faecal material, were subjected to temperature drops from 25 or 30°C to short daylength at 20°C (table 23). Lowering the temperature for 3 weeks during development to 15°C, which is near the threshold for development, caused 5 out of 84 insects to prolong the emergence at 25°C, but no larvae were observed in diapause.

E. Natural Autumn Conditions

i) Diapause in Ephestia elutella reared in outbuildings

Emergence of adults in tanks kept in a slightly heated outbuilding ceased during October in 1970, 1971 and 1972. In the building, the averages of daily mean temperatures during October in these years were 17.0, 17.5 and 16.5°C respectively.

Larvae of the laboratory stock set up at 25°C and moved a week after hatching to an unheated outbuilding at various times during September, either died as larvae, developed as far as the pupal stage, or entered diapause. A few adults appeared during November in a deformed condition, but most pupae formed failed to complete development. There was no evidence of pupation occurring in parallel batches of the field stock, and the overall mortality was only half that obtained in the laboratory stock (table 24).

ii) Diapause in Plodia interpunctella reared in outbuildings

Adult emergence in tanks ceased at the beginning of October in a slightly heated outbuilding in 1971 and 1972. In the building, the averages of daily mean temperatures during September in these years were 18.5 and 18.0°C respectively.

Larvae of the laboratory and field stocks moved a week after egg laying to an unheated outbuilding during mid September either died or entered diapause (table 24).

F. Induction of Diapause in Pieris brassicae, and Bombyx mori

All larvae of Pieris brassicae reared under an 8-hour daylength at 20°C gave rise to diapausing pupae. All larvae reared under a 16-hour daylength at 25°C gave rise to pupae that developed without an arrest.

Diapause in the eggs of the univoltine race of Bombyx mori was averted by immersing first-day eggs in 6 N hydrochloric acid for 6 minutes at 30°C. Mortality after treatment was less than 15%, and eggs hatched at 25°C in 11 to 16 days.

4. DURATION AND TERMINATION OF DIAPAUSE

Of the four Pyralid species, only Plodia interpunctella and Ephestia elutella entered diapause. The duration of diapause was generally shortened by exposure to long photoperiods, by raising the temperature, or by exposure to low temperatures. For each stock, the relative importance of each of these factors in terminating diapause differed widely from one combination of experimental conditions to another. Diapause in E. elutella was much longer than in P. interpunctella and, in each species, was longer in the field stock than in the laboratory stock. The method of induction influenced the subsequent intensity of diapause under certain conditions.

The diapauses of Pieris brassicae and Bombyx mori required extended periods of cold for rapid termination at 25°C. Fumigation speeded the termination of diapause in P. brassicae and E. elutella.

A. Light and Constant Temperature

The mean durations of diapause in E. elutella and P. interpunctella under all

TABLE 23. Attempts to induce diapause in Ephestia cautella by various conditions of acclimation pressure. (At 20°C, all samples maintained under an 8-hour daylength.) ND = No diapause

NO. OF LARVAE PER g FOOD	DAYS AT REARING TEMPERATURES 30°C →	25°C →	20°C →	15°C	NO. INSECTS IN BATCH	% MORTALITY
6 (Food comprised a 1:1 food/faecal pellet mixture)	-	7	STAY	-	72	22.9(ND)
	-	14	STAY	-	72	27.8(ND)
	-	21	STAY	-	72	25.0(ND)
6	10	0	STAY	-	72	15.3(ND)
	10	7	STAY	-	72	16.7(ND)
10	10	0	STAY	-	120	22.5(ND)
6	10	0	21	21 (then to 25°C)	96	16.7(ND)*

*5 insects prolonged the tail of the emergence by 2 or 3 weeks

TABLE 24. Induction of diapause in batches of Ephestia elutella and Plodia interpunctella by natural autumn conditions

SPECIES	STOCK	DATE MOVED TO NATURAL CONDITIONS	TOTAL NO. IN BATCH	% MORTALITY	% DIAPAUSE
E. elutella	LABORATORY	2.9.71	288	20.1	79.2
		9.9.71	144	18.7	80.6
		16.9.71	288	20.8	79.2
	FIELD	2.9.71	288	10.4	89.6
		9.9.71	288	9.4	90.6
		16.9.71	144	12.5	87.5
P. interpunctella	LABORATORY	16.9.71	288	16.7	83.3
	FIELD	16.9.71	288	9.7	90.3

light systems tested at each temperature permitting the induction of diapause, are set out in table 25. The reaction of laboratory and field stocks to temperature and light varied. In the field stock of E. elutella, diapause lasted longer at 20°C than at 15°C. This was not true of the laboratory stock in which diapause lasted longer in continual light at 15°C than at 20°C.

At 25°C, very few larvae of the laboratory stocks of either species entered diapause. In the field stocks, diapause was shorter at 25°C than at 15°C or 20°C under all light systems tested. With a 16-hour daylength, the mean duration of diapause at 25°C was in both species less than a quarter the value at 20°C. Results at each test temperature are now considered in more detail.

i) Ephestia elutella at 15°C

At this temperature, the emergence of insects that had entered diapause was extremely protracted and mortality during diapause was high (fig. 28). In both stocks pupation occurred earlier, and was better synchronised, in continual light than in darkness. Although differences were not significant at the 5% level, it was observed that the duration of diapause was a little less in the laboratory stock than in the field stock under both lighting conditions. The mean duration of diapause and standard deviation in continual light was 171 ± 36 days in the laboratory stock and 195 ± 69 days for the field stock and, under darkness, 253 ± 67 and 282 ± 87 days respectively. Batches in which distribution of pupation times failed the test for normality (cf. Methods Section 3. C.iv) were reanalysed omitting late stragglers. Recalculated means and ~~standard~~ S.D.'s were 187 ± 33 days for the field stock in continual light and, in darkness, 251 ± 50 days, and 270 ± 63 days, for laboratory and field stocks respectively.

ii) Ephestia elutella at 20°C

As very few individuals of the laboratory stock entered diapause at 20°C under 16 or 24-hour daylengths, batches were moved to these conditions from an 8-hour

daylength on the first day adults were seen.

Emergence under all lighting systems tested at 20°C was extremely protracted (table 26), and in darkness the average duration of diapause was longer than at 15°C. Results with an 8-hour daylength at 20°C closely resembled those for darkness, but with a 16-hour daylength, or continual light, diapause was terminated at a faster rate. The effect was more marked in the laboratory stock than in the field stock.

iii) Plodia interpunctella at 20°C

Results for P. interpunctella at 20°C did not closely resemble those for E. elutella. In both stocks emergence was more delayed, and mortality was higher, in darkness than in the presence of light (table 27). A 16-hour daylength was more effective than an 8-hour, or a 24-hour daylength in synchronising emergence. Under all conditions tested, the duration of diapause was less in the laboratory stock than in the field stock.

iv) Field stocks of Ephestia elutella and Plodia interpunctella at 25°C

In both species, diapause was terminated most rapidly at 25°C under a 16-hour daylength (table 28). Apart from a few stragglers, about three months were sufficient for pupation in E. elutella and about 6 weeks were sufficient for P.interpunctella. In the batch of P. interpunctella under a 16-hour daylength, pupation was assessed daily for the first 7 weeks. Continual light was more effective than an 8 or 13-hour daylength in reducing the duration of diapause in E. elutella, while in P. interpunctella diapause lasted a little longer under continual light than under an 8-hour daylength, the means being significantly different at the 5% level. The distribution of pupation times was often extended by a long tail of late stragglers.

TABLE 25. Effect of temperature and daylength on the mean time (days) between cessation of feeding and pupation in diapausing larvae

STOCK	HR LIGHT PER DAY	Ephestia elutella				Plodia interpunctella	
		15°C	20°C	25°C	30°C	20°C	25°C
LAB.	0	253	254	-	-	79	-
	8	-	253	-	-	56	-
	16	-	137	-	-	47	-
	24	171	148	-	-	62	-
FIELD	0	282	301	231	87	108	76
	8	-	286	204	-	73	69
	13	-	-	206	-	-	-
	16	-	237	55	51	68	15
	24	195	233	114	-	89	83

Fig. 28. Times for pupation of diapausing larvae of laboratory (L) and field (F) stocks of Ephestia elutella in light and darkness at 15°C. (Mortality was 40 and 30% in continual light, and 36 and 44% in darkness in L and F stocks respectively).

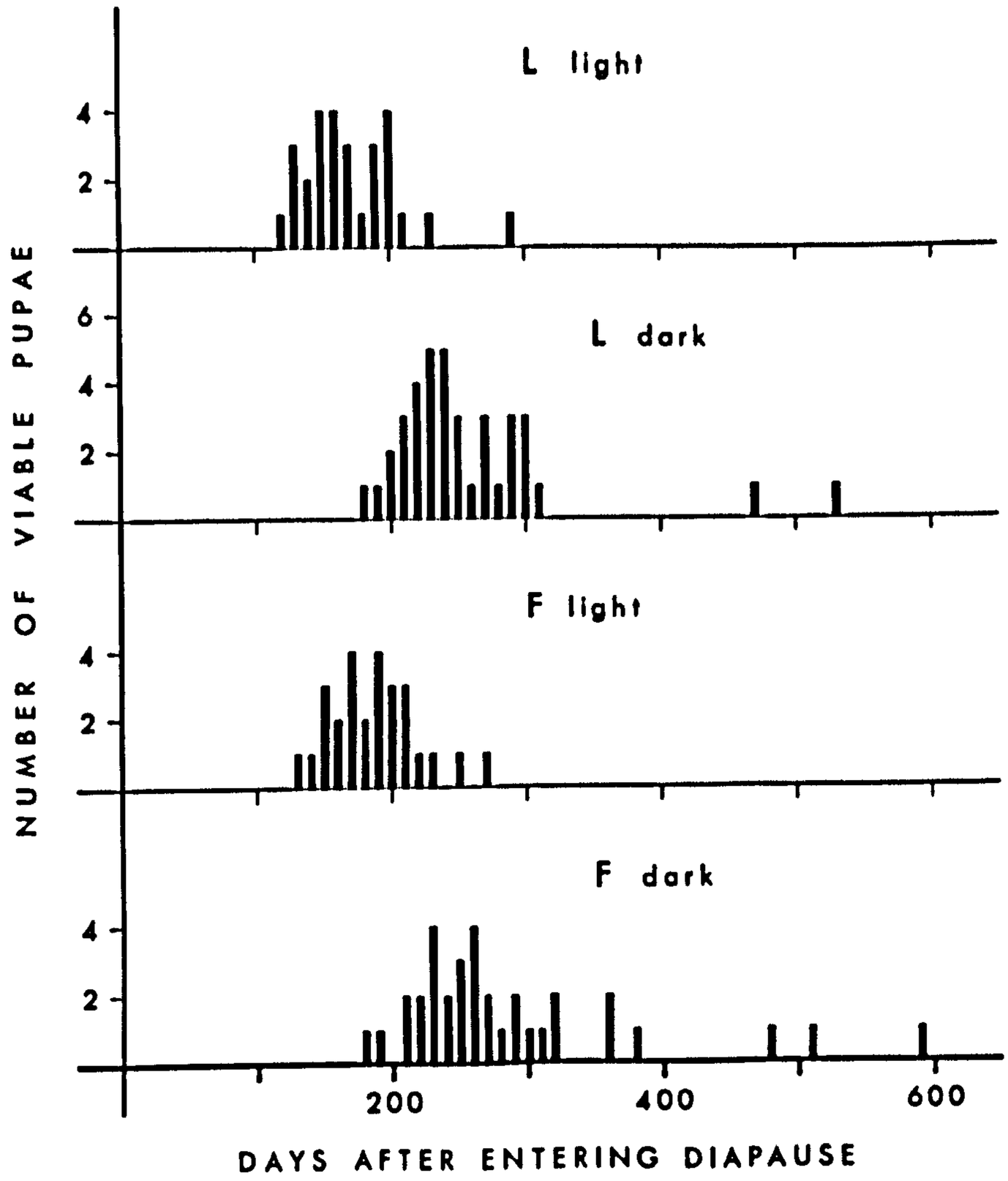

L light
L dark
F light
F dark
NUMBER OF VIABLE PUPAE
4
2
6
4
2
4
2
4
2
200
400
600
DAYS AFTER ENTERING DIAPAUSE

TABLE 26. Days required for pupation of diapausing larvae of Ephestia elutella under various light conditions at 20°C (based on counts performed at least three times a week)

STOCK	HOURS LIGHT PER DAY	NO. IN BATCH	% MORTALITY	RANGE OF PUPATIONS	MEAN AND S. D.	50% PUPATION	MIN. RANGE FOR 80% PUPATION
LAB	0	44	32	77-508	254 ± 64	252	190-318
	8	63	30	88-471	253 ± 60	248	190-310
	16	49*	29	49-301	137 ± 40	136	104-185
	24	33*	27	57-275	148 ± 34	142	93-198
FIELD	0	60	35	159-536	301 ± 88	284	194-419
	8	52	33	135-507	286 ± 85	269	183-400
	16	61	28	110-463	237 ± 66	229	173-313
	24	67	31	119-495	233 ± 62	226	181-322

* Diapause firstly induced under an 8-hour daylength at 20°C

TABLE 27. Days required for pupation of diapausing larvae of Plodia interpunctella under various lighting conditions at 20°C (based on counts performed at least three times a week)

STOCK	HOURS LIGHT PER DAY	NO. IN BATCH	% MORTAL-ITY	RANGE OF PUPATIONS	MEAN AND S. D.	50% PUPA-TION	MIN. RANGE FOR 80% PUPATION
LAB	0	46	48	34-115	79 ± 25	84	38-103
	8	76	26	17-106	56 ± 21	49	38-94
	16	33*	15	15-91	47 ± 19	43	22-73
	24	40*	20	21-109	62 ± 23	56	32-94
FIELD	0	52	25	41-244	108 ± 51	101	52-190
	8	60	8	41-162	73 ± 28	59	49-120
	16	50*	6	36-169	68 ± 22	57	40-92
	24	37	8	38-169	89 ± 37	78	46-144

* Diapause firstly induced under an 8-hour daylength at 20°C

TABLE 28. Days required for pupation of diapausing larvae of Ephestia elutella and Plodia interpunctella under various light conditions at 25°C (based on counts performed at least three times a week)

SPECIES	HOURS LIGHT PER DAY	NO. IN BATCH	% MORTAL-ITY	RANGE OF PUPATIONS	MEAN AND S. D.	50% PUPA-TION	MIN. RANGE FOR 80% PUPATION
EPHESTIA	0	87	48	94-454	231 ± 73	223	148-316
(FIELD	8	59	32	86-369	204 ± 72	197	124-301
STOCK)	13	59	36	85-375	206 ± 75	198	121-302
	16	65*	6	18-201	55 ± 29	49	26-78
			Mean and S.D. recalculated ignoring last 3 stragglers:		53 ± 22		
	24	46*	33	46-278	114 ± 50	112	72-177
PLODIA	0	48	18	18-207	76 ± 32	72	33-102
(FIELD	8	61	12	7-159	69 ± 26	66	31-97
STOCK)	16	43*	5	5-54	15 ± 8	12	8-20
			Mean and S.D. recalculated ignoring last 3 stragglers:		13 ± 5		
	24	34	12	30-176	83 ± 31	78	51-122

* Diapause firstly induced under short daylength at 25°C

v) The field stock of Ephestia elutella at 30°C

At 30°C, no diapause occurred in either of the stocks of P. interpunctella, or in the laboratory stock of E. elutella. In the field stock of E. elutella (fig. 29), diapause induced at 30°C ended rapidly under a 16-hour photoperiod, but was slow to terminate in darkness. The mean and standard S. D. for the duration of diapause in darkness was 87 ± 27 days, and under the 16-hour photoperiod was 51 ± 22 days.

B. Light and Temperature Increases

After an increase in temperature, the termination of diapause was hastened in all stocks. The effect of the temperature rise itself on batches of the field stock was found by comparison of the duration of diapause in samples reared continuously at the temperature to which the batches were raised. In E. elutella, no differences in the duration of diapause at 25° or 30°C caused by a temperature rise were significant at the 5% level. In P. interpunctella, a temperature rise proved instrumental in terminating diapause at 25°C under all light systems tested. Results for each species are now considered in more detail.

i) Ephestia elutella

For experiments on temperature rises, both stocks were reared under an 8-hour daylength at 20°C. In both stocks, a 5°C rise to darkness at 25°C reduced the mean duration of diapause, but achieved no more synchronisation of pupation than switching to a 16-hour daylength at 20°C (tables 29 and 30). A 10°C rise to darkness at 30°C reduced diapause duration to a greater extent, but was not so effective in synchronising emergence as a temperature rise of 5°C to a 16-hour daylength at 25°C. In the laboratory stock, but not in the field stock, continual light at 25°C was almost as effective as a 16-hour daylength in terminating diapause. The duration of diapause in both stocks was reduced to a minimum in batches raised 10°C to a 16-hour daylength at 30°C.
In the field stock, the duration of diapause in batches raised 5°C to a 16-hour daylength or continual light at 25°C (table 30), was a little less than in

batches reared continuously at 25°C (table 28). Under continual darkness, the duration of diapause was short in some individuals experiencing a 5°C rise, and the total range for pupation times was greater than in batches reared continuously in darkness at 25°C. Comparing samples lifted 10°C to 30°C, with those reared continuously at 30°C, revealed only slight differences in the duration of diapause. No differences caused by a temperature rise were found to be significant at the 5% level, firstly because of the very wide standard errors resulting from a general lack of synchronisation in pupation time, and secondly because of the very small differences in mean pupation time occurring between samples exposed to a 16-hour daylength, the only light condition affording a reasonable amount of synchronisation.

ii) Plodia interpunctella

For experiments on temperature rises, diapause in P. interpunctella was induced in darkness at 20°C. Although diapause in P. interpunctella was of much shorter duration than in E. elutella, the overall pattern of response in the two laboratory stocks was similar. Long daylength at high temperature proved the most powerful stimulus for the termination of diapause (table 31). A 10°C rise to a 16-hour daylength at 30°C terminated diapause most rapidly. Exposure to only four 16-hour photoperiods at 25°C greatly reduced the duration of diapause in larvae of the laboratory stock in darkness at 20°C. Darkness at 30°C was less effective than a 16 or 24-hour daylength at 25°C in terminating diapause ($p = < 0.001$).

In contrast with the results for the field stock of E. elutella, in the field stock of P. interpunctella a temperature rise of 5°C proved instrumental in hastening the termination of diapause under all the light conditions tested (table 32). Batches brought to 25°C from 20°C completed pupation in about half the time required by equivalent batches reared continuously at 25°C (tables 28, 32).

The most notable difference between laboratory and field stocks occurred in the

Fig. 29. Effect of a 16-hour daylength on the time before pupation at 30°C in diapausing larvae of Ephestia elutella (field stock). (Mortality in darkness was 22%, and under the 16-hour daylength was 9%).

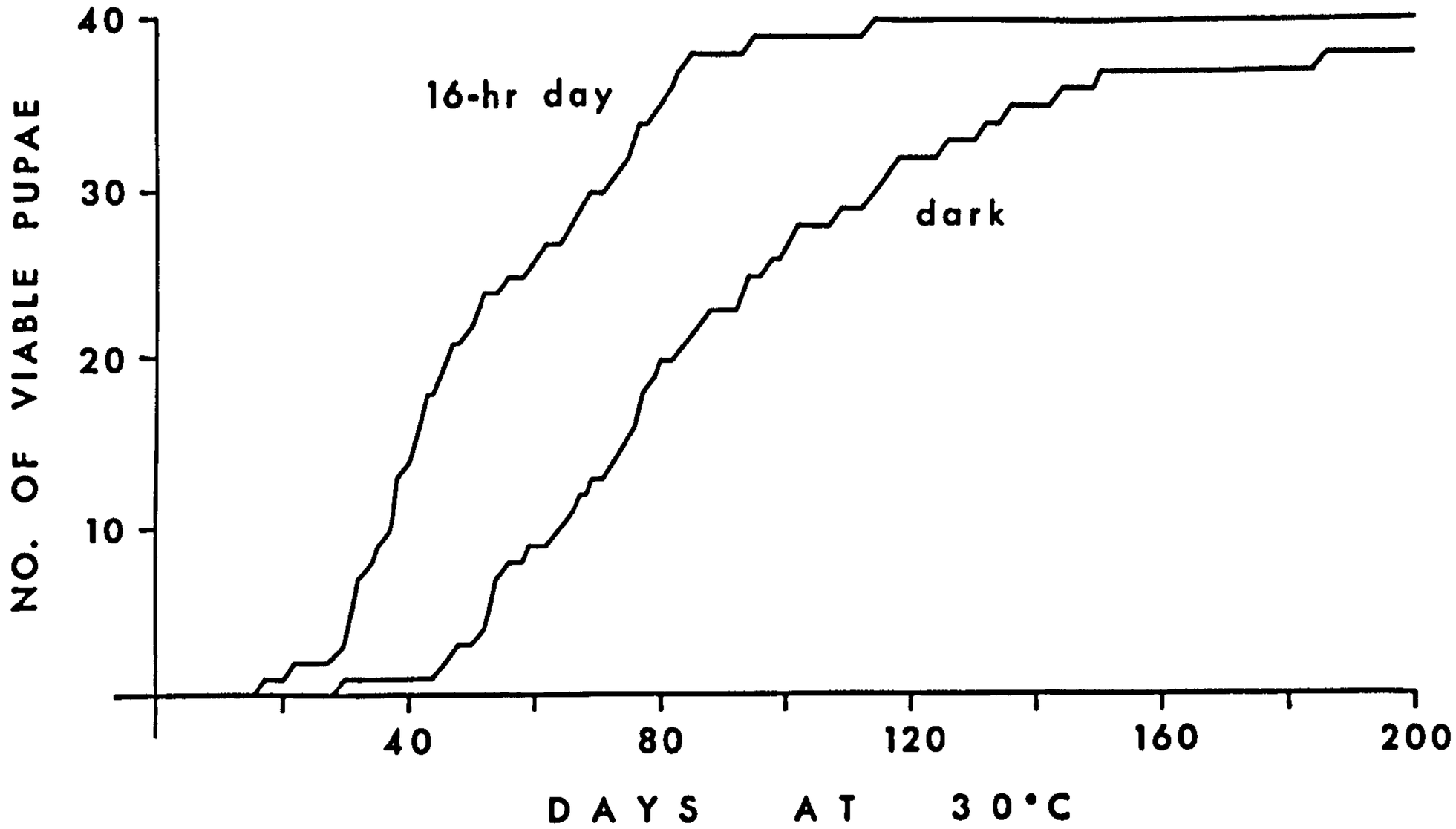

NO. OF VIABLE PUPAE
40
30
20
10
16-hr day
dark
40
80
120
160
200
DAYS AT 30°C

TABLE 29. Ephestia elutella (laboratory stock). Days required under various lighting conditions for pupation of diapausing larvae subjected to 5 and 10°C rises from an 8-hour daylength at 20°C (based on counts performed at least three times a week).

TEMP. RISE °C	HOURS LIGHT PER DAY	NO. IN BATCH	% MORT-ALITY	RANGE OF PUPATIONS	MEAN AND S. D.	50% PUPA-TION	MIN. RANGE FOR 80% PUPATION
0	8	63	30	88 - 471	253 ± 60	248	190 - 310
	16	49	29	49 - 301	137 ± 40	136	104 - 185
5	0	56	27	25 - 321	83 ± 58	69	25 - 120
	Mean and S.D. recalculated ignoring last 3 stragglers :				81 ± 35		
	16	195	8	13 - 67	38 ± 10	38	22 - 49
	24	44	9	19 - 83	39 ± 13	36	23 - 51
10	0	59	25	7 - 107	32 ± 24	26	13 - 49
	Mean and S.D. recalculated ignoring last 4 stragglers :				29 ± 12		
	16	45	22	9 - 49	24 ± 7	25	17 - 34

TABLE 30. Ephestia elutella (field stock). Days required under various light conditions for pupation of diapausing larvae subjected to 5 and 10°C rises from an 8-hour daylength at 20°C (based on counts performed at least three times a week).

TEMP. RISE °C	HOURS LIGHT PER DAY	NO. IN BATCH	% MORT-ALITY	RANGE OF PUPATIONS	MEAN AND S.D.	50% PUPA-TION	MIN. RANGE FOR 80% PUPATION
0	8	52	33	135 - 507	286 ± 85	269	183 - 400
	16	56	25	118 - 482	238 ± 51	229	186 - 308
5	0	50	30	50 - 431	202 ± 99	195	55 - 294
	16	232	8	19 - 217	53 ± 26	48	29 - 76
		Mean and S.D. recalculated ignoring last 7 stragglers :			51 ± 20		
	24	47	38	28 - 255	105 ± 46	100	64 - 142
		Mean and S.D. recalculated ignoring last straggler :			101 ± 37		
10	0	42	19	13 - 206	83 ± 40	84	28 - 114
	16	45	16	15 - 104	48 ± 17	46	29 - 64

TABLE 31. Plodia interpunctella (laboratory stock). Days required under various light conditions for pupation of diapausing larvae subjected to 5 and 10°C rises from darkness at 20°C (based on counts performed daily at 25°C or 30°C, and at least three times a week at 20°C).

TEMP. RISE °C	HOURS LIGHT PER DAY	NO. IN BATCH	% MORT-ALITY	RANGE OF PUPATIONS	MEAN AND S.D.	50% PUPA-TION	MIN. RANGE FOR 80% PUPATION
0	0	46	48	34 - 115	79 ± 25	84	38 - 103
* 5	16	44	18	3 - 112	32 ± 22	29	3 - 54
5	0	50	22	8 - 52	27 ± 10	24	16 - 42
5	16	82	12	3 - 21	9.4 ± 4.2	9	3 - 14
5	24	36	6	4 - 20	9.3 ± 3.9	9	4 - 14
10	0	38	11	9 - 24	13 ± 3	12	10 - 17
10	16	35	3	3 - 11	5.2 ± 1.5	5	4 - 7

* Kept at 25°C, 16 hr light, for 4 days only, then returned to darkness at 20°C.

TABLE 32. Plodia interpunctella (field stock). Days required under various light conditions for pupation of diapausing larvae subjected to 5 and 10°C rises from darkness at 20°C (based on counts performed daily at 25 or 30°C, and at least three times a week at 20°C).

TEMP. RISE °C	HOURS LIGHT PER DAY	NO. IN BATCH	% MORT-ALITY	RANGE OF PUPATIONS	MEAN AND S. D.	50% PUPA-TION	MIN. RANGE FOR 80% PUPATION
0	0	52	25	41 - 244	108 ± 51	101	52 - 190
5	0	46	7	7 - 105	30 ± 22	17	7 - 42
	Mean and S.D. recalculated ignoring last 5 stragglers :				23 ± 12		
	16	49	4	5 - 21	10 ± 3	9	7 - 14
	24	43	9	5 - 91	30 ± 24	18	7 - 49
	Mean and S.D. recalculated ignoring last 3 stragglers :				25 ± 17		
10	0	47	15	4 - 28	12 ± 6	11	5 - 16
	Mean and S.D. recalculated ignoring last straggler :				12 ± 5		
	16	41	5	3 - 12	6.3 ± 1.7	6	4 - 8

duration of diapause of batches raised to continual light at 25°C. In the field stock , the result was similar to raising to continual darkness at 25°C, while in the laboratory stock, the result was similar to raising to a 16-hour daylength.

C. Method of Inducing Diapause

i) Various population densities in the field stock of Plodia interpunctella

Histograms of the pupation occurring each week in batches reared at 2 larval densities in darkness at 25°C, after moving to a 16-hour daylength, are shown in fig. 30. Based on observations made at least three times a week, the mean and ~~standard~~ S. D. of the time required for pupation was 14 ± 7 days for both groups.

ii) Different combinations of temperature and light in the field stock of Plodia interpunctella

Diapause induced at 25°C was of greater duration at 25°C than diapause induced at 20°C (table 33). Movement to a 16-hour daylength at 25°C terminated diapause more rapidly, and synchronised emergence better, in samples reared in darkness at 20°C than in those reared in darkness at 25°C ($p = <0.001$). Large standard errors reduced the significance of difference between samples reared under 8-hour daylengths at the two temperatures, but a 'p' value of <0.01 was obtained when stragglers requiring over twice the first calculated mean time for pupation were omitted. No significant difference existed under the 16-hour daylength at 25°C between the means calculated for samples reared under an 8-hour daylength or in darkness at either 20 or 25°C.

In darkness, the mean duration of diapause in the sample reared and held at 25°C was significantly longer than in samples reared at 20°C, and which experienced a temperature rise ($p = <0.001$). Of the batches transferred to

a 16-hour daylength at 20°C, the batch subjected to a 10°C drop from 30°C took significantly longer than other batches to pupate ($p = <0.01$). Because of the low numbers of insects entering diapause under a 16-hour daylength at 20°C, even after a 10°C temperature drop, the size of this batch was small.

It was noted that of larvae reared at 20°C, in all tests those reared under an 8-hour daylength consistently had slightly longer mean pupation times than those reared in darkness. Under a 16-hour daylength at 20°C, the difference was significant ($p = <0.05$).

iii) Different combinations of temperature and light in the laboratory stock of P. interpunctella

The durations of diapause under a 16-hour daylength at 25°C of groups reared in an 8-hour daylength at 20°C after 6 or 15 days from egg laying at 25°C, or in darkness at 20°C after 15 days at 25°C, were very similar (fig. 31).

The means and ~~standard~~ S.D.'s for the duration of diapause in the three groups at 25°C under a 16-hour daylength was as follows:

15 days at 25°C, then 20°C dark	:	9.5 ± 4.2 days
15 days at 25°C, then 20°C 8 hr light	:	9.6 ± 4.3 days
6 days at 25°C then 20°C 8 hr light	:	9.7 ± 4.0 days

iv) Light and temperature in the field stock of Ephestia elutella

Batches of diapausing larvae were prepared under an 8-hour or 16-hour daylength, or in darkness at 20°C, and under a 13-hour daylength at 25°C.

Pupation under a 16-hour daylength at 25°C was mostly highly synchronised in the batch reared at 20°C in darkness, and was completed by the 16th week (fig. 32). In other groups, pupation was not completed until between the 27th and 31st weeks. A comparison of mean pupation times in all groups

Fig. 30. Pupation under a 16-hour daylength at 25°C of diapausing larvae of Plodia interpunctella (field stock) prepared in darkness at 25°C, at 2 population densities. (Mortality after entering diapause was less than 4% in both groups).

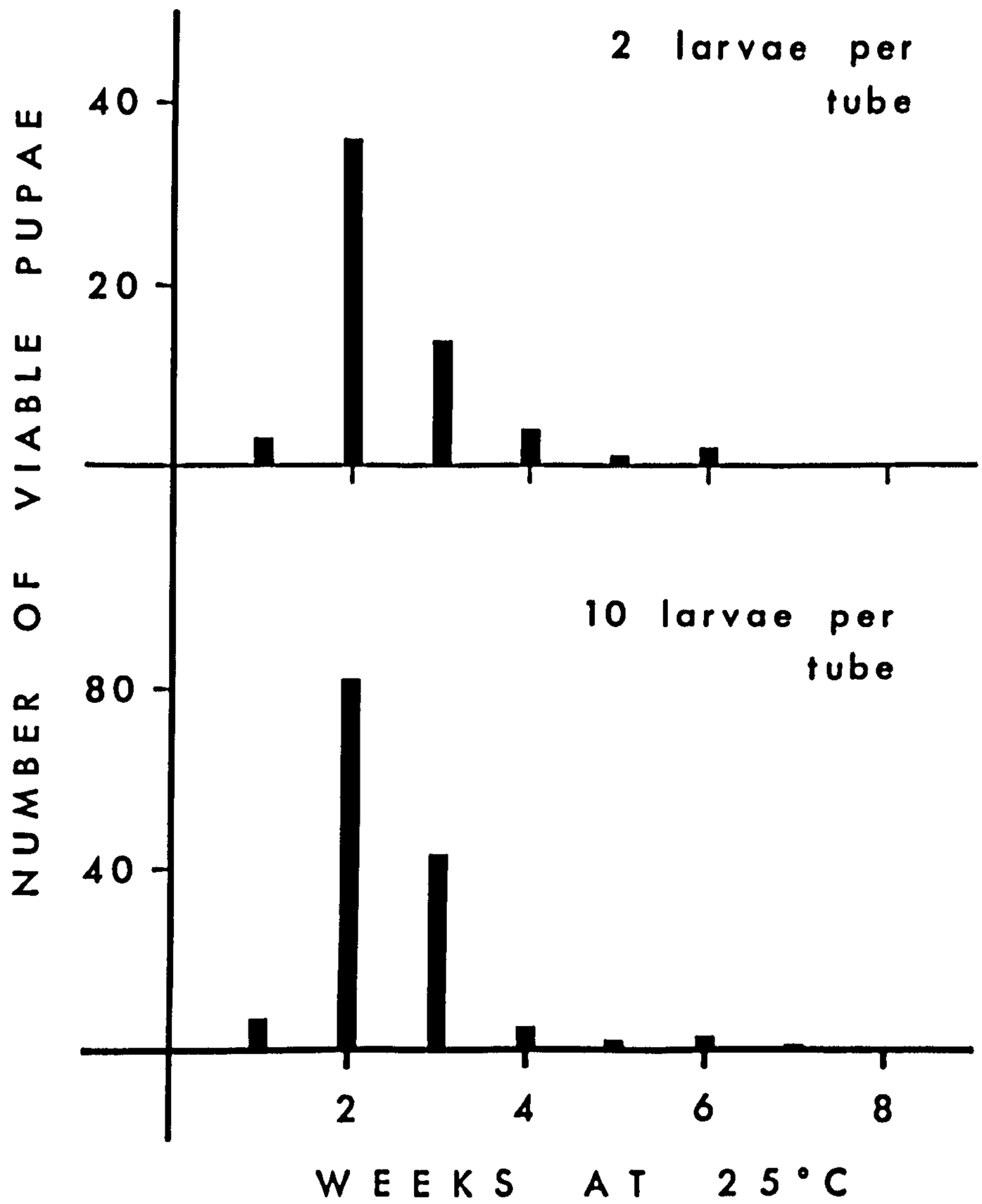

2 larvae per tube
10 larvae per tube
NUMBER OF VIABLE PUPAE
40
20
80
40
2
4
6
8
WEEKS AT 25°C

TABLE 33. Plodia interpunctella (field stock). Days required at 20 or 25°C under a 16-hour daylength, or at 25°C in darkness, for 10% to 90% of diapausing larvae reared under various temperature and light conditions (based on counts performed daily at 25 or 20°C, or at least three times a week at 20°C).

REARED AT		MOVED TO		NO. IN BATCH	% MORTALITY	RANGE OF PUPATIONS	MEAN AND S.D.	50% PUPATION	10% - 90% PUPATION FOR 80% PUPATION
TEMP. °C	LIGHT HR/DAY	TEMP. °C	LIGHT HR/DAY						
20	0	25	16	49	4	5 - 21	10 ± 3	9	7 - 14
	8			48	4	5 - 58	13 ± 9	10	7 - 18
				Mean and S.D. recalculated ignoring last 2 stragglers :			11 ± 5		
25	0			62	3	5 - 41	14 ± 7	12	8 - 19
				Mean and S.D. recalculated ignoring last 4 stragglers :			13 ± 5		
	8			43	2	5 - 54	15 ± 8	12	8 - 20
				Mean and S.D. recalculated ignoring last 3 stragglers :			14 ± 5		
20	0	25	0	46	7	7 - 105	30 ± 22	17	7 - 42
				Mean and S.D. recalculated ignoring last 5 stragglers :			23 ± 12		
	8			47	4	5 - 204	38 ± 49	15	5 - 49
				Mean and S.D. recalculated ignoring last 7 stragglers :			19 ± 14		
25	0			48	19	18 - 207	76 ± 32	72	33 - 102
20	0	20	16	51	6	39 - 128	60 ± 16	54	48 - 78
				Mean and S.D. Recalculated ignoring last straggler:			58 ± 12		
	8			50	6	36 - 169	68 ± 22	57	40 - 92
20*	16			24	4	16 - 182	89 ± 33	92	60 - 112
				Mean and S.D. recalculated ignoring last straggler:			86 ± 26		

* Batch dropped to 20°C after a week at 30°C.

Fig. 31. Effect of temperature and light conditions during rearing on the pupation time of diapausing larvae of Plodia interpunctella (laboratory stock) under a 16-hour daylength at 25°C. (Mortality after entering diapause was less than 8% in all groups).

a. 15 days at 25°C, then 20°C, dark.

b. 15 days at 25°C, then 20°C, 8-hour daylength.

c. 6 days at 25°C, then 20°C, 8-hour daylength.

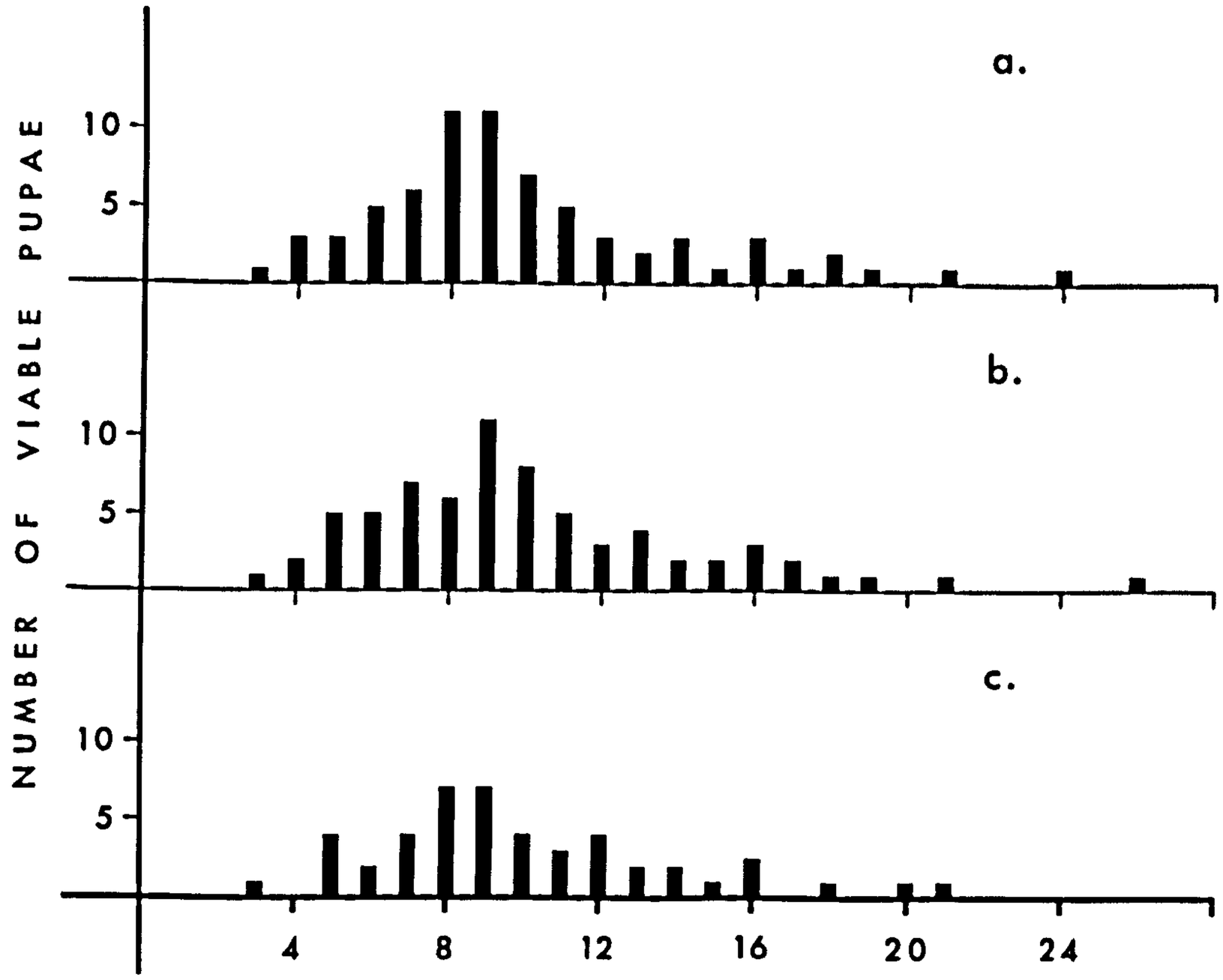
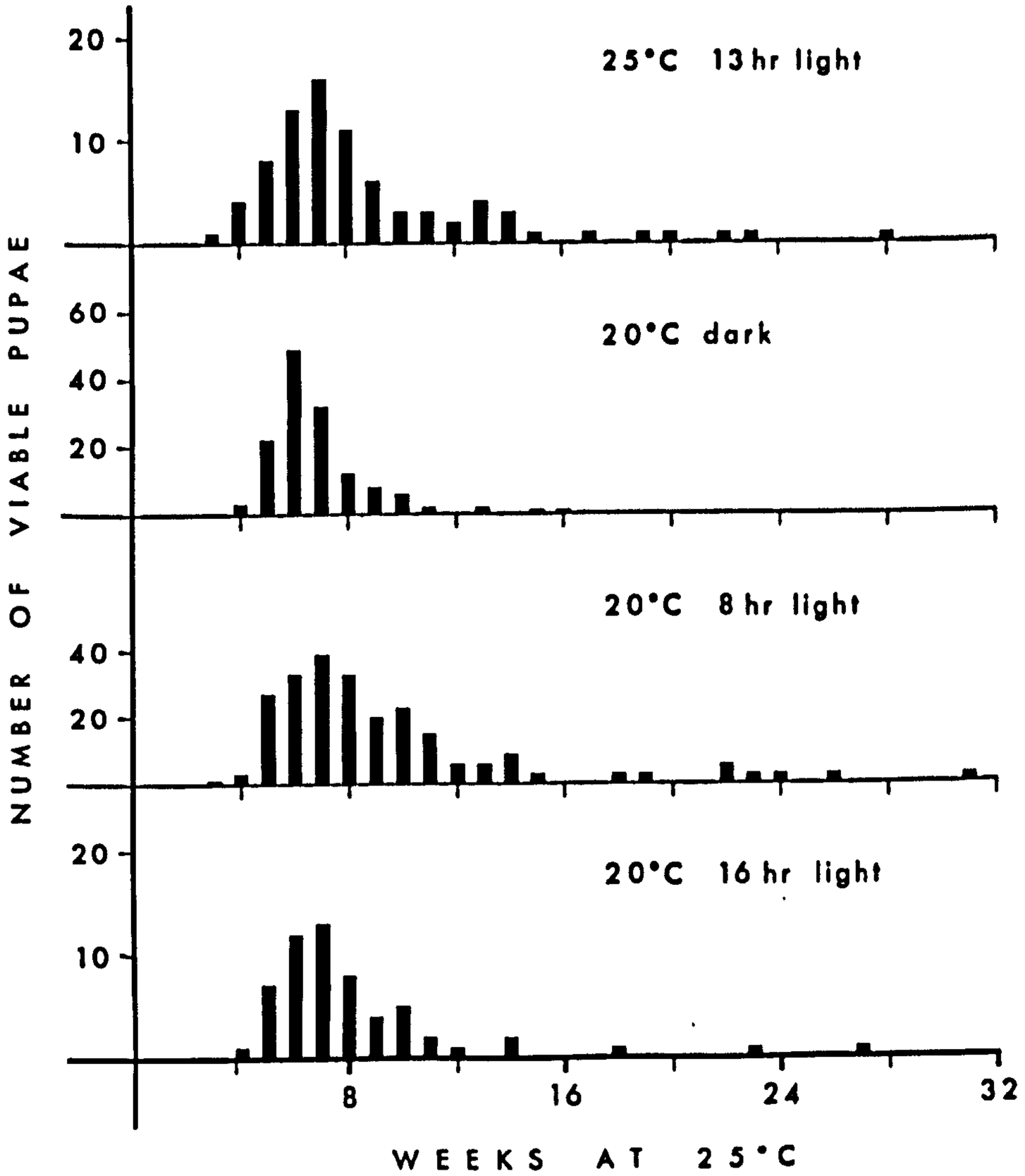

a.
b.
c.
10
5
10
5
10
5
NUMBER OF VIABLE PUPAE
4
8
12
16
20
24
DAYS AT 25°C

Fig. 32. Effect of temperature and light conditions during rearing on the pupation time of diapausing larvae of Ephestia elutella (field stock) under a 16-hour daylength at 25°C. (Mortality after entering diapause was less than 10% in all groups).


25°C 13 hr light
20°C dark
20°C 8 hr light
20°C 16 hr light
NUMBER OF VIABLE PUPAE
20
10
60
40
20
40
20
20
10
8
16
24
32
WEEKS AT 25°C

revealed a significance of difference at the 2% level between the sample reared in darkness at 20°C and any of the other groups. The means and S. D.'s for pupation in the four groups under the 16-hour daylength at 25°C was as follows:-

25°C, 13 hours light	:	58 ± 31 days
20°C, darkness	:	45 ± 13 days
20°C, 8 hours light	:	53 ± 26 days
20°C, 16 hours light	:	55 ± 29 days

In all batches other than the one reared in darkness at 20°C means and S. D.'s were recalculated omitting pupations occurring beyond the time equal to twice the first calculated mean. The recalculated results were as follows:-

25°C, 13 hours light	:	52 ± 20 days
20°C, 8 hours light	:	51 ± 20 days
20°C, 16 hours light	:	49 ± 15 days

A comparison of the recalculated means for these batches with the batch reared at 20°C in darkness, revealed that differences remained significant at the 2% level for the batches reared under an 8-hour daylength at 20°C, or a 13-hour daylength at 20°C. In the batch reared under a 16-hour day-length at 20°C, the significant of difference was reduced to between 5 and 10%. The number of insects present in this batch was, however, lower than in the others.

v) Various light conditions in the laboratory stock of Ephestia elutella

At 20°C, batches of diapausing larvae were prepared in darkness, under an 8-hour daylength, and under a daylength progressively shortening from 12 hours by 4 minutes a day. The duration of diapause in each batch under a 16-hour daylength at 25°C, as assessed by summating the number of pupae appearing each week, is illustrated in fig. 33. The means and ~~standard~~ S.D.'s for the times required for pupation in each batch, calculated from observations made three times a week, were as follows:

20°C, daylength shortening from 12 hours	:	39 ± 12 days
20°C, daylength 8 hours	:	35 ± 10 days
20°C, darkness	:	33 ± 10 days

No significant difference was found between the means of the latter two groups, but in both of these, the duration of diapause was significantly shorter ($p \approx 1$-2%) than in the group reared under a shortening daylength.

vi) Different humidities in Ephestia elutella

Accumulative curves of pupation in batches of the laboratory and field stocks of E. elutella reared and maintained at 25 and 70% RH under continual light at 15°C, are illustrated in fig. 34. High mortality during early development in batches of the laboratory stock, (table 19) greatly reduced the number of insects that subsequently entered diapause. At 25% RH, only 5 insects developed to the adult stage, three of which did so after entering diapause. All insects of the field stock that subsequently completed development at 15°C, entered diapause. In the calculation of mean pupation times for the field stock batches, the start of diapause was taken as the first day an adult of the laboratory stock was seen. This was 137 days after oviposition and 121 days before the first pupation in batches of the field stock. At 70% RH, the mean pupation time for the field stock was 196 ± 45 days, and at 25% RH, was 225 ± 65 days. If, however, the start of diapause at 25% RH was taken as the date for the first emergence of the laboratory stock at this humidity, the mean pupation time

Fig. 33. Effect of light conditions during rearing at 20°C on the pupation time of diapausing larvae of Ephestia clutella (laboratory stock) under a 16-hour daylength at 25°C. (Mortality in all groups was less than 10%).

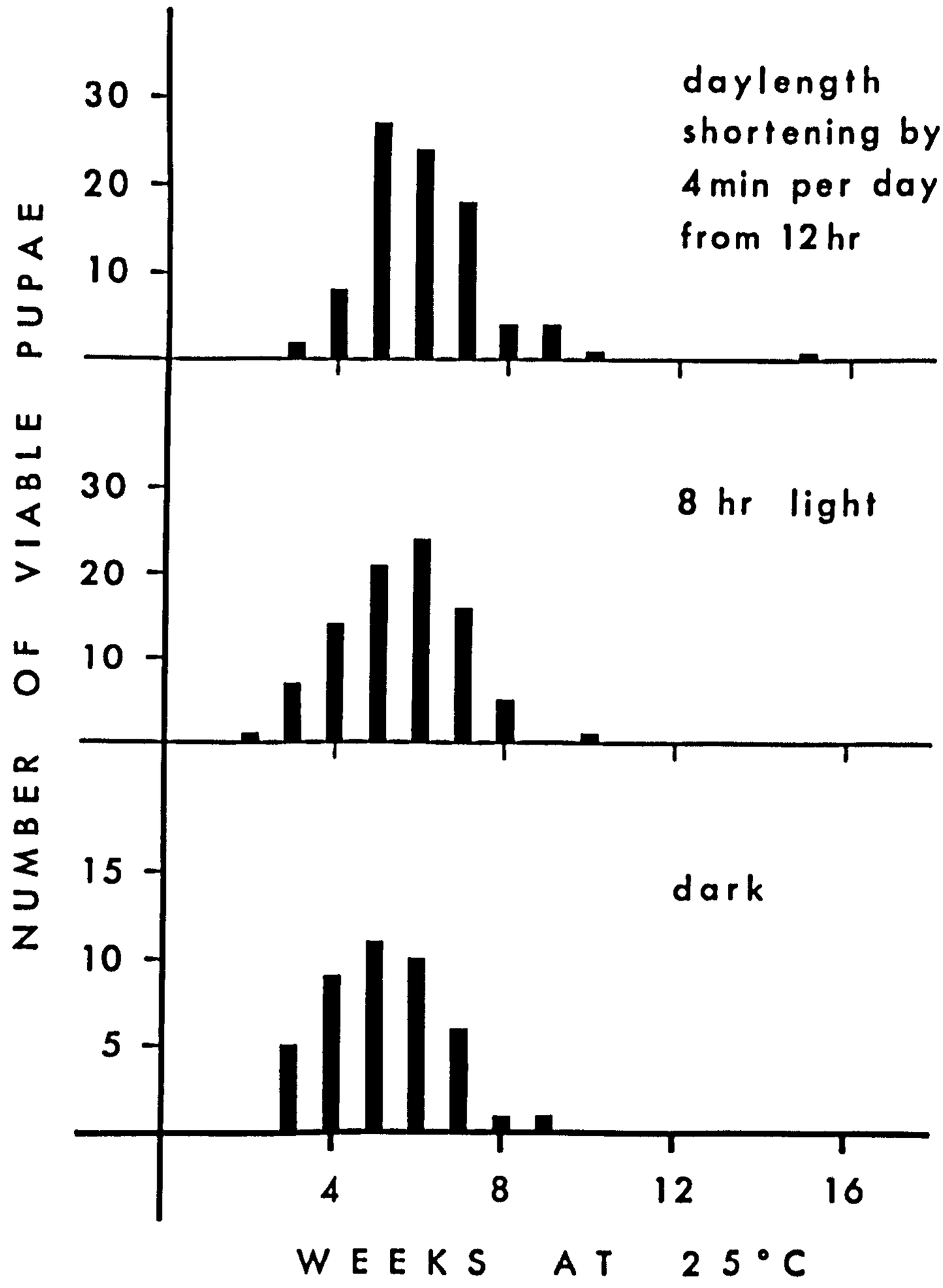
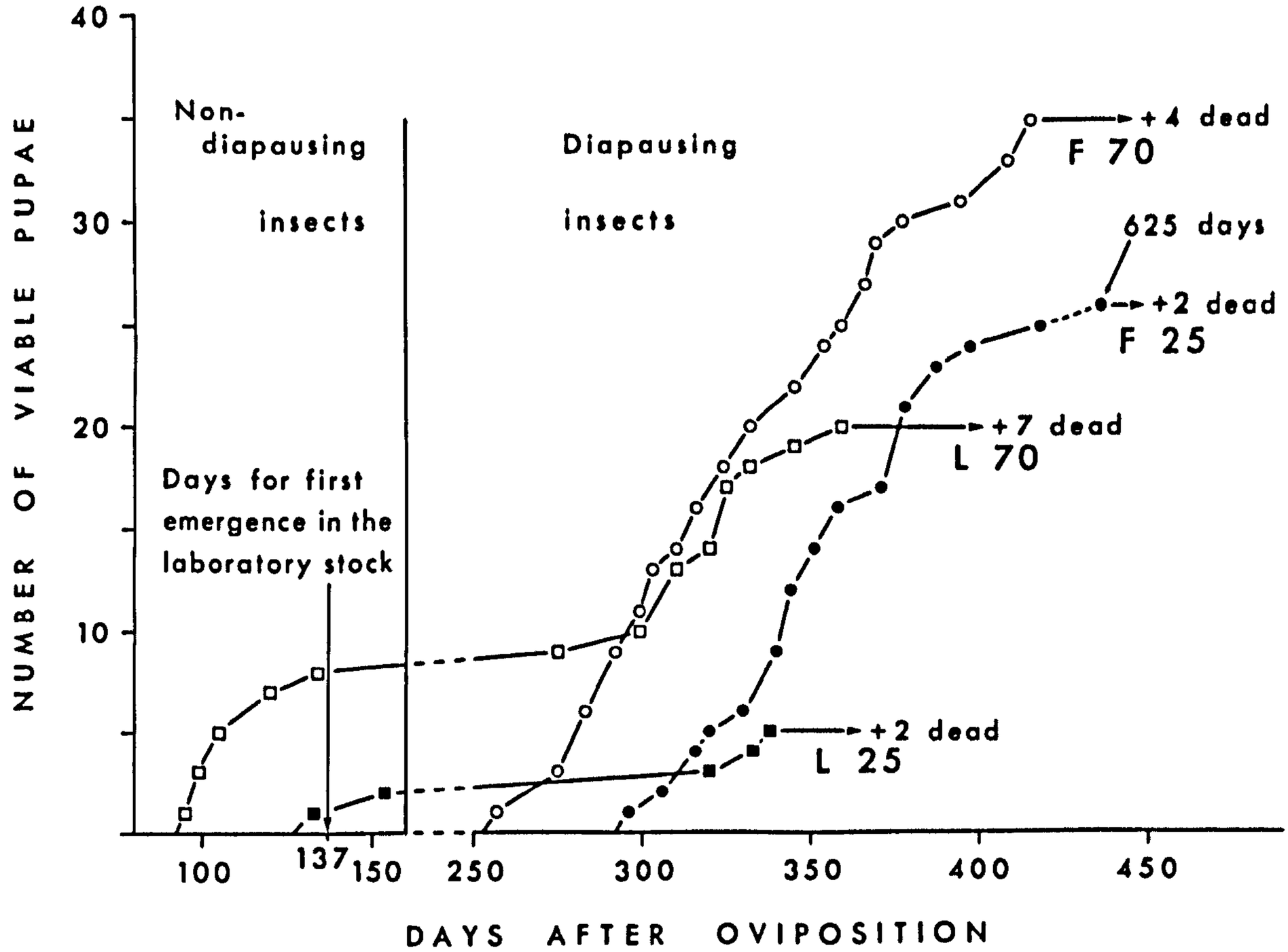

daylength
shortening by
4 min per day
from 12 hr
8 hr light
dark
PUPAE
NUMBER OF VIABLE
30
20
10
30
20
10
15
10
5
4
8
12
16
WEEKS AT 25°C

Fig. 34. Induction and duration of diapause in laboratory (L) and field (F) stocks of Ephestia elutella at 25 and 70% RH in continual light at 15°C.


NUMBER OF VIABLE PUPAE
40
30
20
10
Non-
diapausing
insects
Diapausing
insects
Days for first
emergence in the
laboratory stock
+4 dead
F 70
625 days
+2 dead
F 25
+7 dead
L 70
+2 dead
L 25
100
137
150
250
300
350
400
450
DAYS AFTER OVIPOSITION

was lowered to 185 ± 65 days. It has been shown (Section 1.A.iii, table 5) that development at 15°C proceeds slower at lower humidity, and results assessed from the later date are likely to be the more accurate.

D. Time Already Spent in Diapause

i) Ephestia elutella

In the field stock, but not in the laboratory stock, holding in diapause for increasing lengths of time under an 8-hour daylength at 20°C progressively reduced the time required for pupation under a 16-hour daylength at 25°C (table 34). A batch of the laboratory stock held for about 3 months after entry into diapause, required a longer exposure at 25°C to terminate diapause than batches held for shorter periods of time. However, after 17-18 weeks at 20°C, the mean pupation time at 25°C was significantly less than in all other samples ($p = <0.01$). In both stocks, increasing exposure to 20°C progressively increased the synchronisation of pupation times at 25°C.

ii) Plodia interpunctella

As in E. elutella, samples held for extra periods of time at 20°C, pupated sooner under long daylength at 25°C than control batches (table 35). In the laboratory stock, an extra 10 weeks in darkness at 20°C was significantly more effective in synchronising and hastening the termination of diapause at 25°C than 6-week exposures at 20°C in darkness or under an 8-hour daylength ($p = 0.01$-0.05). In the field stock, an extra 6 weeks at 20°C under an 8-hour daylength significantly reduced the duration of diapause at 25°C ($p = <0.01$). A long tail of late pupations in the field stock batches gave rise to wide standard errors, and means were recalculated as described in previous sections.

E. Low Temperature

i) Plodia interpunctella (laboratory stock)

Samples reared under an 8-hour daylength at 20°C were maintained in darkness for selected periods at 2.5, 7.5, 10 and 15°C, and results for the duration of

diapause under a 16-hour daylength at 25°C, are summarised in table 36. In general, a period at low temperature tended to hasten and synchronise emergence after return to 25°C. A six-week exposure to 10°C or below, synchronised and hastened diapause termination more effectively than a 2-week exposure, or no exposure to low temperature at all ($p = <0.05$). Also, mean pupation times after 6-week exposures to 2.5 or 10°C, were significantly shorter than after a 6-week exposure to 20°C ($p = <0.05$). The duration of diapause was longest after 6 or 10-week exposures to 15°C, but there was no significant difference at the 5% level from results for the sample moved straight to 25°C.

ii) Plodia interpunctella (field stock)

Batches of larvae reared at 20°C in darkness (table 37), or under an 8-hour daylength (table 38), were exposed to low temperature for selected periods. As in the laboratory stock, periods at low temperature hastened pupation at 25°C. In darkness, samples returned to 25°C after a 10-week exposure at 10°C pupated much more rapidly than samples raised to 25°C without a chilling period (table 37) ($p = <0.001$). Even more notable was the increase in pupation rate in darkness at 20°C after 10 weeks at 10°C, the total range of pupation times reducing from 41-244 days to 13-74 days.

Samples reared under an 8-hour daylength at 20°C responded similarly to samples reared in darkness when returned to darkness at 25°C after 10 weeks at 10°C. Larvae returned after chilling to long daylength at 20°C showed a high degree of synchronisation in pupating, and although the mean pupation time was not significantly different from the batch returned to darkness at 25°C, it was concluded that long daylength was more effective than increased temperature in terminating diapause after exposure to cold.

Of the samples returned to a 16-hour daylength at 25°C, 6 and 10-week exposures to 10°C proved equally effective in reducing mean pupation time and synchronising emergence. A 2-week exposure at 10°C, however, gave results for pupation rate

TABLE 34. Days required for pupation at 25°C under a 16-hour daylength of diapausing larvae of Ephestia elutella held under an 8-hour daylength at 20°C for various lengths of time after induction (based on counts performed three times a week).

STOCK	WEEKS AT 20°C	NO. IN BATCH	% MORTAL-ITY	RANGE OF PUPA-TIONS	MEAN AND S.D.	50% PUPA-TION	MIN. RANGE FOR 80% PUPATION
LAB	3-4	114	7	16-67	39 ± 11	39	23-52
	7-8	81	9	13-61	37 ± 10	36	22-49
	12-13	60	8	23-65	46 ± 8	45	37-58
	17-18	61	15	8-57	32 ± 8	31	21-42
FIELD	3-4	149	7	25-217	57 ± 27	52	31-76
	Mean and S.D. recalculated ignoring last 5 stragglers :				54 ± 20		
	7-8	83	10	19-164	49 ± 25	43	26-71
	Mean and S.D. recalculated irnoring last 2 stragglers :				48 ± 20		
	14-15	73	11	22-127	45 ± 17	40	29-57

TABLE 35. Days required for pupation under a 16-hour daylength at 25°C of diapausing larvae of Plodia interpunctella held in darkness or under an 8-hour daylength at 20°C for selected periods (based on daily counts).

STOCK	EXTRA WEEKS AT 20°C	NO. IN BATCH	% MORTAL-ITY	RANGE OF PUPATIONS	MEAN AND S.D.	50% PUPATION	MIN. RANGE FOR 80% PUPATION
LAB	0	68	7	3-26	9.9 ± 5.1	9	5-16
	6 (DARK)	51	12	2-20	9.5 ± 4.4	9	4-15
	10 (DARK)	33	9	2-15	7.5 ± 3.0	7	4-12
	6 (8 HR L)	42	10	3-14	9.1 ± 2.7	10	6-12
FIELD	0	48	4	5-58	13.1 ± 9.0	10	7-18
	Mean and S.D. recalculated ignoring last 2 stragglers :				12.2 ± 5.3		
	6 (8 HR L)	54	4	2-34	8.5 ± 5.9	7	4-15
	Mean and S.D. recalculated ignoring last 3 stragglers :				7.5 ± 4.1		

TABLE 36. Days required at 25°C under a 16-hour daylength for pupation of diapausing larvae of Plodia interpunctella (laboratory stock) held for selected periods at low temperatures in darkness (based on daily counts)

EXPOSURE: TEMP. °C	EXPOSURE: TIME WEEKS	NO. IN BATCH	% MORTAL-ITY	RANGE OF PUPATIONS	MEAN AND S.D.	50% PUPATION	MIN. RANGE FOR 80% PUPATION
2.5	6	49	4	4-20	7.7 ± 3.4	7	5-12
7.5	6	48	8	5-22	8.1 ± 3.5	7	5-12
10	2	64	11	4-25	10.2 ± 4.8	10	6-16
	6	42	5	5-17	7.6 ± 2.8	7	5-11
15	6	43	21	5-25	10.6 ± 5.5	10	5-19
	10	58	21	4-21	10.9 ± 5.3	11	5-17
20	6	51	12	2-20	9.5 ± 4.4	9	4-15
STRAIGHT TO 25°C		68	7	3-26	9.9 ± 5.1	9	5-16

TABLE 37. Days required for pupation in darkness of diapausing larvae of Plodia interpunctella (field stock) reared in darkness at 20°C, showing the effect of exposure to 10°C for 10 weeks (based on counts performed daily for first 4 weeks, and thereafter at least three times a week).

EXPOSURE:		AFTER	NO. IN	%	RANGE	MEAN AND	50%	MIN. RANGE
TEMP. °C	TIME WEEKS	EXPOSURE KEPT AT:	BATCH	MORTAL -ITY	OF PUPA -TIONS	S.D.	PUPA -TION	FOR 80% PUPATION
10	10	25°C DARK	41	5	7-39	15 ± 7	12	10-21
		Mean and S.D. recalculated ignoring last 2 stragglers :				13 ± 5		
STRAIGHT TO		25°C DARK	46	7	7-105	30 ± 22	17	7-42
		Mean and S.D. recalculated ignoring last 5 stragglers :				23 ± 12		
10	10	20°C DARK	45	18	13-74	31 ± 15	25	15-46
REMAIN AT		20°C DARK	52	25	41-244	108 ± 51	101	52-190

TABLE 38. Plodia interpunctella (field stock). Days required under selected temperatures and light conditions for pupation of diapausing larvae reared under an 8-hour daylength at 20°C, and exposed to low temperatures for selected periods of time (based on counts performed daily for first 4 weeks, and thereafter at least three times a week).

EXPOSURE: TEMP. °C	EXPOSURE: TIME WEEKS	AFTER EXPOSURE KEPT AT:	NO. IN BATCH	% MORTAL-ITY	RANGE OF PUPATIONS	MEAN AND S.D.	50% PUPA-TION	MIN. RANGE FOR 80% PUPATION
10	2	25°C 16 HR L	69	6	5-56	14 ± 10	12	6-20
			Mean and S.D. recalculated					
			ignoring last 3 stragglers :			13 ± 6		
	6		42	0	5-14	7.3 ± 2.2	7	5-10
	10		45	9	5-13	7.3 ± 2.3	7	5-11
15	6		44	7	5-28	8.1 ± 4.0	7	5-10
			Mean and S.D. recalculated					
			ignoring last 2 stragglers :			7.4 ± 2.0		
20	6		54	4	2-34	8.5 ± 5.9	7	4-15
			Mean and S.D. recalculated					
			ignoring last 3 stragglers :			7.5 ± 4.1		
STRAIGHT TO		25°C 16 HR L	48	4	5-58	13 ± 9	10	7-18
			Mean and S.D. recalculated					
			ignoring last 2 stragglers :			12 ± 5		
10	10	25°C DARK	46	15	7-35	15 ± 6	14	9-21
STRAIGHT TO		25°C DARK	47	4	5-204	38 ± 49	15	5-49
			Mean and S.D. recalculated					
			ignoring last 7 stragglers :			19 ± 14		
10	10	20°C 16 HR L	44	0	10-25	14 ± 3	13	12-18
STRAIGHT TO		20°C 16 HR L	50	6	36-169	68 ± 22	57	40-92

similar to unchilled samples. In contrast with the laboratory stock, 6-week exposures to 15 or 20°C were not significantly different from a similar exposure to 10°C in reducing mean pupation time, although the ranges of pupation times were much greater. Ranges were greatly extended by 2 or 3 individuals in each batch requiring much longer periods for pupation.

iii) Ephestia elutella (laboratory stock)

Larvae were reared under an 8-hour daylength at 20°C, and after exposure to low temperature were placed under a 16-hour daylength at 20 or 25°C. As in P. interpunctella, samples exposed to low temperatures in general showed an increase in synchronisation of pupation, and a reduction in the duration of diapause (table 39). The effect was more marked after longer exposures. No significant difference occurred between samples exposed to 15°C or below for two weeks and samples moved straight to 25°C. However, 4 or 6-week exposures to low temperatures did significantly shorten the mean time for pupation at 20 or 25°C ($p = <0.05$). A 4-week exposure at 10°C proved much more effective than a 4-week exposure at 2.5°C in hastening pupation under a 16-hour daylength at 20°C ($p = <0.001$). Exposures to low temperatures were conducted as far as possible in darkness.

iv) Ephestia elutella (field stock)

Larvae were reared under an 8-hour daylength at 20°C, and after exposure to low temperatures, were transferred to a 16-hour daylength at 25°C (table 40). An exposure of 10 weeks to 5°C, preceded by 3 weeks at 15°C, greatly increased the synchronisation of emergence at 25°C, and reduced the average duration of diapause when compared with a batch held for 14-15 weeks at 20°C ($p = <0.01$). Shorter exposures to low temperatures, were less effective in limiting the period over which pupation occurred, but even so, an exposure of 6 weeks at 7.5°C was significantly better in shortening mean pupation time than 3 or 4 extra weeks at 20°C ($p = <0.01$).

A comparison was made of the emergence of males and females in the batch chilled for 10 weeks at 5°C, and in the batch exposed for only 2 weeks at 15°C (table 41). In the batch chilled for the longer period, emergence of the sexes proceeded in parallel, while in the other sample, males appeared, on average, a little earlier than females ($p =< 0.1$). This was partly because females contributed most of the later emergences. From the emergence records, an estimate was obtained of the mean pupation time for males and females by subtracting 14 days from each emergence date.

F. Natural Conditions

i) The field stock of Ephestia elutella overwintering from 1970 to 1971 in a slightly heated outbuilding.

Batches of tubes containing newly-hatched larvae were placed in the outbuilding in early September. During the colder months, the temperature in the outbuilding was about 5-10°C above ambient. Measurements were made on a thermograph which gave a trace of temperature for each week. The averages of daily mean temperatures during the autumn and winter months in the outbuilding were as follows: October, 17.0°C; November, 17.0°C; December, 14.0°C; January 13.5°C; February 14.5°C; March, 14.5°C. The lowest temperatures were recorded during the last week of December and the first week of January, when night temperatures fell to about 6.0°C. Fig.35 shows simplified accumulative curves of pupation in batches brought back to the laboratory at monthly intervals from December to March, and kept in darkness or under a 16-hour daylength at 25°C. A fuller account of the results is presented in table 42.

The longer samples were kept in the outbuilding, the shorter was the duration of diapause in the laboratory. However, the effect was much more pronounced in the samples returned to darkness at 25°C. In the December sample, the mean pupation time was 228 days, over four times the value under a 16-hour daylength. Mean pupation time was reduced in the January dark sample, but the ~~standard~~ S. D. was

TABLE 39. Ephestia elutella (laboratory stock). Days required under a 16-hour daylength at 20 or 25°C for pupation of diapausing larvae reared under an 8-hour daylength at 20°C and exposed to low temperatures for selected periods (based on counts performed three times a week).

EXPOSURE: TEMP. °C	EXPOSURE: TIME WEEKS	AFTER EXPOSURE KEPT AT:	NO. IN BATCH	% MORTAL-ITY	RANGE OF PUPATIONS	MEAN AND	50% PUPA-TION	MIN. RANGE FOR 80% PUPATION
2.5	2	20°C 16 HR LIGHT	49	14	65-212	126 ± 29	123	90-167
	4		52	15	80-231	120 ± 28	110	88-162
10	2		47	6	28-210	131 ± 26	124	107-174
	4		46	9	58-159	91 ± 28	81	63-130
STRAIGHT TO			49	29	49-301	137 ± 40	136	104-185
7.5	2	25°C 16 HR LIGHT	46	9	17-64	34 ± 8	33	26-43
	6		61	11	15-55	32 ± 7	34	25-40
15	2		48	6	21-59	37 ± 9	35	25-45
20	3-4*		114	7	16-67	39 ± 11	39	23-52

* Held under an 8-hour daylength

TABLE 40. Ephestia elutella (field stock). Days required under a 16-hour daylength at 25°C for pupation of diapausing larvae reared under an 8-hour daylength at 20°C and exposed to low temperatures for selected periods (based on counts performed three times a week).

EXPOSURE: TEMP. °C	EXPOSURE: TIME WEEKS	NO. IN BATCH	% MORTAL-ITY	RANGE OF PUPATIONS	MEAN AND S.D.	50% PUPA-TION	MIN. RANGE FOR 80% PUPATION
7.5	6	68	4	19-125	47 ± 18	43	27-59
		Mean and S.D. recalculated ignoring last 2 stragglers :			45 ± 12		
15	2	75	7	22-185	53 ± 25	46	34-63
		Mean and S.D. recalculated ignoring last 2 stragglers :			49 ± 13		
15 + 5	4 + 4	44	9	25-114	47 ± 15	43	37-58
		Mean and S.D. recalculated ignoring last straggler :			45 ± 10		
15 + 5	3 +10	44	11	24-65	38 ± 9	36	30-48
20	3-4	149	7	25-217	57 ± 27	52	31-76
		Mean and S.D. recalculated ignoring last 5 stragglers :			54 ± 20		
20	14-15	73	11	22-127	45 ± 17	40	29-57

TABLE 41. Ephestia clutella (field stock). Emergence of males and females under a 16-hour daylength at 25°C after exposure of diapausing larvae to 15°C for 2 weeks and to 15 and 5°C for 3 and 10 weeks respectively.

Weeks at 25°C (16 HR LIGHT)	2 weeks at 15°C		3 weeks at 15°C + 10 weeks at 5°C	
	MALE	FEMALE	MALE	FEMALE
6	1	0	0	1
7	2	1	10	7
8	9	4	5	7
9	12	16	2	2
10	3	7	2	1
11	1	2	1	0
12	2	1	0	1
13	2	1	0	0
14	1	1	0	0
15	1	0	0	0
19	0	1	0	0
22	0	1	0	0
28	0	1	0	0
TOTAL NO's EMERGING	34	36	20	19
MEAN PUPATION TIME	62 ± 15	72 ± 29 65 ± 15*	53 ± 9	52 ± 9

* Mean and standard deviation recalculated ignoring last 2 stragglers.

Fig. 35. Ephestia elutella (field stock): Pupation at 25°C under a 16-hour daylength, or in darkness, of larvae induced to enter diapause by autumn and winter conditions in a slightly heated outbuilding, and moved to the laboratory at monthly intervals.

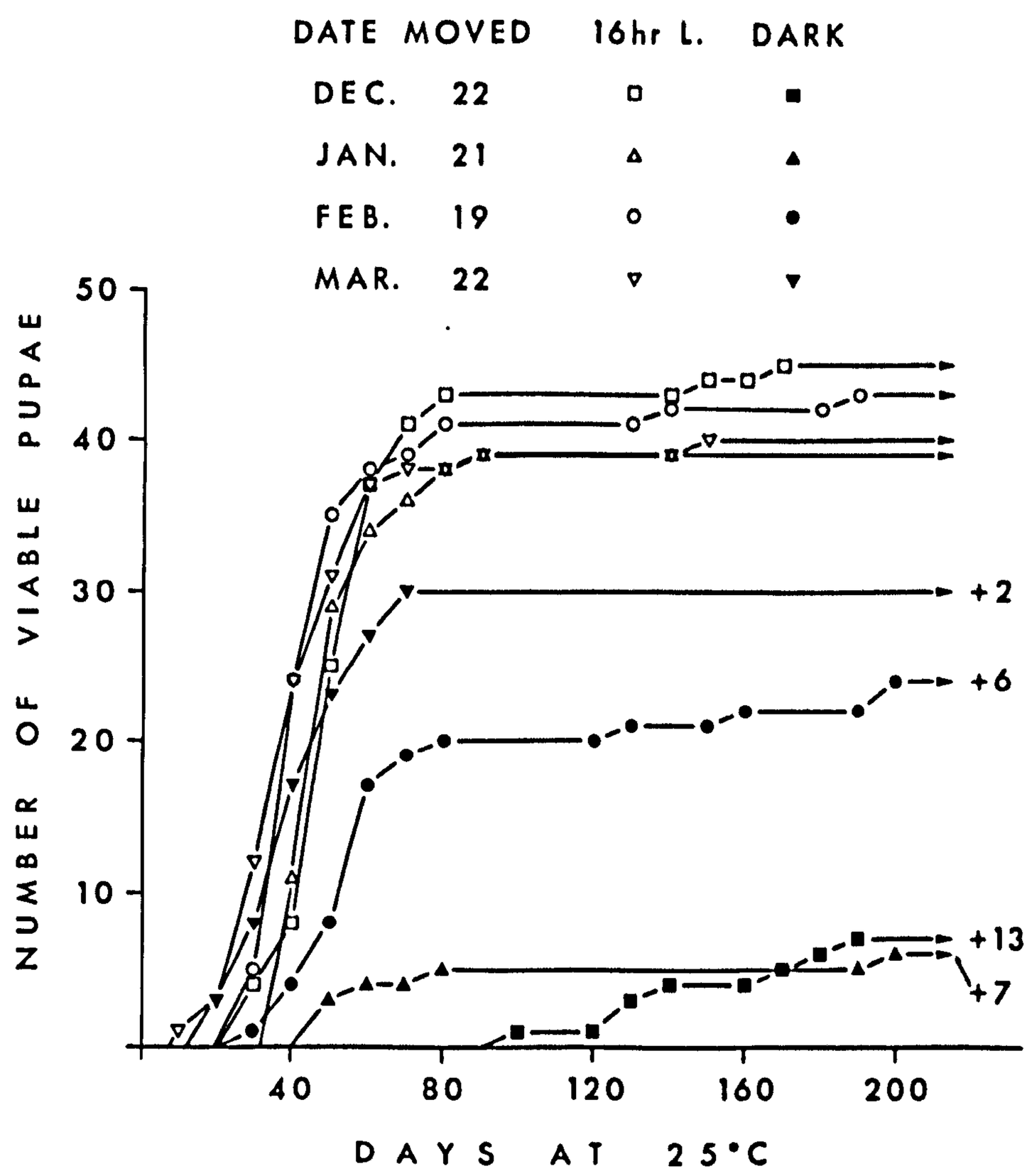

DATE MOVED
16hr L.
DARK
DEC. 22
JAN. 21
FEB. 19
MAR. 22
NUMBER OF VIABLE PUPAE
50
40
30
20
10
+2
+6
+13
+7
40
80
120
160
200
DAYS AT 25°C

TABLE 42. Ephestia (field stock). Days required for pupation in batches of diapausing larvae moved at monthly intervals to 25°C in darkness or long daylength from an outbuilding running at 5 - 10°C above ambient during the winter of 1970-1971 (based on counts performed three times a week)

DATE MOVED TO 25°C	HOURS LIGHT AT 25°C	NO. IN BATCH	% MORTAL-ITY	RANGE OF PUPATIONS	MEAN AND S.D.	50% PUPA-TION	MIN. RANGE FOR 80% PUPATION
DEC 22	0	46	57	94-354	228 ± 75	226	121-310
	16	46	2	22-162	53 ± 25	49	30- 65
		Mean and S.D. recalculated ignoring last 2 stragglers :			48 ± 12		
JAN 21	0	40	68	43-289	169 ± 99	210	43-266
	16	41	5	32- 82	47 ± 13	42	32- 60
FEB 19	0	40	25	25-328	104 ± 81	59	33-204
		BATCH SPLIT: PUPAE UP TO 104 (25 - 74) DAYS:			51 ± 12		
		PUPAE AFTER 104 (123 - 328) DAYS:			210 ± 53		
	16	45	4	21-181	47 ± 30	40	26- 54
		Mean and S.D. recalculated ignoring last 2 stragglers :			41 ± 12		
MAR 22	0	42	24	11-283	53 ± 58	38	23- 64
		Mean and S.D. recalculated ignoring last 2 stragglers :			39 ± 14		
	16	44	9	9-143	41 ± 22	36	22- 53
		Mean and S.D. recalculated ignoring last 2 stragglers :			37 ± 12		

increased. This was caused by a few individuals pupating in a relatively short time. The February sample showed two peaks of pupation, one approximately coinciding with pupation under long daylength, and the other relating closely to pupation in the sample moved to darkness at 25°C in December. The sample returned to darkness inthe laboratory in March differed little from the sample returned to long daylength, particularly when the last two stragglers in each batch were ignored. Mortality in the samples returned to long daylength at 25°C tended to increase slightly with exposure to winter conditions, while much lower mortalities were encountered in the samples returned to darkness in February and March, than those returned in January or December.

ii) Both stocks of Ephestia elutella overwintering 1971 to 1972 in an unheated outbuilding

Batches of tubes containing newly hatched larvae were placed in the outbuilding in mid August. The averages of daily mean temperatures during the autumn and winter months in the outbuilding, as assessed from the traces of a thermograph, were as follows: September, 15.5°C; October, 13.5°C; November 10.0°C; December, 10.0°C; January, 7.0°C; February, 10.0°C; March, 11.5°C. This year mean temperatures were 3-7°C lower than temperatures the previous year in the slightly heated outbuilding. The lowest temperature recorded was-3°C, early in the morning of January 31st, 1972. Batches of both stocks were removed from the outbuilding at monthly intervals from the winter solstice, and were exposed at 25 or 30°C in darkness, or at 25°C under a 16-hour daylength (tables 43,44). Lines joining the times required for 5 and 95% pupation in each batch presented in fig. 36 provide a visual impression of the relative efficiencies of various conditions in terminating diapause after various periods in the outbuilding.

In both stocks, a progressive reduction in the duration of diapause under each system tested in the laboratory was seen the longer material had been left in the outbuilding. When transferred to 25°C, batches of the field stock required much

less time to break diapause than equivalent batches moved from the slightly heated outbuilding during the winter of 1970-1971. In samples moved to the laboratory in December, diapause in both stocks was terminated more rapidly under a 16-hour daylength at 25°C than in darkness at 25 or 30°C. From January, diapause in batches of the laboratory stock (table 43) was terminated most rapidly by darkness at 30°C. In the field stock, however, long daylength at 25°C remained the best agent for synchronising the termination of diapause (table 44), and from January, diapause was terminated under this system as rapidly in the field stock as in the laboratory stock. In both stocks, darkness at 25°C was the least effective in terminating diapause in all samples, but the periods over which pupation occurred in the February and March samples placed under this system, were less than a ninth of those required by the December samples.

Mortality in all batches of the laboratory stock was generally higher than in the field stock, and varied little from one set of diapause-terminating conditions to another. In the field stock, mortality was noticeably lower under long daylength at 25°C than in darkness at 25 or 30°C.

The batch of the field stock returned to a 16-hour daylength at 25°C in March (table 44) was analysed to compare the termination of diapause in males and females. As in the batch chilled at 5°C in the laboratory for 10 weeks (section 4.E.iv), no obvious difference was found between the rates of pupation for the two sexes.

iii) Plodia interpunctella.

Overwintering in an unheated outbuilding until 22nd December 1971 and 22nd March 1972

In both stocks, pupation in batches retained under natural conditions from September to March, proceeded more rapidly under a 16-hour daylength at 25°C than in batches removed in December (fig. 37). Mortality was, however, higher in

TABLE 43. Ephestia elutella (laboratory stock). Days required for pupation of diapausing larvae moved at monthly intervals during the winter of 1971-2 from an unheated outbuilding to selected conditions in the laboratory (based on counts performed three times a week)

DATE MOVED TO LAB.	TEMP. °C	HOURS LIGHT	NO. IN BATCH	% MORTAL-ITY	RANGE OF PUPATIONS	MEAN AND S.D.	50% PUPATION	MIN. RANGE FOR 80% PUPATION
DEC 23	25	0	38	29	20-385	104 ± 71	76	41-166
			Mean and S.D. recalculated ignoring last straggler :			94 ± 46		
		16	35	20	12- 47	30 ± 9	30	17- 40
	30	0	38	24	10-108	36 ± 22	29	17- 45
			Mean and S.D. recalculated ignoring last 3 stragglers :			29 ± 9		
JAN 24	25	0	41	22	19-156	42 ± 30	32	19- 44
			Mean and S.D. recalculated ignoring last 2 stragglers :			35 ± 13		
		16	37	22	16- 51	29 ± 9	28	20- 43
	30	0	38	13	10- 41	21 ± 7	20	12- 27
FEB 22	25	0	38	26	15- 55	29 ± 8	26	22- 38
		16	40	20	16- 41	23 ± 5	22	16- 28
	30	0	39	21	11- 26	18 ± 4	17	13- 23
MAR 22	25	0	38	21	18- 56	29 ± 8	29	20- 36
		16	39	31	14- 42	25 ± 6	24	20- 34
	30	0	38	29	12- 30	19 ± 4	19	14- 23

TABLE 44. Ephestia elutella (field stock). Days required for pupation of diapausing larvae moved at monthly intervals during the winter of 1971-2 from an unheated outbuilding to selected conditions in the laboratory (based on counts performed three times a week).

DATE MOVED TO LAB.	TEMP. °C	HOURS LIGHT	NO. IN BATCH	% MORTALITY	RANGE OF PUPATIONS	MEAN AND S.D.	50% PUPATION	MIN. RANGE FOR 80% PUPATION
DEC 23	25	0	46	30	43 - 453	198 ± 92	201	43-244
		16	42	5	21 - 92	39 ± 17	32	24- 48
			MEAN AND S.D. RECALCULATED IGNORING LAST 2 STRAGGLERS :			37 ± 12		
	30	0	43	19	11 - 202	92± 61	90	18-144
JAN 24	25	0	42	10	16 - 212	51 ± 39	36	29- 58
			MEAN AND S.D. RECALCULATED IGNORING LAST 3 STRAGGLERS :			41 ± 18		
		16	42	0	15 - 79	32 ± 12	28	17- 38
			MEAN AND S.D. RECALCULATED IGNORING LAST STRAGGLER :			31 ± 10		
	30	0	45	13	15 - 181	31 ± 27	23	15- 40
			MEAN AND S.D. RECALCULATED IGNORING LAST STRAGGLER :			27 ± 12		
FEB 22	25	0	43	16	15 - 57	32 ± 11	29	22- 49
		16	43	9	17 - 36	24 ± 5	24	17- 29
	30	0	41	12	13 - 38	23 ± 5	22	18- 28
MAR 22	25	0	40	10	15 - 56	32 ± 10	31	19- 42
		16	44	0	8 - 36	20 ± 6	20	14- 28
			21MALES	0	8 - 36	20 ± 7	20	14- 32
			23FEMALES	0	11 - 34	21 ± 6	21	14- 28
	30	0	43	9	6 - 46	22 ± 8	21	14- 33

Fig. 36. Ephestia elutella: Lines joining the times required for 5 and 95% of diapausing larvae to pupate in batches placed under selected conditions in the laboratory after overwintering in an unheated outbuilding until December, January, February and March.

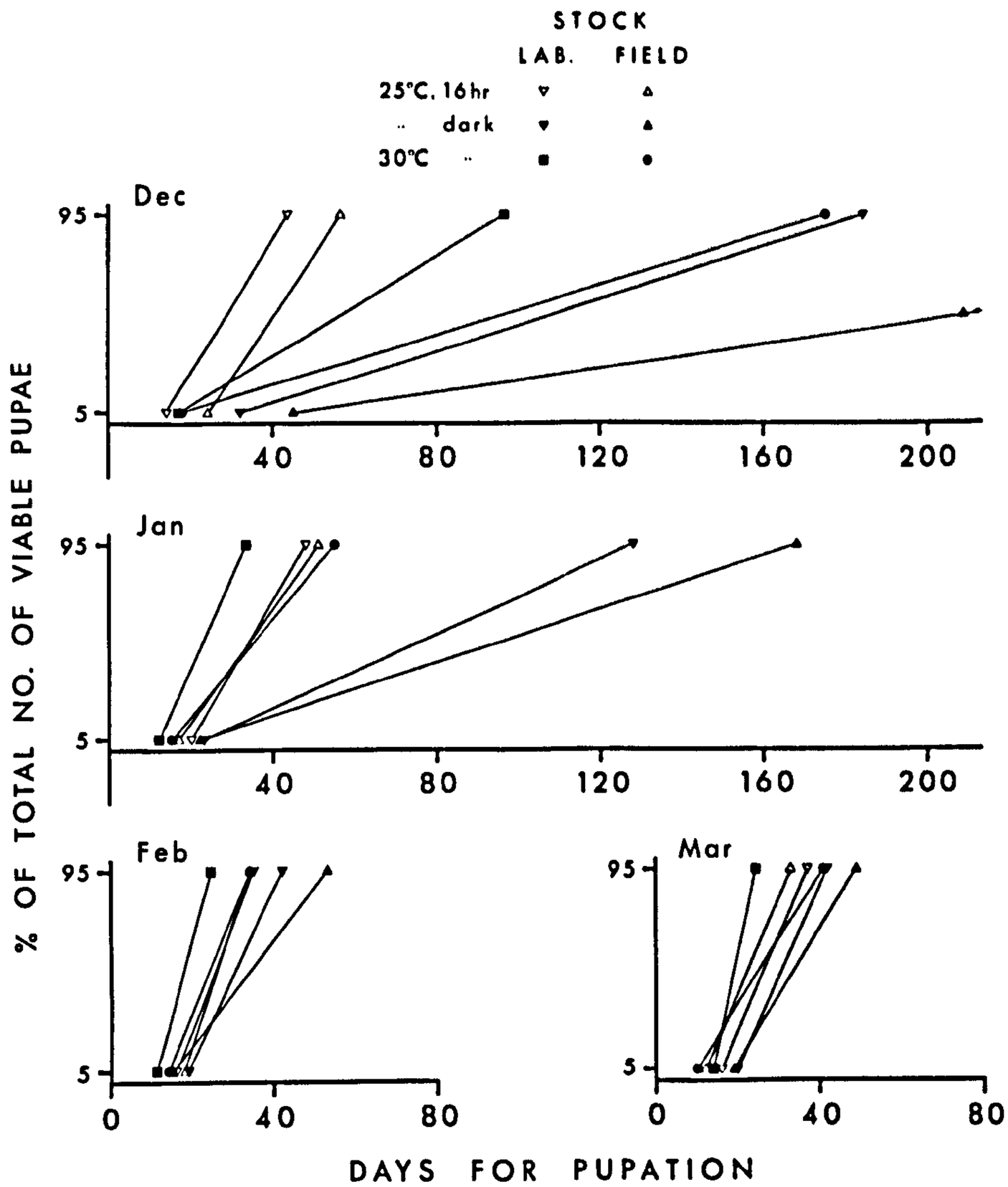
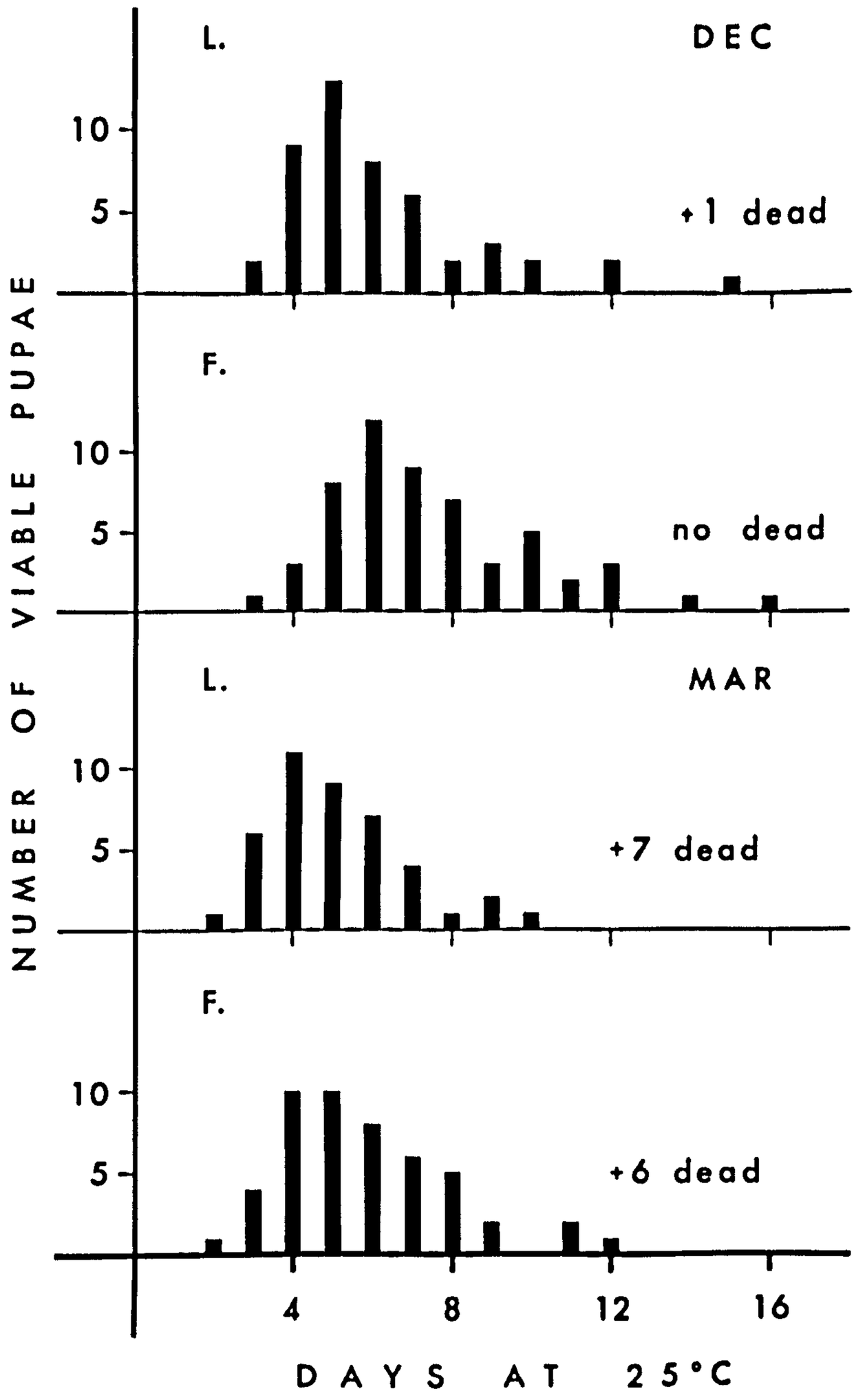

STOCK
LAB. FIELD
25°C. 16hr
" dark
30°C "
Dec
Jan
Feb
Mar
95
5
0
40
80
120
160
200
% OF TOTAL NO. OF VIABLE PUPAE
DAYS FOR PUPATION

Fig. 37. Plodia interpunctella: Pupation in December and March under a 16-hour daylength at 25°C of batches of the laboratory (L.) and field (F.) stocks entering diapause in autumn and overwintering in a slightly heated outbuilding.


L.
DEC
10
5
+1 dead
F.
10
5
no dead
L.
MAR
10
5
+7 dead
F.
10
5
+6 dead
NUMBER OF VIABLE PUPAE
4
8
12
16
DAYS AT 25°C

the March batches. All samples showed a much higher degree of synchronisation, and a faster pupation rate ($p = < 0.001$), than samples reared in the laboratory and placed under a 16-hour daylength at 25°C soon after diapause induction (tables 31, 32). The means and ~~standard~~ S.D.'s for times to pupate in the overwintering batches were as follows:-

December 22nd samples :	Laboratory stock	:	5.7 ± 2.5 days
	Field stock	:	7.0 ± 2.6 days
March 22nd samples :	Laboratory stock	:	4.7 ± 1.8 days ($p = < 0.05$)
	Field stock	:	5.4 ± 2.2 days ($p = < 0.01$)

iv) Emergence of Ephestia elutella and Plodia interpunctella during the spring and summer of 1972 in slightly heated and unheated outbuildings exposed to natural daylight.

The averages of daily mean temperatures in the unheated outbuilding during the spring and summer months were as follows; March, 11.5°C; April, 13.5°C; May, 14.5°C; June, 16.5°C; July, 19.0°C; August, 18.5°C; and in the slightly heated outbuilding were : February, 15.0°C; March, 16.0°C; April, 17.5°C; May, 18.0°C; June, 19.0°C; July, 21.5°C; August, 21.0°C. Emergence of both stocks of E. elutella in the unheated building occurred predominately during July (table 45). In the laboratory stock, the emergence of both sexes commenced in the first week of July, reaching a peak by the third week. The last emergences occurred about three weeks later. In the field stock, the emergence of males began in the last week of June and reached a peak during the first two weeks of July. None emerged after the 2nd of August. Females started to appear in the first week of July, reaching a peak during the second and third weeks. A few continued to appear throughout August. Emergence of the sexes resembled the batch in section E.iv) which had been held for 2 weeks at 15°C, rather than the one which experienced 10 weeks at 5°C, or the batch in F.ii) which had been returned to long daylength at 25°C after overwintering in the outbuilding until 22nd March.

In the field stock of P. interpunctella, the emergence of both sexes began in late June, reached a peak in early July, and ended 2 weeks later. Very few adults of the laboratory stock emerged, but those that did, appeared near the time for peak emergence in the field stock.

Emergence in the building receiving some heat from an adjacent room running at 25°C occurred much earlier than in the unheated building (table 46). The temperature in the slightly heated building was 7°C above ambient on colder days, but the difference reduced as the ambient temperature approached 25°C on warmer days. Emergence of the laboratory stock of E. elutella in the current experimental batch, and also in cultures, commenced in the third week of May and finished in the third week of July, while in the field stock, emergence started a week earlier and ended 4 weeks later. The span of emergence was greater than in the unheated building, and females of both stocks were responsible for the majority of the later emergences. In both stocks, most of the emergence occurred in June, but, even taking into account the smaller batch size, there was less evidence of a peak period than in the unheated building. Emergence of P. interpunctella occurred from the 4th week in February to the 2nd week in May, the range for the laboratory stock being a little less than for the field stock. Less difference was evident in the emergence of the sexes than in E. elutella.

G. Pieris brassicae and Bombyx mori.

i) Duration at 10°C in darkness and at 20°C under an 8-hour daylength

The duration of the pupal diapause of P. brassicae at 10°C was not significantly longer than at 20°C(table 47). Diapause in eggs of B. mori lasted 5-8 months at 20°C, while no eggs survived a 12-month exposure to 10°C. However, samples of eggs lifted from 10°C after an 8-month exposure, and placed at 25°C, showed less than 20% mortality. Results at 25°C are described in G.iii.

TABLE 45. Emergence of males and females of Ephestia elutella and Plodia interpunctella in the spring and summer of 1972 after overwintering as diapausing larvae in an unheated outbuilding

SPECIES	STOCK	SEX	TOTAL NO. ADULTS	NUMBER EMERGING IN 7-DAY PERIODS ENDING ON : 28.6	5.7	12.7	19.7	26.7	2.8	9.8	16.8	23.8	30.8
EPHESTIA	LAB	Male	26	0	3	7	8	5	3	0	0	0	0
(70 INSECTS)		Female	30	0	2	6	9	7	5	1	0	0	0
	FIELD	Male	33	2	11	9	7	3	1	0	0	0	0
(79 INSECTS)		Female	36	0	6	11	8	4	3	1	0	2	1
PLODIA	LAB	Male	3	2	1	0	0	0	0	0	0	0	0
(61 INSECTS)		Female	6	2	2	2	0	0	0	0	0	0	0
	FIELD	Male	26	6	17	2	1	0	0	0	0	0	0
(65 INSECTS)		Female	24	1	16	6	1	0	0	0	0	0	0

TABLE 46. Emergence of males and females of Ephestia elutella and Plodia interpunctella in the spring and summer of 1972 after overwintering as diapausing larvae in a slightly heated outbuilding.

SPECIES	STOCK	SEX	TOTAL NO. ADULTS	NUMBERS EMERGING IN 7-DAY PERIODS ENDING ON : 24.5	31.5	7.6	14.6	21.6	28.6	4.7	11.7	18.7	25.7	2.8	9.8	16.8
EPHESTIA	LAB	Male	17	2	1	4	2	3	3	2	0	0	0	0	0	0
(46 INSECTS)		Female	19	1	1	3	4	2	4	2	1	1	0	0	0	0
	FIELD	Male	21	1	2	2	4	4	4	2	1	0	1	0	0	0
(52 INSECTS)		Female	20	1	1	2	3	4	2	1	1	2	1	0	1	1
				23.2	1.3	8.3	15.3	22.3	29.3	5.4	12.4	19.4	26.4	3.5	10.5	17.5
PLODIA	LAB	Male	16	0	1	1	2	3	1	1	3	2	1	1	0	0
(83 INSECTS)		Female	15	0	1	0	3	3	1	1	3	2	1	0	0	0
	FIELD	Male	35	1	4	6	7	6	3	3	1	1	2	0	1	0
(91 INSECTS)		Female	38	1	3	6	8	7	4	3	2	1	1	1	1	0

TABLE 47. Days required at 10 and 20°C for emergence of adults from diapausing pupae of Pieris brassicae, and of larvae from diapausing eggs of Bombyx mori (based on counts performed three times a week)

SPECIES (AND STAGE IN DIAPAUSE)	TEMP. °C	NO. IN BATCH	% MORTAL -ITY	RANGE OF EMERGENCE	MEAN AND S.D.	50% EMERG -ENCE	MIN. RANGE FOR 80% EMERGENCE
PIERIS	10	84	47	220-361	303 ± 38	298	242-333
(PUPAE)	20	72	42	234-322	276 ± 25	269	249-305
BOMBYX	10	603	100 (AFTER 12 MONTHS)				
(EGGS)	20	318	30	153-244	191 ± 29	185	160-224

ii) Effect of chilling periods on diapausing pupae of Pieris brassicae

An 11-week exposure at 5-10°C had little effect on the duration of diapause under a 16-hour daylength at 25°C, but emergence was markedly advanced and synchronised after a 16-week exposure (fig. 38). The means and ~~standard~~ S.D.'s for pupation times in the three batches were as follows:-

0 weeks at or below 10°C : 53 ± 18 days

11 " " " " " : 53 ± 16 days

16 " " " " " : 27 ± 11 days

iii) Effect of chilling periods on diapausing eggs of Bombyx mori

The batches described here were primarily controls for fumigation experiments, and were able to provide only limited data on the importance of the timing and duration of chilling periods in the determination of diapause. Mortality remained below 20% in samples retained for 35 weeks or less at 10 or 7.5°C, after 8 weeks from laying at 25°C (table 48). No eggs hatched at 25°C after a full year at 10°C. Batches retained for 25 or 35 weeks at 10°C, or for 12 or 16 weeks at 7.5°C, were highly synchronised in their hatching at 25°C. Hatch was rather slower in a batch retained for 15 weeks at 10°C.

H. Fumigation

i) Diapausing larvae of Ephestia elutella

In batches of both stocks exposed to phosphine, pupation under a 16-hour daylength at 25°C reached a peak a week earlier than in the controls (fig. 39). In the field stock, the last stragglers pupated by the 16th week in batches fumigated with phosphine, and by the 31st week in control batches. After fumigation with methyl bromide, pupation in the laboratory stock again occurred a week earlier than in the controls, but the effect was less apparent in the field stock. The means and ~~standard~~ S.D.'s for pupation time in the batches were as follows:-

Laboratory stock, controls	:	39 ± 9 days
" " phosphine fumigated	:	33 ± 9 days ($p = < 0.001$)
" " methyl bromide fumigated	:	32 ± 8 days ($p = < 0.001$)
Field stock, controls	:	56 ± 27 days
" " phosphine fumigated	:	42 ± 16 days ($p = < 0.001$)
" " methyl bromide fumigated	:	55 ± 34 days ($p =$ 0.7-0.9)

The wide range (fig. 39) for the methyl bromide sample of the field stock was largely caused by three late pupations, two in the 30th week, and one in the 36th week after exposure to long daylength at 25°C. A recalculated mean and S. D. for this sample without these three stragglers was 49 ± 16 days. A revised estimate for the control mean and ~~standard~~ S. D., ignoring the last 9 stragglers, was 51 ± 17 days. The difference between the recalculated means was not significant.

ii) Diapausing pupae of Pieris brassicae

In samples chilled for 11 weeks, the time for peak emergence under a 16-hour daylength at 25°C occurred three weeks earlier in the samples fumigated with methyl bromide than in the control (fig. 40). Doses giving over 10% mortality were largely responsible for the advance. The 100 survivors from doses giving over 10% mortality, and the remaining 137 insects, which emerged from doses giving less than 10% mortality, were analysed separately for comparison with the control. Means and ~~standard~~ S. D.'s were also calculated for a parallel sample of pupae fumigated in a test with phosphine which gave very little kill in any exposure, and for a later test with phosphine, in which all doses gave an appreciable level of kill after a chilling period of 16 weeks. Values for the significance of difference between fumigated and control batches are included in the following summary of mean pupation times and S. D.'s:-

11-week chilled samples:	CONTROL	53 ± 16 days
	Methyl bromide doses giving over 10% kill	29 ± 18 days ($p =< 0.001$)
	Methyl bromide doses giving under 10% kill	48 ± 24 days ($p =< 0.05$)
	Phosphine doses giving no significant kill	52 ± 15 days ($p = 0.5 - 0.7$)
16-week chilled samples:	CONTROL	28 ± 13 days
	Phosphine doses giving over 20% kill	23 ± 8 days ($p =< 0.02$)

Fig. 38. Emergence of Pieris brassicae under a 16-hour daylength at 25°C after exposure of diapausing pupae to low temperatures.

a. 16 weeks at or below 10°C.

b. 11 weeks at or below 10°C.

c. Control

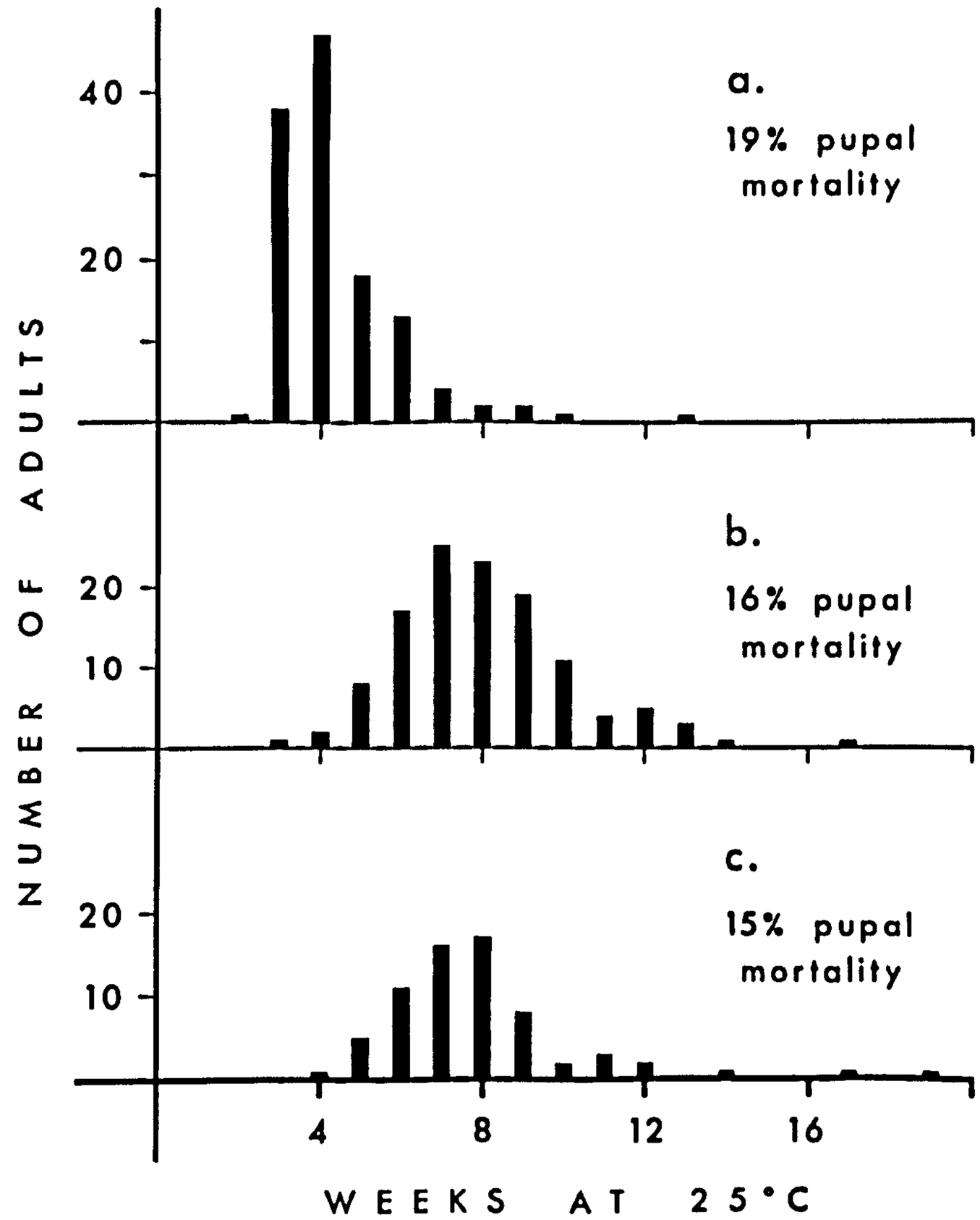

a.
19% pupal
mortality
b.
16% pupal
mortality
c.
15% pupal
mortality
NUMBER OF ADULTS
40
20
20
10
20
10
4
8
12
16
WEEKS AT 25°C

TABLE 48. Days required for hatching at 25°C of diapausing eggs of Bombyx mori exposed to low temperatures for various periods (based on daily counts)

INITIAL TIME AT 25°C (WEEKS)	EXPOSURE TO COLD: WEEKS	°C	NO. IN BATCH	% MORTAL -ITY	RANGE OF HATCHINGS	MEAN AND S.D.	50% HATCH	MIN. RANGE FOR 80% HATCH
8	15	10	390	12	16-25	18.8 ± 2.8	18	16-22
8	25	10	345	10	11-18	13.9 ± 1.6	14	12-16
8	35	10	520	19	11-17	14.0 ± 1.4	14	12-15
8	52	10	603	100	-	-	-	-
12	12	7.5	504	8	12-18	14.3 ± 1.8	14	12-16
8	16	7.5	353	14	12-18	14.3 ± 1.7	14	12-16

Fig. 39. Effect of fumigating diapausing larvae of laboratory (L) and field (F) stocks of Ephestia elutella with phosphine or methyl bromide on pupation under a 16-hour daylength at 25°C.

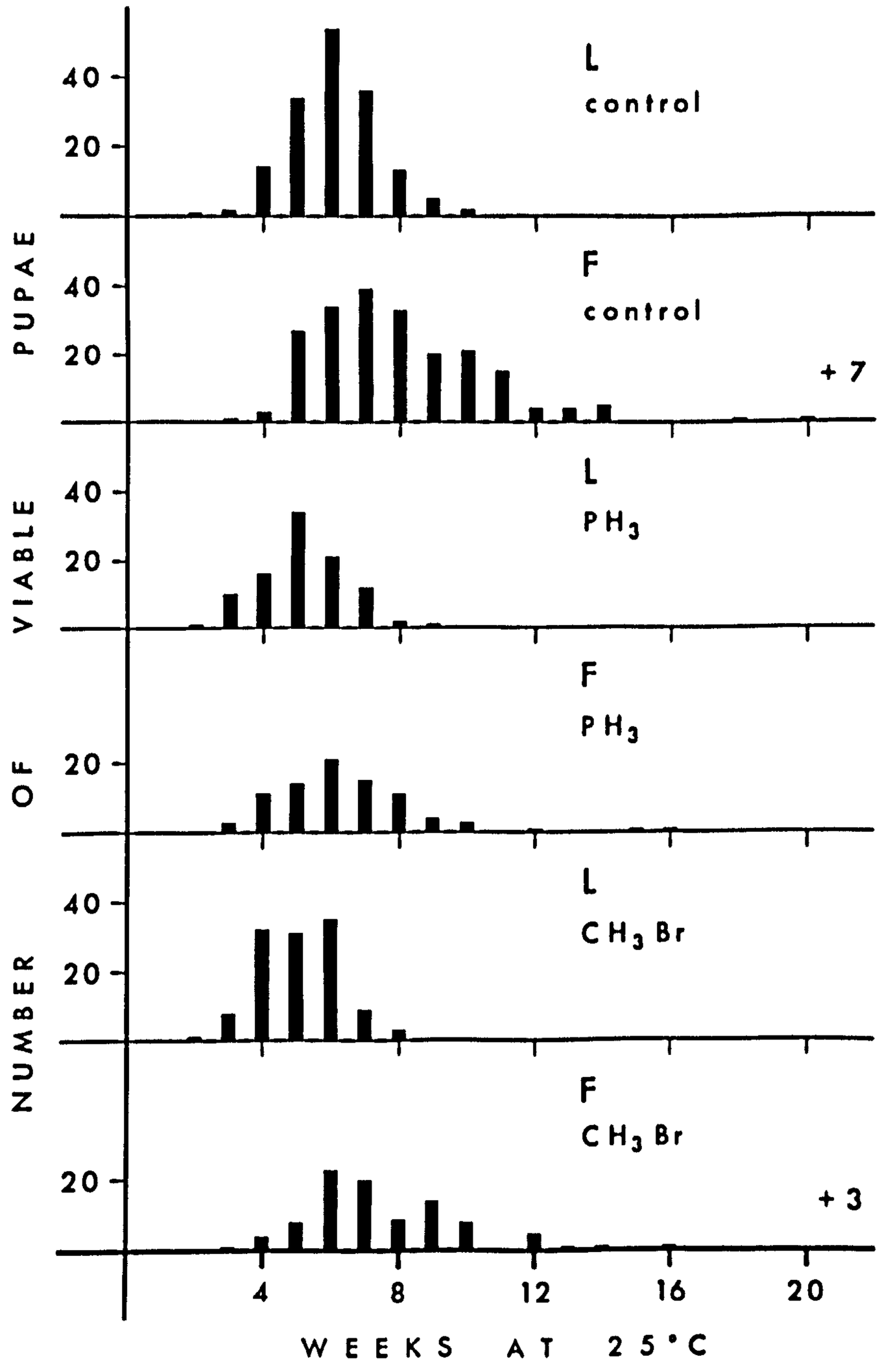
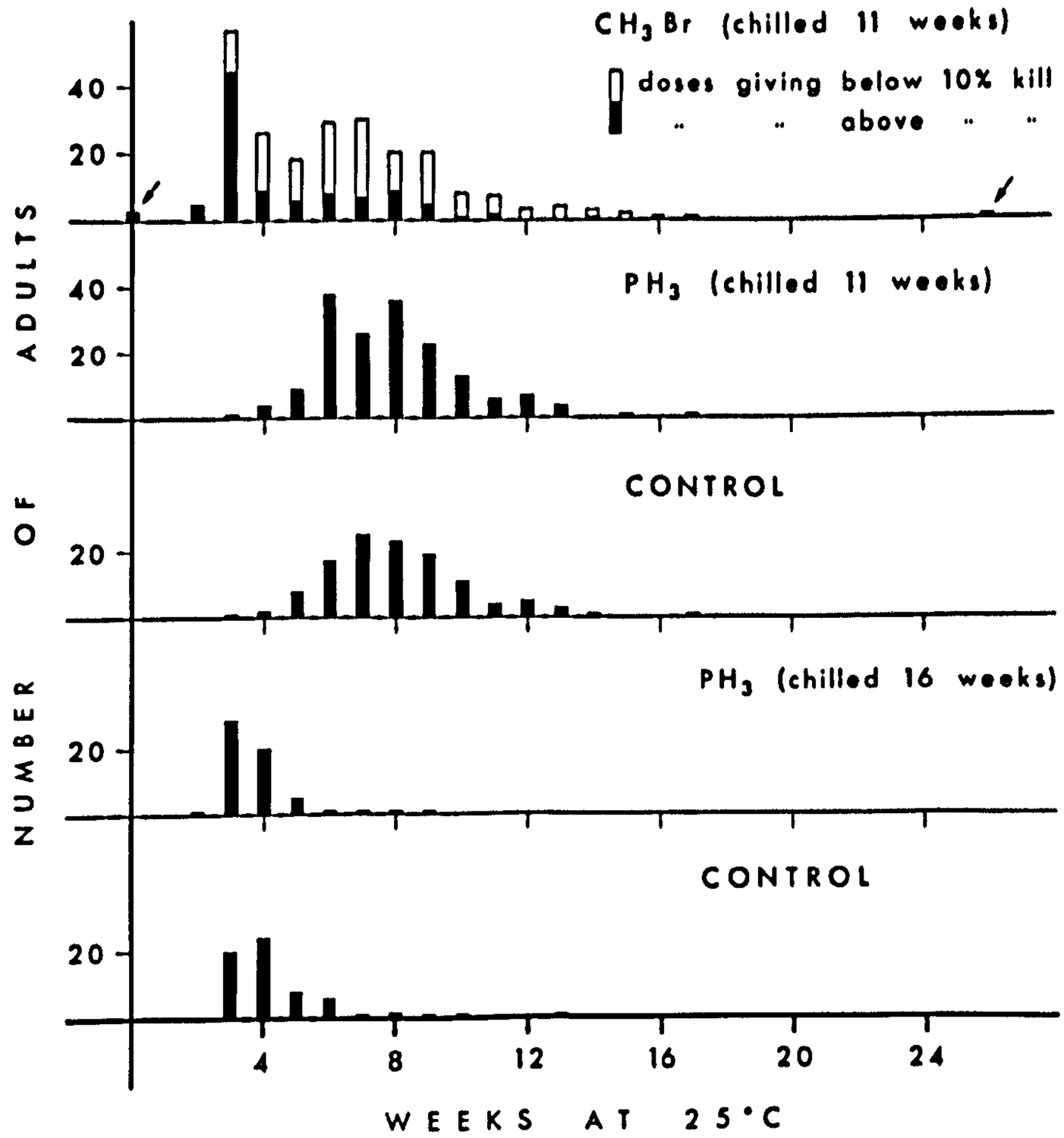

PUPAE
VIABLE
OF
NUMBER
40
20
L
control
40
20
F
control
+ 7
40
20
L
PH3
20
F
PH3
40
20
L
CH3 Br
20
F
CH3 Br
+ 3
4
8
12
16
20
WEEKS AT 25°C

Fig. 40. Emergence of Pieris brassicae under a 16-hour daylength at 25°C after fumigating diapausing pupae with methyl bromide or phosphine.


CH_3Br (chilled 11 weeks)
doses giving below 10% kill
" " above " "
PH_3 (chilled 11 weeks)
CONTROL
PH_3 (chilled 16 weeks)
CONTROL
ADULTS
OF
NUMBER
40
20
20
20
20
4
8
12
16
20
24
WEEKS AT 25°C

FUMIGATION RESULTS

1. PHOSPHINE : ALL STAGES EXCEPT DIAPAUSING LARVAE

In all 4 Pyralid species, a markedly tolerant phase was observed in the egg stage, and by comparison, pupae and larvae were very susceptible. Even more susceptible were adults, which failed to survive the lowest concentration-time (CT) products tested.

A. 25°C

i) Stages surviving fumigation

Cultures were set up by adding batches of eggs at weekly intervals. Two cultures of each species were presented for each treatment, one containing all the later developmental stages, and the other containing the earlier ones. All exposure periods included the period during which phosphine was released from the solid formulation. A concentration peak was reached in about 24-36 hours. After a 2-day exposure to a concentration rising to 0.016 mg/l, some survival occurred in both cultures of all species (figs. 41 and 42).

An examination of the control emergence pattern, and the time required at 25°C for the completion of the various stages of development, revealed that a high proportion of eggs, younger pupae, and prepupae had survived fumigation. Older pupae produced adults which died a few hours after emergence. Larval survivors were identified in the older culture of E. kuehniella and in both cultures of E. cautella, but not in cultures of E. elutella. A single adult of P. interpunctella which emerged between 17 and 20 days after fumigation, was identified as being present under gas as a larva. No adults survived fumigation in any species. In the interests of clarity, the emergence of survivors arising from eggs laid by adults emerging just before fumigation, adults from pupae which emerged and died within a few hours, and adults emerging before fumigation which died under gas, are not included in figs. 41 and 42.

ii) Effect of concentration and exposure period

All stages other than eggs failed to develop into viable adults after exposure to CT products of 3.7 mg h/l or above (table 49). Some pupae developed into adults after exposure to CT products of up to 5 mg h/l, but these died within a few hours of emergence with the wings not fully expanded (fig. 43). Results demonstrated the advantage of longer exposures over shorter ones. In 2-day exposures, eggs of all four species survived a CT product of 38 mg h/l, and those of E. elutella survived 142 mg h/l. In 4-day exposures, no eggs hatched after exposure to a CT product of 9.3 mg h/l. Furthermore, percentage survival after 2-day exposures to phosphine, bore little relationship to concentration.

B. 15°C

Cultures were prepared as in the tests at 25°C, and were fumigated at 15°C for 2, 4, 8, and 16 days at concentrations rising in 36-48 hours to about 0.1, 0.2 and 0.4 mg/l (tables 50 and 51). All stages other than eggs were killed by a 2-day CT product of 13.8 mg h/l. At 15°C, development virtually ceased in P. interpunctella, and in larvae and pupae of E. cautella. In E. kuehniella and E. elutella, development proceeded at less than a third the rate at 25°C. Mortality in eggs of P. interpunctella was increased after exposure to 15°C, but survival was obtained in 2 and 4-day exposures when the period at 15°C was reduced to 10 days. Survival of eggs was recorded in exposures of up to 8 days in other species, and CT products up to 288 mg h/l failed to give 100% kill. The tolerance of eggs to phosphine is further examined in section 2.

A single pupa of E.kuehniella survived a 4-day CT product of 8.5 mg h/l, while pupae of the other three species succumbed to a 2-day CT product of 7.2 mg h/l. Eggs and young pupae of E. kuehniella and E. elutella survived a 2-day CT product of 3.5 mg h/l (fig. 44). In both species, emergence in cultures containing older stages started about 5 days before the test, or 2 days before lowering to 15°C, and eggs were laid before the test. For greater clarity, the emergence of survivors from these eggs is not included in fig. 44.

Fig. 41. Effect of a 2-day exposure at 25°C to a phosphine concentration rising to 0.016mg/l on the emergence under a 16-hour daylength at 25°C in cultures of A. Ephestia kuehniella and B. Ephestia elutella set up to contain all non-diapausing stages of the life cycle.

KEY : i) Older cultures set up weekly with eggs from 5½ to 2½ weeks (E.kuehniella), or 6 to 3 weeks (E.elutella), before fumigation.

ii) Younger cultures set up weekly with eggs from 2 weeks before, to the day of the fumigation.

EXPECTED = Expected emergence based on hatch in control cultures.

CONTROL = Actual control emergence.

FUMIGATED = Emergence in fumigated cultures.

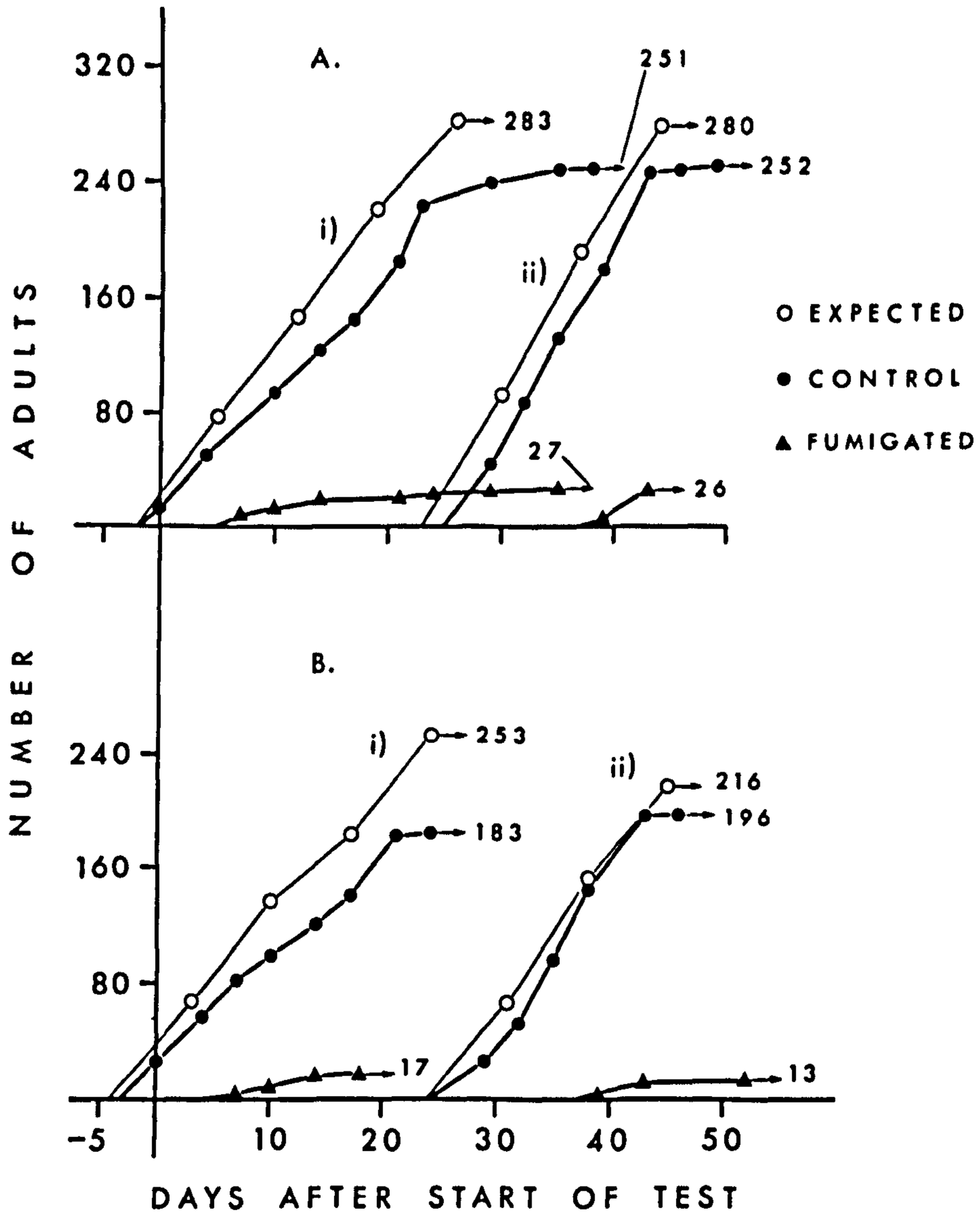

A.
320
240
160
80
283
251
280
252
i)
ii)
27
26
O EXPECTED
● CONTROL
▲ FUMIGATED
B.
240
160
80
253
183
216
196
17
13
i)
ii)
-5
10
20
30
40
50
NUMBER OF ADULTS
DAYS AFTER START OF TEST

Fig. 42. Effect of a 2-day exposure at 25°C to a phosphine concentration rising to 0.016 mg/l on the emergence under a 16-hour daylength at 25°C in cultures of A. Ephestia cautella and B. Plodia interpunctella set up to contain all non-diapausing stages of the life cycle.

KEY : i) Older cultures set up weekly with eggs from 5 to 3 weeks (E.cautella), or 4½ to 2½ weeks (P.interpunctella), before fumigation.

ii) Younger cultures set up weekly with eggs from 2 weeks before, to the day of the fumigation.

EXPECTED = Expected emergence based on hatch in control cultures

CONTROL = Actual control emergence

FUMIGATED = Emergence in fumigated cultures.

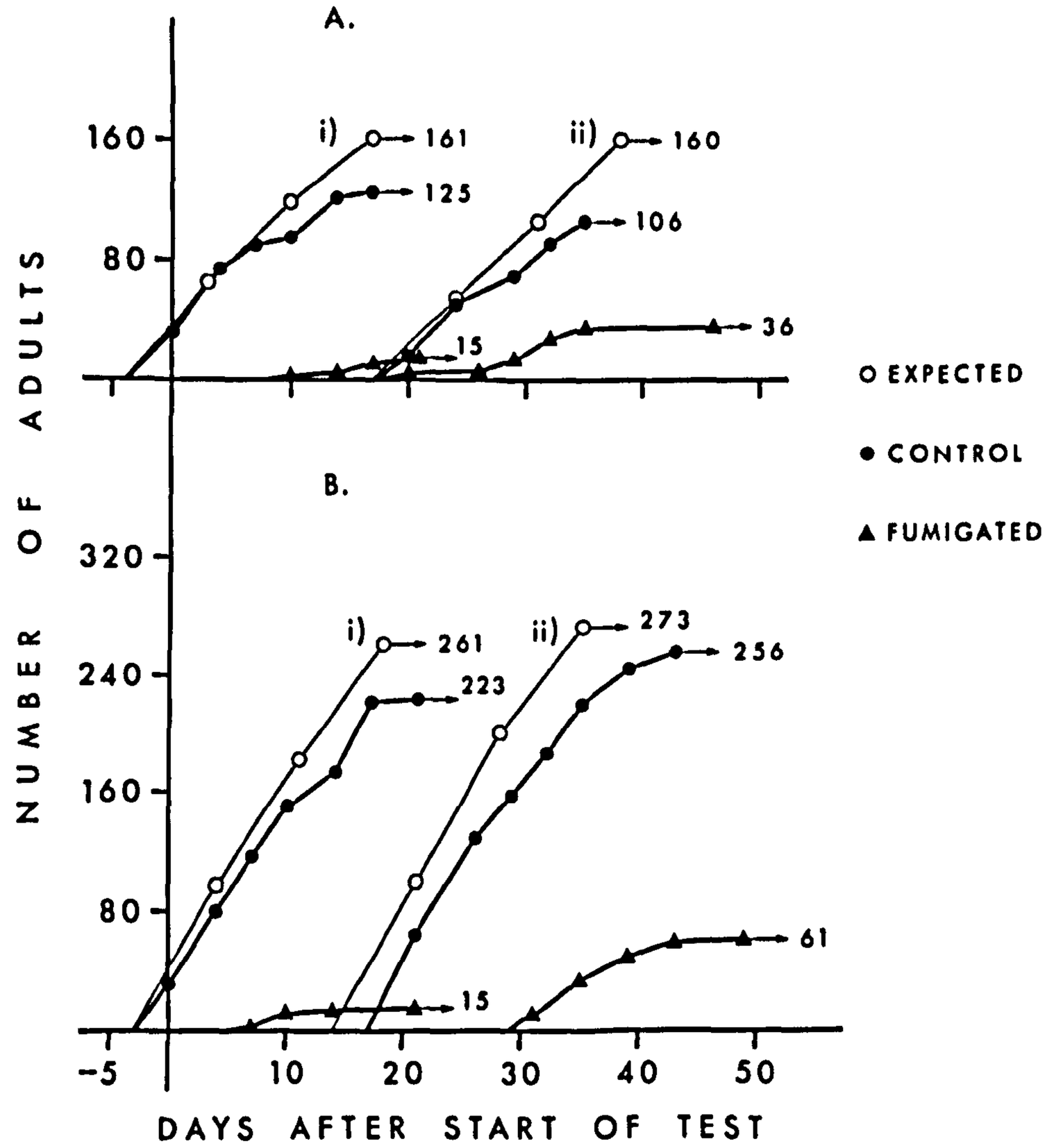

A.
160
80
i)
161
125
ii)
160
106
36
15
O EXPECTED
● CONTROL
▲ FUMIGATED
B.
320
240
160
80
i)
261
223
ii)
273
256
61
15
-5
10
20
30
40
50
NUMBER OF ADULTS
DAYS AFTER START OF TEST

TABLE 49. Toxicity of phosphine to 4 species of stored product moths in tests on all stages at 25°C, 70% RH (% survivals to adult stage based on emergence in controls)

DOSE (TABLETS/ 15 cu.m.	EXPOSURE PERIOD (DAYS)	CT PRODUCT (mg h/l)	Ephestia elutella 1	Ephestia elutella 2	Ephestia kuehniella 1	Ephestia kuehniella 2	Ephestia cautella 1	Ephestia cautella 2	Plodia interpunctella 1	Plodia interpunctella 2
¼	2	0.7	21	6	38	10	62	15	100	4
	4	1.4	1	*	7	*	3	9	68	*
	7	2.2	0+	*	4	*	1.5	1	35	*
	10	2.8	0+	*	0.5	*	0.6	5	13	*
½	2	1.3	35	3	8	5	4	1	70	*
	4	2.5	0+	*	0+	*	?	0	5	0
	7	3.7	0	*	0	0	0	0	0+	0
	10	4.8	0	0	0	0	0	0	0+	0
1	2	2.6	30	2	2	*	?	*	8	*
	4	5.0	0	*	0+	*	0+	0	0+	0
	7	7.6	0	0	0	0	0	0	0	0
	10	9.4	0	0	0	0	0	0	0	0
2	2	5.0	12	*	8	*	8	0	6	0
	4	9.3	0	0	0	0	0	0	0	0
	7	13.6	0	0	0	0	0	0	0	0
	10	16.3	0	0	0	0	0	0	0	0
4	2	10.5	7	0	9	0	4	0	16	0
	4	19	0	0	0	0	0	0	0	0
	7	27	0	0	0	0	0	0	0	0
	10	31	0	0	0	0	0	0	0	0
8	2	19	20	0	8	0	5	0	10	0
	4	35	0	0	0	0	0	0	0	0
	7	53	0	0	0	0	0	0	0	0
	10	63	0	0	0	0	0	0	0	0
16	2	38	2	0	5	0	2	0	12	0
	4	87	0	0	0	0	0	0	0	0
	7	124	0	0	0	0	0	0	0	0
32	2	77	4	0	0	0	0	0	0	0
	4	158	0	0	0	0	0	0	0	0
64	2	142	2	0	0	0	0	0	0	

KEY: HEADINGS: 1 = eggs 2 = larvae and pupae.
* Adults emerge but die in a few hours.
0+ Eggs hatch, but no survival to the adult stage.
? No survival from added egg sample, but survival among egg laid in culture.

Fig. 43. Adults of Ephestia kuehniella emerging from pupae fumigated with phosphine, and dying within hours of emergence.

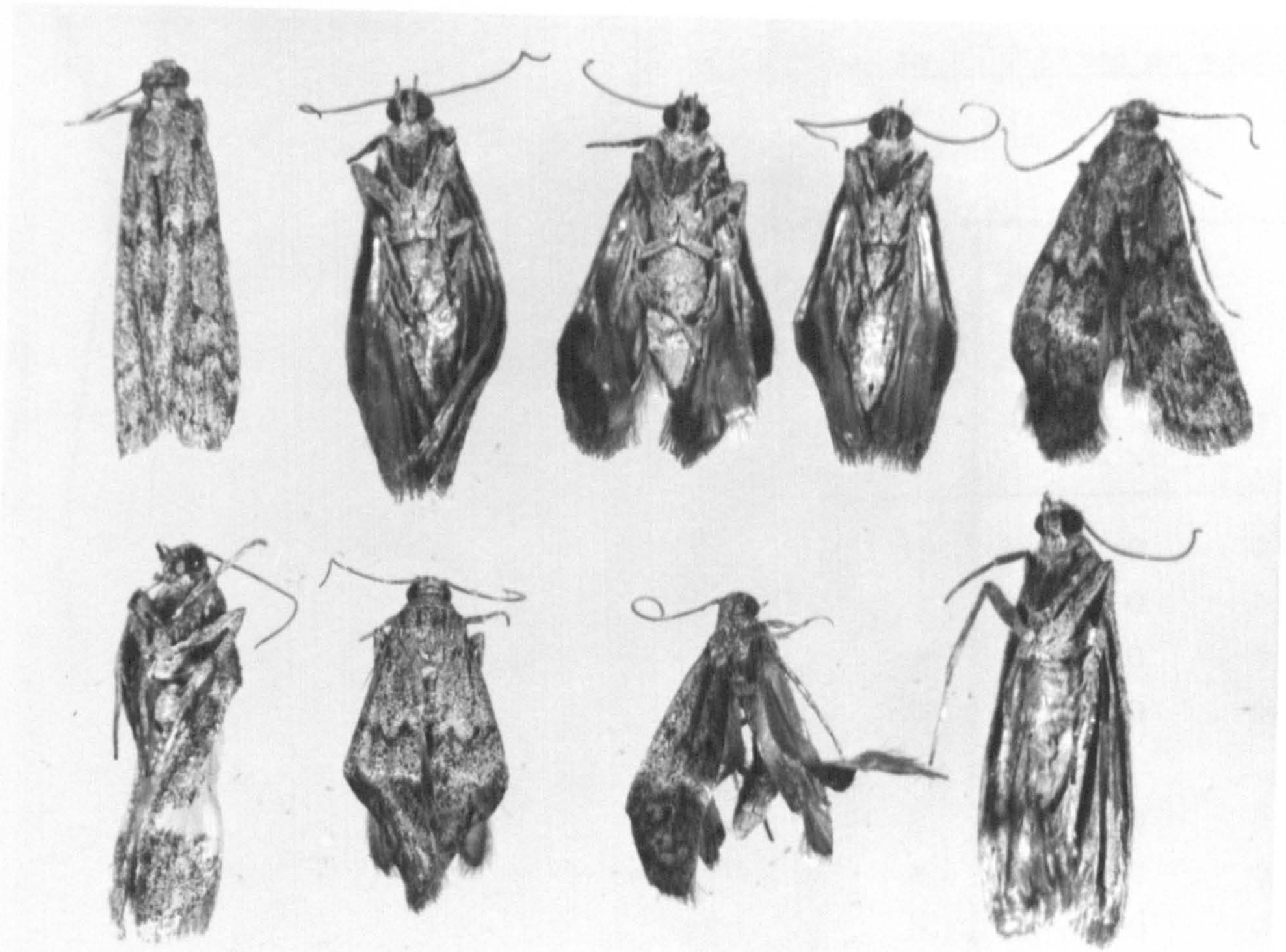


TABLE 50. Toxicity of phosphine to Ephestia elutella and Ephestia kuehniella at 15°C, 70% RH, in tests on cultures containing all stages of active development (% survivals to adult stage at 25°C based on emergence in the control)

DOSE (PELLETS /1700l)	EXPOSURE PERIOD (DAYS)	CT PRODUCT (mg h/l)	Ephestia elutella			Ephestia kuehniella		
			1	2	3	1	2	3
1	2	3.5	40	0	12	26	0	12
	4	8.5	38	0	*	15	0	1
	8	17.9	3	0	0	9	0	0
	16	35.7	0	0	0	0	0	0
2	2	7.2	28	0	*	39	0	8
	4	16.8	32	0	0	24	0	0
	8	34.8	1	0	0	?	0	0
	16	69.3	0	0	0	0	0	0
4	2	13.8	38	0	*	32	0	*
	4	34.9	24	0	0	33	0	0
	8	74.4	?	0	0	0+	0	0
	16	150	0	0	0	0	0	0

KEY:

HEADINGS: 1 = Eggs, 2 = Larvae, 3 = Pupae

* Adults emerge but die in a few hours

0+ Eggs hatch, but no survival to the adult stage

? No survival from added egg sample but survival among eggs laid in culture

TABLE 51. Toxicity of phosphine to Ephestia cautella and Plodia interpunctella at 15°C, 70% RH, in tests on cultures containing all stages of active development (% survivals to adult stage at 25°C, based on emergence in the controls)

DOSE (PELLETS /1700l)	EXPOSURE PERIOD (DAYS)	CT PRODUCT (mg h/l)	Ephestia cautella			Plodia interpunctella		
			1	2	3	1	2	3
1①	2	3.5	7	0	2	0+	0	1
	4	8.5	2	0	0	0+	0	0
	8	17.9	0+	0	0	0	0	0
	16	35.7	0	0	0	0	0	0
2②	2	7.2	10	0	*	4	0	*
	4	16.8	10	0	0	1	0	0
	8	34.8	0.2	0	0	0	0	0
	16	69.3	0	0	0	0	0	0
4②	2	13.8	12	0	0	8	0	0
	4	34.9	8	0	0	?	0	0
	8	74.4	0	0	0	0	0	0
	16	150	0	0	0	0	0	0

① Retained at 15°C for 18 days

② Material kept at 15°C for 10 days, except for 16-day exposure

KEY

As for table 50.

Fig. 44. Effect of a 2-day exposure to phosphine at 15°C (CT product 3.5 mg h/l) on emergence under a 16-hour daylength at 25°C in cultures of A. Ephestia kuehniella and B. E. elutella set up to contain all non-diapausing stages of the life cycle. (All cultures kept at 15°C for 3 days before and 16 days after fumigation).

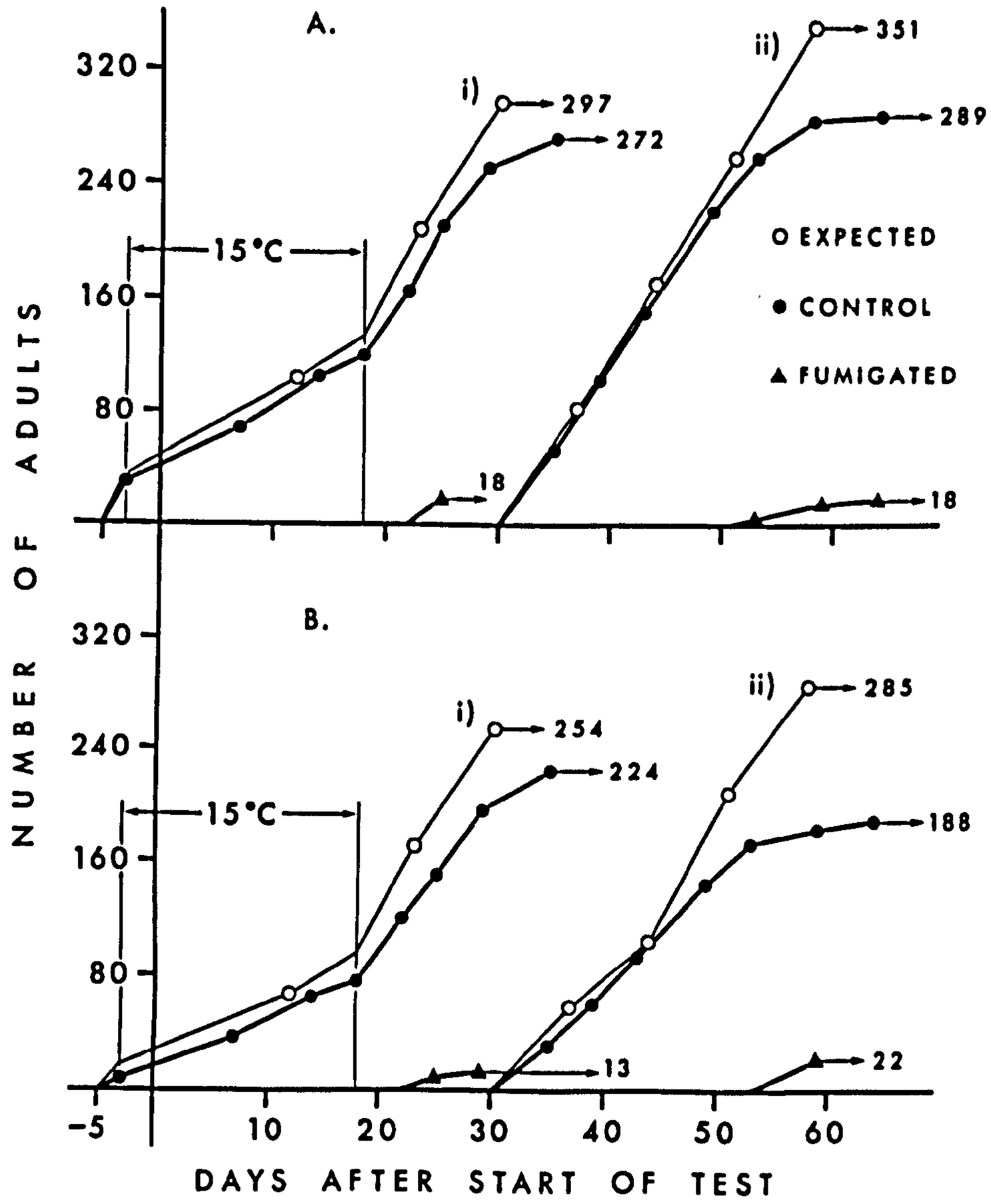
KEY : i) Older cultures set up weekly with eggs from 6 to 4 weeks before fumigation

ii) Younger cultures set up weekly with eggs from 3 weeks before, to the day of the fumigation.

EXPECTED = Expected emergence based on hatch in control cultures

CONTROL = Actual control emergence

FUMIGATED = Emergence in fumigated cultures.


A.
320
240
160
80
15°C
i)
297
272
ii)
351
289
O EXPECTED
● CONTROL
▲ FUMIGATED
18
18
B.
320
240
160
80
15°C
i)
254
224
ii)
285
188
13
22
-5
10
20
30
40
50
60
NUMBER OF ADULTS
DAYS AFTER START OF TEST

2. EGGS

A. Phosphine

i) Exposures and CT products required for complete kill

Eggs of Pyralid moths proved highly tolerant to phosphine for the first 35-45% of the developmental period. In shorter exposure periods, it was very difficult to achieve 100% kill (table 52). In longer exposures, complete kill was obtained in all species by very low CT products. Low temperature, which slowed development, lengthened the exposures required for a complete kill. However, eggs of all species were killed by prolonged exposure at 10°C (see Biological Results, section 1.B.). At 15°C, 0-1 day-old eggs of P. interpunctella did not give rise to adults after a 14-day exposure, and were more susceptible to phosphine than the other 3 species at this temperature. Eggs of E. elutella showed the highest tolerance, surviving CT products of 288 mg h/l over 8 days at 15°C, and 142 mg h/l over 2 days at 25°C.

ii) Effect of age of eggs

All tests were conducted with a phosphine concentration stabilised at about 0.2 mg/l. Susceptibility increased with age, the highest levels of survival occurring amongst eggs of the youngest age group.

At 25°C, a clear relationship was observed between the age of eggs at the end of fumigation, and survival to the adult stage (table 53). No survival occurred in batches of eggs aged 3 days or more at the end of fumigation.

At 15°C in the three species of Ephestia, eggs of the youngest age group showed little mortality after fumigation for 2 or 4 days (table 54). The older the age group, the smaller was the percentage survival. No eggs of E. kuehniella survived the 8-day exposure, but E. elutella and E. cautella required the 9-day exposure for complete kill. Results for P. interpunctella have been considered separately (table 55), as new-laid eggs were adversely affected by exposure at 15°C, and different mortalities after fumigation were

obtained when samples were held at 15°C for various lengths of time.

iii) Effect of exposure period and temperature: CT product or concentration held constant

Results for tests with a constant CT product of about 9-10 mg h/l are summarised in tables 56 and 57, and for tests with a constant concentration of about 0.4 mg/l, in tables 58 and 59. In all tests, eggs aged 0-1 days at 25°C were fumigated. It was apparent that temperature, and not concentration, controlled the maximum time eggs were able to survive fumigation, and the level of survival after any given exposure period. With a constant CT product, a drop in temperature of 5°C between 30 and 15°C, in all 4 species approximately doubled the exposure period permitting survival. At 15°C, however, very low concentrations during the longer exposures permitted survival beyond the exposure range tested.

When concentration rather than CT product was held constant (tables 58, 59), the pattern of survival was little affected apart from there being no survival in the longer exposures at 15°C. Maximum exposure periods tested that permitted survival were 1 day at 30°C, 2 days at 25°C, 3 to 4 days at 20°C, and 5 to 8 days at 15°C. At 10°C, a substantial survival among eggs of E. elutella and E. kuehniella occurred after an 8-day exposure, but all eggs were killed after 16 days.

iv) Susceptibility of laboratory and field stocks

For each species, the susceptibilities of eggs aged 0-1 days in a laboratory stock, and in a recently-obtained field stock, were compared at a range of concentrations and exposure periods at various temperatures (tables 60, 61 and 62). In all species, variation between stocks was not great. However, field stocks often survived long exposures at very low concentrations rather better than laboratory stocks. In P. interpunctella at 15°C, the higher cold tolerance of the field stock may have enhanced this effect.

TABLE 52. Concentration-time products (mg h/l) for various exposures at 5 temperatures giving complete kill of the egg stage of 4 species of stored product moth pests (2 stocks per species tested).

TEMP. °C	EXPOSURE PERIOD (DAYS)	Ephestia elutella	Ephestia kuehniella	Ephestia cautella	Plodia interpunctella
10	8	138	138	-	-
	16	< 36.8	< 36.8	-	-
15	4	-	-	-	126
	6	-	> 207	> 207	52.8
	7	-	248	248	62.9
	8	> 288	74.4	74.4	34.8
	9	20.1	11.7	20.1	20.1
	16	< 16.9	< 16.9	< 16.9	< 16.9
20	3	> 157	157	> 157	> 157
	3¾	-	35.8	35.8	-
	4	> 89.0	9.6	9.6	89.0
	5	10.3	< 4.0	< 4.0	10.3
25	1¾	-	> 80.0	> 80.0	80.0
	2	> 142	77.0	77.0	77.0
	3	3.9	3.9	3.9	3.9
	4	2.5	2.5	3.3	3.3
30	1	> 9.4	> 9.4	> 9.4	> 9.4
	2	< 2.4	< 2.4	< 2.4	< 2.4

KEY

< Complete kill at this, the lowest CT product tested.

> Survival at this, the highest CT product tested.

– Not tested at this exposure, or only at CT products below those giving survival in a longer exposure.

TABLE 53. Effect of fumigating three age groups of eggs of 4 species of stored product moths at 25°C with a phosphine concentration stabilized at 0.2 mg/l (100 eggs per treatment).

SPECIES	AGE FROM LAYING (DAYS)	% CONTROL MORTALITY		MORTALITIES FOR 2 REPLICATES AT EACH EXPOSURE CORRECTED FOR AVERAGE CONTROL MORTALITY					
				24 HOURS		48 HOURS		72 HOURS	
		1	2	1	2	1	2	1	2
Ephestia clutella	0-1	0	13	15	5	85	86	100	100
	1-2	7	13	92.5	92.9	100	100		
	2-4	10	16	100	100	100	100		
Ephestia kuehniella	0-1	11	5	8	14	98.9	97.8	100	100
	1-2	12	9	91.6	98.8	100	100		
	2-4	3	3	100	100	100	100		
Ephestia cautella	0-1	19	15	5	49	98.9	100	100	100
	1-2	8	21	100	100	100	100		
	2-4	13	25	100	100	100	100		
Plodia interpunctella	0-1	5	3	2	7	90.6	88	100	100
	1-2	4	5	90.5	88	100	100		
	2-4	6	21	100	100	100	100		

TABLE 54. Effect of fumigating several age groups of eggs of three species of Ephestia at 15°C with a phosphine concentration stabilized at about 0.2 mg/l (300 eggs per treatment).

SPECIES	AGE		% CONTROL MORTAL-ITY	MORTALITIES AT EACH EXPOSURE CORRECTED FOR CONTROL MORTALITY			
	DAYS AT 25°C FROM LAYING	DAYS ACCLIMATISED AT 15°C(1)		2 DAYS	4 DAYS	8 DAYS	9 DAYS
Ephestia	0-1	0	21	26	27	98.7	100
		1	26	43	73	100	
elutella	1-2	0	20	90	89	100	
		1	18	100	100	100	
	2-3	0	19	100	100		
Ephestia	0-1	0	3	2	13	100	100
		1	5	5	86	100	
kuehniella	1-2	0	2	83	99.3	100	
		1	6	91	99.6	100	
	2-3	0	10	100	100		
Ephestia	0-1	0	17	2	22	96	100
cautella	1-2	0	16	70	81	100	
	2-3	0	16	100	100		

(1) All samples were allowed at least 1 hour acclimatisation at 15°C before fumigation.

TABLE 55. Effect of fumigating three age groups of eggs of Plodia interpunctella at 15°C with a phosphine concentration stabilized at about 0.2 mg/l. Eggs held at 15°C for various periods of time before returning to 25°C (300 eggs per treatment).

AGE AT FUMIGATION (DAYS AT 25°C)	TOTAL NO. OF DAYS AT 15°C	% CONTROL MORTALITY	MORTALITIES AFTER EACH EXPOSURE TO PHOSPHINE CORRECTED FOR CONTROL MORTALITY		
			2 DAYS	4 DAYS	8 DAYS
0-1	4	12	2	48	-
	9	73	24	71	100
	15	100	100	100	100
1-2	4	10	23	70	-
	9	49	73	90	100
	15	98	100	100	100
2-3	4	12	100	100	-
	9	23	100	100	-
	15	35	-	-	-

TABLE 56. Effect of temperature and exposure period on the susceptibility to phosphine of eggs of Ephestia elutella and Ephestia kuehniella aged 0-1 days at 25°C, with a constant CT product of 9.4 ± 0.9 mg h/l (100-400 eggs per exposure, percentage mortalities assessed on the basis of adult emergence and corrected for control mortality).

SPECIES	EXPOSURE (DAYS)	TEMPERATURE °C 15	20	20	30
Ephestia	1	-	-	5	44
elutella	2	-	0	85	100
	3	-	32	100	-
	4	0	97.0	100	100
	5	-	100	-	-
	8	81	-	-	-
	9	95.8(1)	-	-	-
	10	100(2)	-	100	-
Ephestia	1	-	-	28	94.9
kuehniella	2	-	2.6	97.8	100
	3	-	22	100	-
	4	39	100	100	100
	8	99.4	-	-	-
	9	100(1)	-	-	-

- = Not tested

(1) CT product 11.7 mg h per l

(2) CT product 13.1 mg h per l

TABLE 57. Effect of temperature and exposure period on the susceptibility to phosphine of eggs of Ephestia cautella and Plodia interpunctella aged 0-1 days at 25°C, with a constant CT product of 9.4 ± 0.9 mg h/l (100-400 eggs per exposure, percentage mortalities assessed on the basis of adult emergence and corrected for control mortality).

SPECIES	EXPOSURE (DAYS)	TEMPERATURE °C 15	20	25	30
Ephestia	1	-	-	39	76
cautella	2	-	44	98.9	100
	3	-	90.6	100	-
	4	20	100	100	100
	8	91.4	-	-	-
	9	100(1)	-	-	-
Plodia	1	-	-	14	77
interpunctella	2	-	24	88	100
	3	-	35	100	-
	4	73	98.1	100	100
	5	-	100	-	-
	8	93.8	-	-	-
	9	100(1)	-	-	-

- = Not tested

(1) CT product 11.7 mg h per l

TABLE 58. Effect of temperature and exposure period on the susceptibility to phosphine of eggs of Ephestia elutella and Ephestia kuehniella aged 0-1 days at 25°C at a constant concentration of 0.4 mg/l (100-400 eggs per exposure, percentage mortalities assessed on the basis of adult emergence and corrected for control mortality).

SPECIES	EXPOSURE (DAYS)	TEMPERATURE °C				
		10	15	20	25	30
Ephestia elutella	1	-	-	0	5	44
	2	6	27	16	99.0	100
	3	-	-	85	100	100
	4	0	52	99.2	100	-
	5	-	76	100	-	-
	8	68	99.7	100	-	-
	9	-	100	-	-	-
	16	100	100	-	-	-
Ephestia kuehniella	1	-	-	0	28	94.9
	2	20	10	25	99.3	100
	3	-	-	92.1	100	100
	3¾	-	-	97.3	-	-
	4	31	3	100	100	-
	5	-	48	100	-	-
	6	-	79	-	-	-
	7	-	98.8	-	-	-
	8	45	100	-	-	-
	9	-	100	-	-	-
	16	100	100	-	-	-

- = Not tested

TABLE 59. Effect of temperature and exposure period on the susceptibility to phosphine of eggs of Ephestia cautella and Plodia interpunctella aged 0-1 days at 25°C at a constant concentration of 0.4 mg/l (100-400 eggs per exposure, percentage mortalities assessed on the basis of adult emergence and corrected for control mortality).

SPECIES	EXPOSURE (DAYS)	TEMPERATURE °C 15	20	25	30
Ephestia cautella	1	-	0	39	76
	2	36	31	98	100
	3	-	86	100	100
	3¾	-	100	-	-
	4	56	100	100	-
	5	71	100	-	-
	6	82	-	-	-
	7	92.4	-	-	-
	8	100	-	-	-
	9	100	-	-	-
Plodia interpunctella	1	-	0	14	77
	2	56	19	92.8	100
	3	-	47	100	100
	4	96.3	99.4	100	-
	5	98.8	100	-	-
	6	100	-	-	-
	7	100	-	-	-
	8	100	100	-	-
	9	100	-	-	-

- = Not tested

TABLE 60. Susceptibility of eggs aged 0-1 days of laboratory (LAB) and field stocks of 4 stored product moths to phosphine at 10 and 15°C: percentage mortalities corrected for control mortality; 100-500 eggs per treatment.

TEMP. (°C)	EXPOSURE (HOURS)	CT PRODUCT (mg h/l)	Ephestia elutella		Ephestia kuehniella		Ephestia cautella		Plodia interpunctella	
			LAB	FIELD	LAB	FIELD	LAB	FIELD	LAB	FIELD
10	48	11.7	6		23	20				
	48	15.3	71		36	33				
	96	33.1	0		44	31				
	96	55.0	79		59	85				
	192	74.2	68		45	51				
	192	138	100		100	100				
	384	36.8	100	100	100	100				
15	48	44.4	32	28	17	55	91		85	
	96	126	47	57	23	57	88		100	
	144	52.8			95.3	79	82		100	100
	144	207	90.7	92.2	99.6	99.7	99.1		100	100
	168	62.9			100	98.8	100	92.4	100	100
	192	10.3	81	35	99.4		91.4		93.8	
	192	11.7	94.0		98.4	98.6	89	68	97.5	81
	192	35.2	96.7		99.8		96.3		100	
	192	74.4	100		100		100		100	
	192	288	99.7	100	100	100	100	100	100	
	216	11.7	95.8		100		100		100	
	216	13.2	96.9	63	100	100	100	94.0	100	86
	216	20.1	100	100			100	100	100	100
	240	14.6	100	91.2	100	100	100	100	100	85
	384	16.9	100		100	100	100	100	100	100

TABLE 61. Susceptibility of eggs aged 0-1 days of laboratory (LAB) and field stocks of 4 stored product moths to phosphine at 20°C: percentage mortalities corrected for control mortality; 100-500 eggs per treatment.

TEMP. (°C)	EXPOSURE (HOURS)	CT PRODUCT (mg h/l)	Ephestia elutella		Ephestia kuehniella		Ephestia cautella		Plodia interpunctella	
			LAB	FIELD	LAB	FIELD	LAB	FIELD	LAB	FIELD
20	48	8.5	81	35	99.4		91.4		93.8	
	48	88.0	33	46	50	90	94.6	92.8	96.6	57
	72	8.6	32		22		90.6		35	
	72	13.8	27	39	31	83	92.0	88	14	
	72	26.8	85	92.9	97.5	100	86	95.1	67	78
	72	27.9	78	73	100	92.1	100	98.4	47	64
	72	157	95.5	77	100	100	99.5	100	100	99.5
	90	35.8			97.3		100		92.8	92.5
	91	42.0	98.4	93.3	100	100	100	100	98.8	97.6
	96	3.3	70	22	100	97.3	100	94.6	32	43
	96	9.6	92.6	87	100	100	100	100	99.0	98.1
	96	44.4	99.2	99.2					100	99.4
	120	4.0	100	84	100	100	100	100	66	82
	120	10.3	100	100					100	100

TABLE 62. Susceptibility of eggs aged 0-1 days of laboratory (LAB) and field stocks of 4 stored product moths to phosphine at 25 and 30°C: percentage mortalities corrected for control mortality; 100-500 eggs per treatment.

TEMP. °C	EXPOSURE (HOURS)	CT PRODUCT mg h/l	Ephestia elutella		Ephestia kuehniella		Ephestia cautella		Plodia interpunctella	
			LAB	FIELD	LAB	FIELD	LAB	FIELD	LAB	FIELD
25	24	9.5	5	0	41	28	39	65	27	14
	24	12.5			39	54			9	18
	24	62.5			60	78	81	48	67	75
	42	47.3	83	78	96.2	98.1	97.1	97.4	98.9	95.3
	42	80.0			98.7	100	100	95.6	100	100
	48	16.1	91.8	92.7	100	99.4	100	98.7	100	99.2
	48	18.7	99.0	93.4	99.3	99.5	98.0	100	100	99.4
	48	24.6			95.9	100			94.7	94.4
	48	57.6			98.8	100	99.5	100	100	99.8
	48	77.0	95.1	96.8	100		100		100	
	48	142	98.4		100		100		100	
	72	3.9	100	100	100	100	100	100	100	100
30	24	8.1	44		89		90.0		90.4	
	24	9.4	47		94.9		76		77	
	48	2.4	100	100	100	100	100	100	100	100

v) Effect of including the initial evolution of phosphine in the exposure period.

Exposures of similar length which included or did not include the initial evolution of phosphine from a particular dosage of aluminium phosphide, gave similar levels of kill (table 63). The concentration towards the end of the exposure was therefore considered to control the level of kill obtained.

B. Methyl Bromide

i) CT products required for complete kill

Eggs of Pyralid moths were quite susceptible to methyl bromide at all temperatures (table 64). Variations in susceptibility between species and between age groups were slight, but in general the eggs of E. elutella were a little more tolerant than other species, and older eggs were usually more tolerant than younger ones. The CT product needed to kill all eggs fell from 63 mg h/l at 10 or 15°C, to 21 mg h/l at 30°C.

ii) Effect of concentration and exposure period at 15°C

The laboratory stocks of E. elutella and P. interpunctella were the test insects in this investigation, and three age groups of each were treated. Tests were conducted at concentrations of 4.2, 6.7 and 10.8 mg/l, with exposure periods ranging from 1 to 16 hours. The number of eggs selected per treatment varied from 100 to 500 as it was desired to reduce the difference between weighting factors at doses giving about 50% mortality and those giving higher mortalities. Eggs of P. interpunctella are susceptible to cold, and in all tests, eggs were returned to 25°C after 48 hours at 15°C, this time including an initial acclimatisation period of not less than 1 hour, the actual fumigation itself, and an airing period of at least 24 hours.

As in tests on eggs with phosphine, mortality assessments were based on the number of survivors to the adult stage. Percentage mortalities were calculated from the number of eggs expected to hatch, and were corrected for mortality

between hatching and adult emergence in the controls. Table 65 summarises the results and presents LD 50 and 99.9 values obtained from eye-fitted plots of probit mortality against log. CT product.

To investigate the interaction of concentration and exposure time, the results for each age group of eggs were subjected to a probit analysis in which the concentration and time components of the CT product were treated separately. An account of this method is given by Finney (1952) in his examples 18 and 19. Regression coefficients b_1 for concentration and b_2 for time were calculated using the following equations relating sums of squares and products:

$$Sx_1y = (Sx_1x_1)\ b_1 - (Sx_1x_2)\ b_2$$

$$Sx_2y = (Sx_2x_2)\ b_2 - (Sx_1x_2)\ b_1$$

The values and standard errors calculated for b_1, b_2 and b_1-b_2, are set out in table 66. In all but one of the six age groups tested, the difference between b_1 and b_2 was exceeded by the standard error for this value. Thus the regression coefficients for concentration and exposure time were similar, and it was assumed that the data could be adequately represented by expressing dose in CT products.

The data from each concentration level were therefore combined together with doses expressed as CT products to estimate LD 50, 90, and 99 values (table 67). At the 5% level, heterogeneity was significant in two of the six groups, but the corrected fiducial limits for each LD level remained quite close together.

The eggs of E. elutella proved more tolerant than those of P. interpunctella. At all chosen LD levels, there was no overlap between the fiducial limits of the most susceptible batch of E. elutella and the most tolerant batch of P. interpunctella (p, the probability of batches being similar = <0.001). The youngest eggs of P. interpunctella were significantly more susceptible than older eggs ($p = <0.001$ at all LD levels). In E. elutella, 2-3 day-old

eggs were significantly more tolerant than younger eggs at the LD 50 and 90 levels ($p =< 0.001$), but not at the LD 99 level.

iii) Susceptibility of laboratory and field stocks

Eggs of all stocks proved quite susceptible to methyl bromide. LD 99.9 values obtained from eye-fitted probit lines ranging from 35 mg h per l for 1-2 day-old eggs of the field stock of E. kuehniella, to 72 mg h/l for 0-1 day-old eggs of the laboratory stock of E. elutella, were recorded at 15°C (tables 68-71). At 25°C, the range was 18-30 mg h/l. At 20 and 25°C, slopes of probit lines were higher than at 15°C. All stocks were similar in that eggs aged 2-3 days proved the most tolerant at the LD 50 level, with the exception of the laboratory stock of P. interpunctella at 15°C. In this case, eggs aged 1-2 days appeared very marginally more tolerant than 2-3 day-old eggs. After further analysis (table 67), it was found that the difference was significant at the LD 50 level ($p =< 0.001$), but not at the LD 90 or 99 levels.

Between stocks of the same species, differences in the susceptibility of eggs of similar age were small, and only in the case of E. elutella at 20°C was the difference between stocks greater than the difference between age groups of the same stock. These results were subjected to a mathematical probit analysis (table 72). Eggs of the field stock aged 0-1 or 1-2 days, proved more tolerant at the LD 90 and 99 levels than those of the laboratory stock ($p =< 0.001$). Heterogeneity within batches rendered the difference between batches of 2-3 day-old eggs insignificant, although in both stocks 2-3 day-old eggs were significantly more tolerant than younger eggs at the LD 90 level ($p =< 0.001$).

Calculated LD 50 values closely resembled results obtained by eye-fitted lines, and further analysis of results was considered inappropriate because of the small differences between batches, a low number of degrees of freedom available, and the likelihood of high variation within batches containing eggs differing in age by up to 24 hours.

TABLE 63. Percentage mortalities of eggs aged 0-1 days exposed to phosphine concentrations rising from zero with the breakdown of Phostoxin pellets, and to stabilized concentrations.

TEMP. °C	EXPOSURE (HOURS)	CT PRODUCT mg h/l	Ephestia elutella LAB	Ephestia elutella FIELD	Ephestia kuehniella LAB	Ephestia kuehniella FIELD	Ephestia cautella LAB	Ephestia cautella FIELD	Plodia interpunctella LAB	Plodia interpunctella FIELD
20	72	27.9 R	85	92.9	97.5	100	86	95.1	67	78
		26.8 S	78	73	100	92.1	100	98.4	47	64
25	48	16.1 R	91.8	92.7	100	99.4	100	98.7	100	92.8
		18.7 S	99.0	93.4	99.3	99.5	93.0	100	100	99.2
30	24	8.1 R	44		90.6		89		90	
		9.4 S	47		77		94.9		76	

R = RISING : Concentration rising from zero at start of test

S = STABLE : Concentration at maximum at start of test

TABLE 64. Concentration-time products (mg h/l) of methyl bromide required for complete kill of different age groups of storage moth eggs at a range of temperatures (2 stocks per species tested.

SPECIES	AGE IN DAYS AT 25°C	TEMPERATURES °C 10	15	20	25	30
Ephestia	0-1	63	63	53	26	21
elutella	1-2	63	63	42	26	21
	2-3	63	63	53	30	21
	3-4		63	53	30	
Ephestia	0-1	63	53	30	22	17
kuehniella	1-2	63	32	30	22	17
	2-3	63	53	36	26	17
	3-4		53	36	26	
Ephestia	0-1	ALL EGGS	53	36	22	17
cautella	1-2	KILLED BY	53	41	30	21
	2-3	14 DAYS	53	41	30	21
	3-4	AT 10°C	63	41	30	
Plodia	0-1	ALL EGGS	47	29	22	21
interpunctella	1-2	KILLED BY	47	29	22	17
	2-3	10 DAYS	53	35	26	21
	3-4	AT 10°C	53	35	26	

TABLE 65. Variation in the susceptibility of three age groups of Ephestia elutella and Plodia interpunctella eggs with three concentrations of methyl bromide at 15°C.

INSECT	CONCENTRATION mg/l	CT PRODUCT mg h/l	AGE OF EGGS IN DAYS AT 25°C					
			0-1			1-2		2-3		
			% kill	LD50 / LD99.9		% kill	LD50 / LD99.9	% kill	LD50 / LD99.9
PLODIA (LAB STOCK)	4.2	21.1	66			20		28	
		25.3	80	20 / 40		33	27 / 61	46	25 / 57
		33.7	99.2			80		86	
		63.0	100			100		100	
	6.7	20.0	59			13		29	
		26.6	87	19 / 44		38	27 / 48	52	25 / 52
		33.3	98.2			88		88	
		46.6	100			100		99.6	
	10.8	10.8	12			0		0	
		21.5	69	18 / 47		19	27 / 55	23	26 / 53
		26.8	86			36		59	
		32.2	96.8			75		86	
		53.2	100			100		100	
EPHESTIA (LAB STOCK)	4.2	21.1	12			14		0	
		25.3	35	29 / 70		25	29 / 68	3.7	38 / 71
		33.7	65			68		24	
		63.0	100			100		100	
	6.7	20.0	20			10		0	
		26.6	38	29 / 76		28	30 / 70	8.4	37 / 69
		33.3	69			74		23	
		46.6	94.1			92.5		89	
	10.8	21.5	16			11		0	
		26.8	37	29 / 70		31	29 / 68	4.8	38 / 68
		32.2	51			44		18	
		53.2	97.0			98.4		96.0	

(100-500 eggs per exposure, % kill corrected for control mortality)

TABLE 66. A comparison of the regression coefficients b_1 and b_2 calculated for concentration and time of exposure in tests against eggs of Ephestia elutella and Plodia interpunctella with methyl bromide at 15°C.

SPECIES	AGE OF EGGS AT 25°C	CONCENTRATION $b_1 \pm$ S.E.	TIME $b_2 \pm$ S.E.	$b_1 - b_2 \pm$ S.E.	HETEROGENEITY FACTOR
Plodia (Lab. Stock)	0 - 1	7.86 ± 0.55	7.99 ± 0.51	- 0.12 ± 0.25	< 1
	1 - 2	10.28 ± 0.89	10.58 ± 0.84	- 0.30 ± 0.42	2.56
	2 - 3	9.67 ± 0.56	9.60 ± 0.53	+ 0.07 ± 0.25	< 1
Ephestia (Lab. Stock)	0 - 1	7.21 ± 0.59	7.55 ± 0.54	- 0.34 ± 0.42	2.19
	1 - 2	7.71 ± 0.44	8.47 ± 0.40	- 0.75 ± 0.28	< 1
	2 - 3	12.13 ± 0.57	12.38 ± 0.57	- 0.25 ± 0.38	< 1

S.E. = Standard Error

TABLE 67. Probit analysis of the combined results of tests with three concentrations of methyl bromide at 15°C against different age groups of eggs of Plodia interpunctella and Ephestia elutella (LD values calculated as CT products in mg h/l).

TEST INSECT	AGE OF EGGS (DAYS) AT 25°C)	LD VALUES WITH FIDUCIAL LIMITS IN PARENTHESIS			SLOPE b ±S.E.	HETEROG-ENEITY FACTOR	DEGREES OF FREEDOM
		LD 50	LD 90	LD 99			
Plodia (Lab. Stock)	0 - 1	19.3 (20.3, 18.0)	27.2 (28.1, 26.5)	36.1 (38.5, 34.3)	8.55 ±0.59	<1	9
	1 - 2	27.5 (28.5, 26.5)	36.2 (39.0, 34.3)	45.2 (51.3, 41.5)	10.78 ±0.98	2.84	9
	2 - 3	25.5 (26.1, 24.8)	34.5 (35.9, 33.4)	44.2 (47.5, 41.8)	9.74 ±0.55	<1	9
Ephestia (Lab. Stock)	0 - 1	29.6 (30.6, 28.5)	43.5 (45.4, 41.9)	59.5 (64.3, 55.9)	7.67 ±0.38	<1	10
	1 - 2	30.5 (31.9, 28.9)	43.2 (46.3, 40.9)	57.3 (64.7, 52.6)	8.48 ±0.67	2.63	10
	2 - 3	37.8 (38.7, 36.9)	47.7 (49.2, 46.4)	57.7 (60.5, 55.4)	12.69 ±0.58	<1	9

TABLE 68. Susceptibility of eggs of the laboratory and field stocks of Plodia interpunctella to methyl bromide.

		PERCENTAGE MORTALITIES CORRECTED FOR CONTROL MORTALITY					
	STOCK :	LABORATORY			FIELD		
	AGE (DAYS) :	0 - 1	1 - 2	2 - 3	0 - 1	1 - 2	2 - 3
Temp. (°C)	Dose (mg h/l)						
15	10.8	12	0	0	5		
	20.7 ± 0.8*	65	17	27	58	14	11
	26.1 ± 0.8*	84	36	52	90.5	44	22
	33.0 ± 0.8*	98.1	81	87	97.8	88	61
	46.6	100	100	99.6	100	100	99.3
	53.2	100	100	100	100	100	100
	LD 50 (mg h/l) :	19	27	26	19	26	30
	LD 99.9 (mg h/l):	45	55	54	44	49	53
20	11.8	20	10	0	25	31	
	17.6	95.1	66	13	73	66	0
	23.5	100	98.8	79	98.1	90.0	74
	29.3	100	100	100	100	100	98.6
	35.1	100	100	100	100	100	100
	40.9	100	100	100	100	100	100
	LD 50 (mg h/l) :	14	16	21	15	16	22
	LD 99.9 (mg h/l):	22	29	30	29	30	33
25	10.5	36	8	0	24	0	
	14.6	95.2	80	52	72	74	32
	18.2	100	98.7	94.9	89	98.6	86
	21.8	100	100	100	100	100	99.7
	26.1	100	100	100	100	100	100
	LD 50 (mg h/l) :	11	13	14	13	13	16
	LD 99.9 (mg h/l):	19	21	23	24	21	23

* Results for similar CT products in three tests combined for laboratory stock

TABLE 69. Susceptibility of eggs of the laboratory and field stocks of Ephestia kuehniella to methyl bromide.

		PERCENTAGE MORTALITIES CORRECTED FOR CONTROL MORTALITY					
	STOCK :	LABORATORY			FIELD		
	AGE(DAYS):	0 - 1	1 - 2	2 - 3	0 - 1	1 - 2	2 - 3
Temp. (°C)	Dose (mg h/l)						
15	10.8	22	11	6.0	0	1.5	30
	21.5	84	74	18	70	78	30
	26.8	89	95.9	72	80	94.8	63
	32.2	95.7	100	93.8	93.7	100	85
	53.2	100	100	100	100	100	100
	LD 50 (mg h/l) :	17	15	21	21	18	22
	LD 99.9 (mg h/l):	48	38	49	48	35	53
20	12.1	49	52	8.2	61	50	14
	18.2	96.5	96.7	56	93.9	98.7	49
	24.3	100	99.8	91.0	99.7	100	89
	30.3	100	100	99.3	100	100	100
	36.4	100	100	100	100	100	100
	42.4	100	100	100	100	100	100
	LD 50 (mg h/l) :	12	12	18	12	12	18
	LD 99.9 (mg h/l):	25	26	36	26	22	35
25	10.5	67	70	39	42	61	18
	14.6	97.7	98.5	97.8	85	90	60
	18.2	100	100	99.5	99.1	96.5	91.9
	21.8	100	100	100	100	100	99.7
	26.1	100	100	100	100	100	100
	LD 50 (mg h/l) :	9	9	10	11	10	13
	LD 99.9 (mg h/l):	19	18	19	22	23	25

TABLE 70. Susceptibility of eggs of the laboratory and field stocks of Ephestia cautella to methyl bromide.

Temp. (°C)	Dose (mg h/l)	PERCENTAGE MORTALITIES CORRECTED FOR CONTROL MORTALITY					
STOCK :		LABORATORY			FIELD		
AGE (DAYS) :		0 - 1	1 - 2	2 - 3	0 - 1	1 - 2	2 - 3
15	10.8	2.3			0		
	20.0	37	5.9	2.6	59	0	0
	26.7	66	38	33	83	27	10
	33.3	81	87	40	90	68	40
	46.6	99.6	100	95.0	100	98.8	96.4
	53.2	100	100	100	100	100	100
	LD 50 (mg h/l) :	24	27	31	21	32	36
	LD 99.9 (mg h/l):	56	43	58	48	48	54
20 *	12.0 ± 0.2	5			11		
	17.9 ± 0.3	41	32	16	65	33	44
	23.9 ± 0.4	86	78	53	96.1	73	69
	29.8 ± 0.5	98.9	98.9	90	100	97.9	90.8
	35.7 ± 0.6	100	100	99.4	100	99.6	98.6
	41.6 ± 0.7	100	100	100	100	100	100
	52.4						100
	LD 50 (mg h/l) :	19	21	23	16	20	20
	LD 99.9 (mg h/l):	36	36	44	30	38	44
25	10.5	10			21		
	14.6	79	55	0	74	41	0
	18.2	99.4	71	61	95.4	59	50
	21.8	100	99.3	95.0	100	80	93.3
	26.1	100	100	100	100	98.2	96.9
	30.3		100	100		100	100
	LD 50 (mg h/l) :	13	15	18	13	17	18
	LD 99.9 (mg h/l):	20	25	26	23	31	30

* Laboratory and field stocks fumigated in separate tests

TABLE 71. Susceptibility of eggs of the laboratory and field stocks of Ephestia elutella to methyl bromide.

Temp. (°C)	Dose (mg h/l)	PERCENTAGE MORTALITIES CORRECTED FOR CONTROL MORTALITY					
	STOCK :	LABORATORY			FIELD		
	AGE (DAYS) :	0 - 1	1 - 2	2 - 3	0 - 1	1 - 2	2 - 3
15	20.7 ± 0.8	16	12	0	25	8	0
	26.1 ± 0.8	37	28	5.4	46	36	9.4
	33.0 ± 0.8	62	62	22	70	81	25
	46.6	94.1	92.5	89	97.5	94.6	87
	53.2	97.0	98.4	96.0			
	63.0	100	100	100	100	100	100
	LD 50 (mg h/l) :	29	30	38	27	29	37
	LD 99.9 (mg h/l):	72	69	67	67	65	66
20	12.0 ± 0.2	15			2.8		
	17.9 ± 0.3	70	47	14	57	1.9	0
	23.9 ± 0.4	90.9	83	45	74	63	16
	29.8 ± 0.5	99.3	98.6	72	95.9	88	40
	35.7 ± 0.6	100	100	98.5	97.8	94.3	64
	41.6 ± 0.7	100	100	100	97.5	99.1	95.9
	52.4				100	100	100
	LD 50 (mg h/l) :	16	19	25	18	24	30
	LD 99.9 (mg h/l):	36	38	43	50	48	53
25	10.9	34			51		
	14.6	82	64	17	68	41	12
	18.2	97.9	90	69	98.4	74	40
	21.8	99.5	99.3	89	100	98.7	86
	26.1	100	100	99.4	100	100	98.8
	30.3		100	100		100	100
	LD 50 (mg h/l) :	12	14	17	11	16	19
	LD 99.9 (mg h/l):	25	25	29	23	25	30

TABLE 72. Probit analysis to compare the susceptibility of eggs of laboratory (LAB) and field stocks of Ephestia elutella to methyl bromide at 20°C (LD values calculated in mg h/l).

STOCK	AGE OF EGGS (DAYS AT 25°C)	LD VALUES WITH FIDUCIAL LIMITS IN PARENTHESIS			SLOPE b ±S.E.	HETEROG-ENEITY FACTOR	DEGREES OF FREEDOM
		LD 50	LD 90	LD 99			
LAB	0 - 1	16.2 (17.5, 14.6)	23.0 (25.3, 21.3)	30.7 (36.1, 27.5)	8.38 ±0.73	<1	4
	1 - 2	19.0 (20.6, 16.7)	25.4 (27.7, 23.7)	32.1 (38.4, 29.1)	10.21 ±1.07	<1	3
	2 - 3	25.0 (30.7, 2.6)	34.0 (1677, 28.3)	43.8 (>10^5, 33.8)	9.57 ±2.86	9.21	3
FIELD	0 - 1	17.7 (26.0, 13.0)	29.0 (34.2, 25.7)	43.3 (63.7, 36.1)	5.98 ±0.95	2.29	5
	1 - 2	22.6 (24.6, 19.7)	31.3 (33.5, 29.5)	40.8 (47.0, 37.4)	9.10 ±0.96	<1	4
	2 - 3	31.1 (33.5, 27.5)	39.3 (49.7, 36.3)	47.6 (63.7, 42.2)	12.57 ±2.05	3.87	4

C. Observations on Fumigation Survivors

i) Delay of hatching and mortality

In general, it was noted that the hatching of eggs surviving fumigation was delayed. In tests on eggs of E. elutella with phosphine, in which exposure periods ranged from 1 to 8 days, delay was less than the period of exposure, but in tests with methyl bromide, in which exposure periods never exceeded 16 hours, delay was always greater than the time spent under gas (table 73).

Investigations on other species gave comparable results to those obtained for E. elutella. With methyl bromide, exposures giving mortalities of below 50%, in general gave a delay of about 1 day at 25°C, while those giving higher mortalities gave a delay of about 2 days at this temperature. With phosphine, delay was less obviously linked with mortality, and was represented more accurately as one half to threequarters of the exposure period, provided that some kill was recorded.

Counts of hatch alone were inadequate in assessing fumigation efficiency, as fewer adults were produced than would have been expected from the number of eggs hatching. Often, when hatch was low, no adults at all were produced, indicating that mortality occurred during the larval or pupal stages.

ii) Adults produced from surviving eggs

Although no detailed records were made, it was observed that the emergence of adults developing from eggs surviving fumigation, began a day or two later at 25°C than adults in the controls. At lower temperatures, longer delays were encountered. No deformities were observed in the mouthparts or genitalia of adults developing from eggs fumigated with either phosphine or methyl bromide. When a comparison was made with adults obtained from controls, the weight at emergence, and the fecundity of survivors from either fumigant, were normal (table 74). The fertility of eggs laid was normal.

3. NON-DIAPAUSING LARVAE AND PUPAE

A. Phosphine

The tolerance of non-diapausing larvae and pupae to phosphine was low (tables 75 and 76). A CT product of 6.2 mg h/l killed all non-diapausing larvae, and 15.3 mg h/l killed all pupae, under all conditions tested. Tolerance to phosphine was lower the higher the temperature. The longest exposure periods giving the same CT product were the most effective (table 76).

Pupae aged less than 3 days at 25°C were more tolerant than older pupae, which, in the case of E. elutella and E. kuehniella, were destroyed at 25 and 15°C by 2-day CT products of 1.3 mg h/l and 3.5 mg h/l respectively. Some younger pupae (table 76) survived CT products of up to 9.8 mg h/l at 15°C, and 2.8 mg h/l at 25°C.

Of the four species, P. interpunctella showed the lowest tolerance, and E. kuehniella slightly the highest in most instances, particularly at low temperature. Differences between laboratory and field stocks (table 75) were small.

B. Methyl Bromide

Like phosphine, methyl bromide was highly toxic to pupae and non-diapausing larvae (table 77). At 15°C or above, a CT product of 64 mg h/l was sufficient for complete control of both stages. In contrast to results with phosphine, tolerance to methyl bromide was decreased only slightly at higher temperatures. In tests on 0-3 day-old pupae of E. kuehniella (table 78), there was little change in LD 50 values read from eye-fitted probit lines at 15, 20 or 25°C. The LD 99.9 decreased only from 75 mg h/l at 15°C to 62 mg h/l at 25°C.

Pupae older than three days proved less tolerant than younger pupae in all species, and were killed by CT products of 32 mg h/l at 20 and 25°C and 43 mg h/l at 15°C. Younger pupae required higher CT products (tables 77 and 78).

Generally, 0-3 day-old pupae were more tolerant than larvae. At 10°C, however, fully grown larvae of the field stock of E. cautella were more tolerant than pupae of all species and were of equal tolerance to some at 15°C (table 77). Larvae of the laboratory stock of E. cautella were

not tested at 10°C, but at 15°C were much less tolerant than larvae of the field stock. Apart from this isolated case, differences between the susceptibilities of species or stocks to methyl bromide were small.

C. Observations on Survivors from Fumigated Pupae

In contrast with results for eggs, adults of normal appearance and longevity, which emerged from pupae fumigated with either phosphine or methyl bromide at levels giving considerable mortality, generally showed a reduction in fertility. The number of progeny obtained from E. kuehniella adults selected from batches of pupae fumigated with either phosphine or methyl bromide, was less than half the number obtained from an equal number of adults from unfumigated pupae (table 79).

After fumigation with phosphine, many adults successful in emerging failed to expand the wings properly, and died within a few hours (fig.43). Weighing pupae and adults before and after emergence indicated that whereas the weight loss after emergence from unfumigated pupae was about 25 to 40% of the pupal weight, imperfect adults of short longevity emerging from fumigated pupae often showed weight losses in excess of 50% (table 80). With methyl bromide, adults from fumigated pupae often failed to free themselves of the pupal case, remaining with wings, legs, or abdomen trapped. Adults not successfully completing emergence, or dying soon afterwards, were counted as dead in assessing results. Examination of genitalia and mouthparts revealed no deformity in adults emerging from fumigated pupae.

4. FUMIGATION OF LARVAE IN DIAPAUSE

In longer exposures, diapausing larvae were more tolerant to fumigation than other stages of the life cycle. Very high tolerance was shown to

TABLE 73. [illegible] hatching of eggs of Ephestia elutella (laboratory stock) surviving fumigation with phosphine and methyl bromide.

FUMIGANT AND TEMPERATURE	EXPOSURE (HOURS)	NO. TESTED	NO. SURVIVING TO ADULT	NUMBER HATCHING	DAYS FROM OVIPOSITION	
					RANGE FOR HATCHING	MEAN AND S.D.
PH_3 (0.4 mg/l) At 20°C	CONTROL	100	76	82	8 - 11	9.9 ± 0.8
	24	100	77	85	9 - 12	10.3 ± 0.7
	48	100	63	78	9 - 12	10.8 ± 0.8
	72	200	23	68	10 - 13	11.4 ± 0.7
	96	500	3	20	11 - 13	12.4 ± 0.5
MeBr (7.3 mg/l) At 25°C	CONTROL	100	67	86	5 - 7	6.2*
	1½	100	45	73	6 - 8	7.1
	2	200	24	62	6 - 9	7.9
	2½	300	4	31	7 - 9	7.9
	3	300	1	10	8 - 9	8.1

* Standard errors not calculated for eggs at 25°C, as interval between counts (1 day) was too large in relation to the duration of the egg stage.

TABLE 74. Progeny from groups of 4 pairs of Pyralid moths selected from batches of fumigated and unfumigated eggs aged 0-1 days at 25°C (laboratory stocks only).

SPECIES	TREATMENT	MORTALITY IN TREATED BATCH	TOTAL WT OF 4 YOUNG FEMALES (mg) (1)	NO. OF PROGENY
Plodia interpunctella	CONTROL	-	49.5	229
	PH_3	27	49.8	260
	MeBr	36	46.8	218
Ephestia cautella	CONTROL	-	45.2	306
	PH_3	39	42.6	243
	MeBr	79	47.5	318
Ephestia kuehniella	CONTROL	-	53.7	265
	PH_3	41	50.1	182
	MeBr	67	52.8	249
Ephestia elutella	CONTROL	-	41.4	136
	PH_3	83	46.0	201
	MeBr	82	42.2	138

(1) Weighed within 1 day of emergence

TABLE 75. Concentration - time products (mg h/l) in 2-day exposures to phosphine that killed all non-diapausing larvae and pupae of laboratory (LAB) and field stocks of 4 Pyralid moths.

TEMP. °C	STAGE	Ephestia elutella		Ephestia kuehniella		Ephestia cautella		Plodia interpunctella	
		LAB	FIELD	LAB	FIELD	LAB	FIELD	LAB	FIELD
10	LARVAE	1.6/	1.6/	3.5	3.5		6.2		
	PUPAE	11.7	11.7	15.3	15.3		11.7*		
15	LARVAE	1.6	1.6	3.5	3.5	3.5	5.9	1.6	1.6
	PUPAE	7.2	7.2	13.8	8.3	7.2	5.9	5.9	5.9
20	LARVAE	1.0	1.0	1.7	1.4	1.7	1.7	1.0	1.0
	PUPAE	4.0	4.0	4.0	4.0	3.2	2.6	2.3	3.2
25	LARVAE	0.7	0.7	1.3	1.1	1.3	1.3	0.9	0.9
	PUPAE	3.4	2.8	3.4	2.8	2.6	2.0	1.3	2.5

* Adversely affected by prolonged exposure to this temperature

/ 30-hour exposures

TABLE 76. Ephestia elutella and E. kuehniella (laboratory stocks). Exposure periods at selected concentrations of phosphine and percentage mortality of nupae aged 0-3 days at 25°C (100 pupae per batch).

TEMP. °C	EXPOSURE (DAYS)							
	2			4			8	
	CTP mg h/l	E. elutella	E. kuehniella	CTP mg h/l	E. elutella	E. kuehniella	CTP mg h/l	E. kuehniella
15	0	3	6					
	1.6	65	52					
	3.5	88	78	3.9	98	83		
	5.8	99	96	5.4	100	96		
	7.2	100	92	8.4		100	8.3	100
				8.5		99*	8.6	100
	9.8		99	9.4		100		
	13.8		100					
	LD 50 (mg h/l)	1.5	2.0		-	-		-
25	0	4	6					
	0.7	68	60					
	1.3	88	80	1.4	100	100		
	2.6	96	100	2.5	100	100		
	2.8	98	97					
	5.0	100	100	5.0	100	100		
	LD 50 (mg h/l)	0.45	0.65		-	-		

* Estimated result from a test on all stages (see Fumigation Results Section 1).

TABLE 77. Concentration - tire products (gr A/l) in tests with methyl bromide that killed all non-diapausing larvae and pupae of laboratory (LAB) and field stocks of four Pyralid moths.

TEMP.	STAGE	Ephestia elutella		Ephestia kuehniella		Ephestia cautella		Plodia interpunctella	
°C		LAB	FIELD	LAB	FIELD	LAB	FIELD	LAB	FIELD
10	LARVAE	63	48	63	48		110		
	PUPAE	72	72	72	72		72		
15	LARVAE	53	40	40	40	34	64	34	34
	PUPAE	64	64	64	64	54	64	64	54
20	LARVAE	37	27	37	27	37	40	31	35
	PUPAE	64	64	64	53	50	64	50	47
25	LARVAE	32	32	31	24	31	32	24	31
	PUPAE	50	50	53	43	50	50	43	43
30	LARVAE					25	25	25	25
	PUPAE					40	40	35	40

TABLE 78. Percentage mortalities of nymphs aged 0-3 days at 25°C of Ephestia kuehniella (laboratory stock) exposed to about 10 mg/l of methyl bromide at 15, 20 and 25°C (100 nymphs per treatment).

CT PRODUCTS	TEMPERATURE °C		
mg h/l	15	20	25
21.0 ± 0.5	0	0	11
26.5 ± 0.2	13	20	18
32.0 ± 0.3	37	44	43
43.0 ± 0.5	82	92.9	89
53.5 ± 0.3	95.9	96.8	100
64.0	100	100	-
LD 50 (mg h/l)	35	33	31
LD 99.9 (mg h/l)	75	66	62

TABLE 79. Progeny from 4 pairs of Tribolium confusum adults selected from fumigated and unfumigated batches of 100 pupae.

FUMIGANT	CONTROL			FUMIGATED				
				% MORTALITIES				
	% MORTALITY	WT. OF 4 ♀'s (mg)	NO. OF PROGENY	AS PUPAE	AT ADULT ECLOSION	TOTAL	WT. OF 4 ♀'s (mg)	NO. OF PROGENY
PHOSPHINE	0	48.6	112	36	16	52	51.7	127
	5	53.8	207	40	22	62	51.2	104
	0	52.9	286	46	32	78	50.3	91
	5	53.2	231	69	12	81	52.6	38
	8	50.7	155	58	26	84	54.9	49
	TOTALS (5 batches)	259.2	991				260.7	409
METHYL BROMIDE	7	49.6	143	19	6	25	54.1	174
	0	56.8	416	26	11	37	53.5	201
	4	55.5	331	41	4	45	49.7	67
	10	51.2	95	40	10	50	54.2	0
	0	50.3	72	75	7	82	50.9	14
	4	53.1	196	80	9	89	48.9	35
	TOTALS (6 batches)	316.5	1253				311.3	491

TABLE 80. Weight losses of adults emerging from unfumigated pupae, and from pupae fumigated with phosphine.

	Ephestia elutella (LAB. STOCK)		Ephestia kuehniella (LAB. STOCK)	
	CONTROL	FUMIGATED WITH PH_3	CONTROL	FUMIGATED WITH PH_3
NO. OF PUPAE	10	11	5	3
MEAN WT. (μg)	13.56	13.98	18.06	15.40
MEAN WT. OF ADULTS EMERGING (μg)	9.68	7.71	12.88	6.83
% WT. LOSS	28.6	44.9	28.7	55.6
LONGEVITY OF ADULTS (DAYS)	All > 5	All but 2* < 1	All > 5	All < 1

* One survived 4 days, and the other 7 days. The weight losses at emergence were 32.6% and 36.0% respectively. A higher weight loss, 39.0%, was recorded from one individual in the control batch.

methyl bromide in all exposures, particularly at low temperature. Diapausing larvae of E. elutella were more tolerant than those of P. interpunctella, and in both species, field stocks proved more tolerant than laboratory stocks. Apart from population density in P. interpunctella, no factor involved in the induction or termination of diapause greatly influenced tolerance.

A. Phosphine

i) CT products required for complete kill

Although fairly susceptible to phosphine, diapausing larvae were more tolerant than other stages in all exposure periods long enough to span the highly tolerant phase in the egg. Longer exposures proved more effective than shorter ones of equivalent CT product in achieving a particular level of kill (table 81), but the effect was not so marked as in the fumigation of actively developing stages.

In all stocks, increased temperatures generally reduced tolerance, although at 10°C two stocks were less tolerant than at 15°C (table 81). At 20°C or above, all diapausing larvae succumbed to a CT product of 18 mg h/l, even in 2-day exposures. The laboratory stock of P. interpunctella was particularly susceptible.

Mortality of diapausing larvae was often delayed until the pupal stage. Sometimes, the pupation moult was not completed, and a monstrosity was produced (fig.45).

(ii) Effect of exposure period and concentration

The efficiency of phosphine in achieving a particular level of kill declined as concentration was increased and exposure time was correspondingly decreased. However, it was possible to calculate CT products for selected mortality levels, as, over restricted ranges of exposure time, the change in efficiency was small, and results were not significantly heterogeneous at the 5% level. (table 82). These ranges of exposure time varied according to temperature and the species or stock tested. With diapausing larvae of P. interpunctella, which were quite susceptible to phosphine, the ranges were narrow, and as it was often invalid to average the results of tests with concentration levels separated by a factor of two, results at certain temperatures have not been entered in table 82.

At 15°C, diapausing larvae of P. interpunctella were tested at concentrations rising to about 0.1 and 0.2 mg/l (table 83). In the 0.2 mg/l test, some exposures were run from the addition of tablets or pellets to the chamber, while others were begun after the initial evolution of gas. As a result, different concentrations of phosphine were prevalent during different exposures. In the field stock, higher mortality was observed in longer exposures than in shorter ones with a higher CT product, and in the analysis of results based on CT products (table 83), marked heterogeneity occurred and fiducial limits became incalculable. The same peculiarity was observed to a lesser extent in the results for the laboratory stock, but departures from linearity in the regression line were not significant at the 5% level. Because of the obvious influence of exposure time, results for the two stocks at 0.2 mg/l were subjected to a further probit analysis, supplanting log. CT product with log. time (table 84). No significant heterogeneity was detected in results for either stock. For comparative purposes, exposures calculated for LD 50 values were converted to CT products by multiplying by a

concentration of 0.155 mg/l, which was the average concentration of the 40-hour test exposure. LD values calculated in this way in the laboratory stock were a little higher than those calculated directly from CT products, while in the field stock they were lower.

When both stocks of P. interpunctella were tested at 3 concentration levels at 20°C, no significant difference occurred in the results obtained for the laboratory stock at 0.04 or 0.10 mg/l (table 85). At 0.20 mg/l, however, diapausing larvae were significantly more tolerant at the LD 50 and 90 levels than at either 0.10 or 0.04 mg/l ($p = < 0.001$), as the 95% fiducial probability limits calculated for either the LD 50 or the LD 90 in the test at 0.20 mg/l showed no overlap with their counterparts in tests at lower concentrations.

The field stock of P. interpunctella was more tolerant at all three calculated LD levels at 0.1 or 0.2 mg/l, than at 0.04 mg/l ($p = < 0.001$). Although much higher CT products were required at each LD level at 0.2 mg/l than at 0.1 mg/l, fiducial limits overlapped considerably, and the significance of difference between these tests was of a low order. At the LD 90 level, neither set of limits included the LD 90 value calculated in the other test, indicating a significance of difference of $p = < 0.025$. At the 3 concentrations tested (table 85), the field stock of P. interpunctella was significantly more tolerant than the laboratory stock at the LD 90 and 99 levels ($p = < 0.001$).

At 25°C (table 85), each stock was tested at only two concentrations, but once again higher CT products were required at each LD level in the tests at the higher concentrations. In the laboratory stock, differences between tests were significant at the LD 50 and 90 levels ($p = < 0.025$).

In tests against diapausing larvae of E. elutella at 10 and 15°C, (table 86), laboratory and field stocks responded differently. At 10°C, although both stocks showed higher susceptibility at the lower concentration, the

significance of difference was low as fiducial limits were wide. At 15°C, with a concentration rising to about 0.1 mg/l, the laboratory stock was significantly more susceptible than with concentrations rising to about 0.2 or 0.4 mg/l ($p =< 0.001$). No significant difference occurred between results for the field stock at concentrations of 0.1 - 0.4 mg/l.

At 20°C (table 87), the laboratory stock was significantly more tolerant at the LD 50 ($p =< 0.001$) and LD 90 ($p =< 0.025$) levels at 0.2 mg/l than at 0.03, 0.05, or 0.1 mg/l. The field stock was more tolerant at a concentration rising to approximately 0.4 mg/l than at 0.05 or 0.1 mg/l, the significance of difference varying with the LD level selected. At the two concentrations to which both stocks were exposed, the field stock was significantly more tolerant than the laboratory stock (at LD 90, $p =< 0.001$).

In contrast to all previous tests with phosphine, the field stock was most highly tolerant at the lowest concentration tested at 25°C, which reached a maximum of about 0.04 mg/l (fig. 46). The very high LD 90 and 99 values, and wide fiducial limits calculated in this test, occurred because the fourth and fifth exposure periods gave little more kill than the third exposure, indicating that a threshold concentration for mortality had been reached between the 4th and 10th days. By the fourth day of the test, which was conducted in a 14,000l brick-built chamber, the concentration was about 0.03 mg/l, and by the 10th day, it had fallen below 0.02 mg/l.

Of the remaining tests at 25°C (table 87), both stocks of E. clutella were significantly more tolerant at the LD 50 level ($p =< 0.025$) at 0.25 mg/l, than at other doses tested.

iii) Effect of temperature

Because of the changes in susceptibility to phosphine with concentration (see A.ii), a concentration level was chosen that was efficient against diapausing larvae over the whole temperature range to be investigated. The

TABLE 81. Concentration-time products (mg h/l) of phosphine required for complete control of diapausing larvae after selected exposure periods at 10°C - 30°C.

TEMP. °C	EXPOSURE (DAYS)	Ephestia elutella		Plodia interpunctella	
		LABORATORY STOCK	FIELD STOCK	LABORATORY STOCK	FIELD STOCK
10	4	< 8.6	33.1	< 8.6	< 8.6
	8	-	36.3	-	-
	16	-	36.8	-	-
15	2	21.9	44.4	7.7	11.2
	4	17.9	34.9	8.4	8.4
	8	-	18.9	< 8.3	< 8.3
	9	-	20.1	-	< 9.4
	16	< 16.9	< 16.9	-	-
20	2	8.5	18.0	2.3	8.5
	3	7.2	13.8	2.6	7.2
	5	-	12.3	-	5.9
	7	7.9	-	-	-
	10	-	10.8	-	-
25	2	9.1	10.5	1.7	3.8
	3	6.9	8.7	-	-
	4	-	9.3	-	< 3.3
	10	< 6.7	< 6.7	-	-
30	2	7.0	7.0	-	-
	3	6.7	6.7	-	-

KEY

< Complete kill at this, the lowest CT product tested

> Survival at this, the highest CT product tested

- Not tested at this exposure, or only at CT products higher than those giving complete mortality in a shorter exposure.

Fig. 45. Larval-pupal transitionals produced after fumigation of diapausing larvae of Ephestia elutella.

UPPER: The larval skin has split and has moved forward, but the head capsule remains unchanged. During the attempted moult, the larva became stuck to a hair.

LOWER: The larval skin has split (noticeable on metathorax and first 4 abdominal segments) but has not moved, leaving head capsule larval legs and bristles in situ.

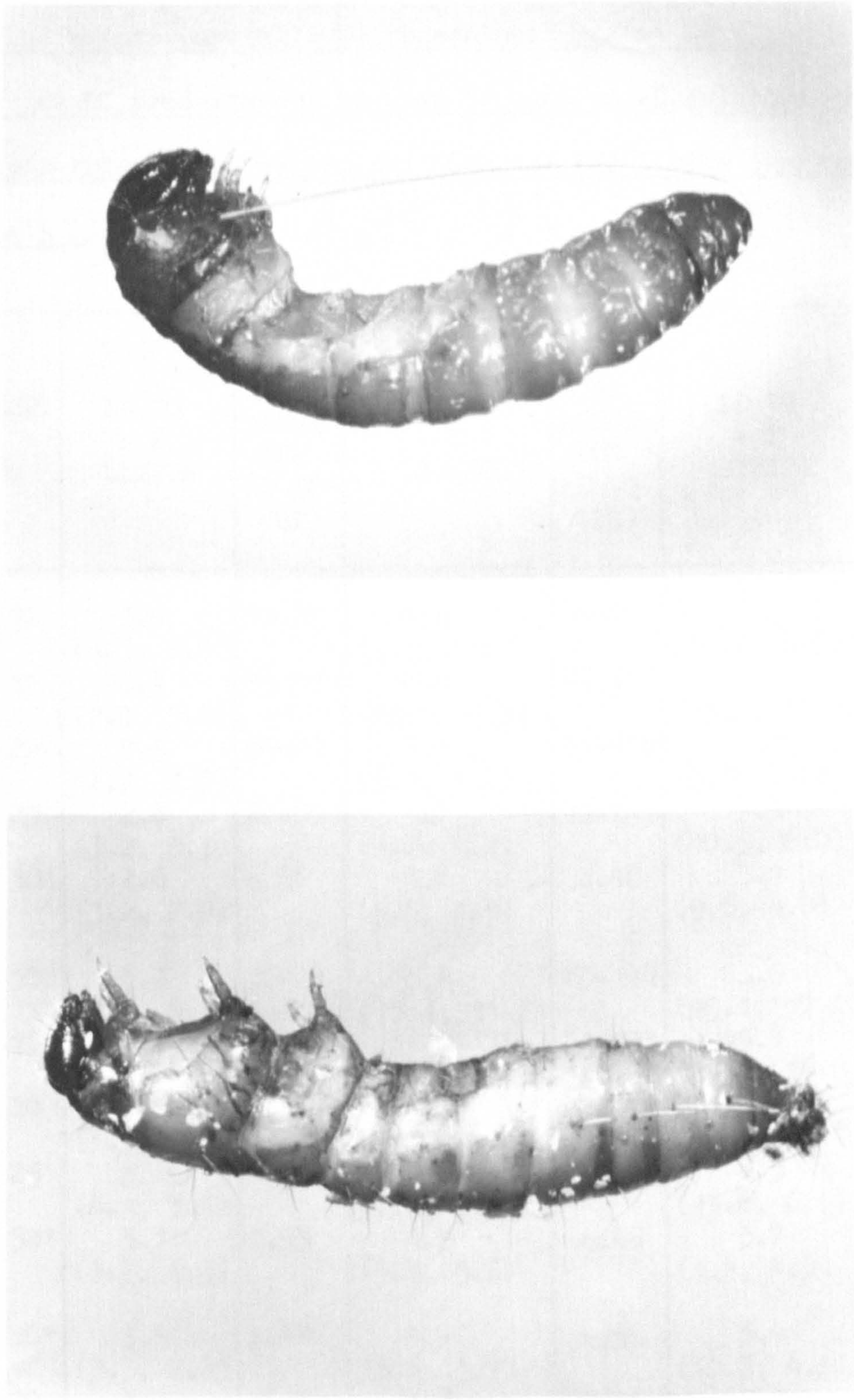


TABLE 82. The susceptibility of diapausing larvae of Ephestia elutella and Plodia interpunctella to phosphine: ranges of exposure times over which CT products can be used to express LD values at various temperatures (LD values and fiducial (F) limits expressed in mg h/l).

SPECIES AND STOCK	TEMP. °C	LD 50 + F LIMITS	EXPOSURE RANGE FOR LD 50 (HR)	LD 90 + F LIMITS	EXPOSURE RANGE FOR LD 90 (HR)	LD 99 + F LIMITS	EXPOSURE RANGE FOR LD 99 (HR)	D/F ≠
EPHESTIA LAB. STOCK	10	3.9 (4.5, 3.0)	37-52	5.9 (7.9, 5.0)	46-70	8.2 (14.2, 6.5)	57-93	8
	15	6.4 (7.0, 5.6)	35-42	10.6 (13.0, 9.3)	46-61	16.0 (23.2, 13.0)	59-87	20
	20	2.5 (2.7, 2.3)	30-86	4.6 (5.2, 4.2)	49-168	7.7 (9.5, 6.7)	77-300	36
	25	2.8 (3.2, 2.3)	27-81	4.7 (5.8, 4.2)	41-149	7.3 (10.5, 6.0)	61-269	15
	30*	2.6 (3.2, 2.0)	c.26	3.8 (5.5, 3.1)	c.40	5.1 (9.6, 4.0)	c.55	6
EPHESTIA FIELD STOCK	10	6.7 (7.9, 5.1)	50-78	13.4 (19.1, 11.2)	83-144	23.5 (49.1, 17.2)	130-258	15
	15	7.3 (7.9, 6.6)	38-86	12.5 (14.3, 11.3)	51-133	19.4 (24.6, 16.6)	67-197	30
	20	5.5 (5.9, 5.0)	58-114	8.2 (9.1, 7.6)	82-175	11.3 (13.5, 10.1)	110-243	24
	25	3.6 (4.1, 3.1)	33-40	5.8 (7.4, 5.1)	50-61	8.5 (13.6, 6.9)	70-88	12
	30*	3.1 (3.7, 2.5)	c.33	4.3 (5.9, 3.6)	c.46	5.7 (9.4, 4.5)	c.61	5
PLODIA LAB. STOCK	10*	3.1 (3.5, 2.2)	c.44	4.2 (5.6, 3.7)	c.55	5.4 (10.3, 4.5)	c.65	8
	20	1.2 (1.3, 1.1)	16-34	1.6 (1.8, 1.5)	22-46	2.1 (2.5, 1.9)	27-59	18
PLODIA FIELD STOCK	10*	2.9 (3.4, 2.1)	c.42	4.3 (5.7, 3.7)	c.56	5.9 (10.8, 4.8)	c.70	8
	25	1.4 (1.6, 1.3)	17-20	2.2 (2.5, 2.0)	23-28	3.2 (3.8, 2.8)	30-38	27

* Only one test analysed at these temperatures

≠ D/F = Degrees of freedom

TABLE 83. Susceptibility of diapausing larvae of Plodia interpunctella to phosphine at 15°C: 1. LD values calculated as CT products in mg h/l.

STOCK	NOMINAL CONCN. (mg h/l)	LD VALUES WITH FIDUCIAL LIMITS IN PARENTHESIS			SLOPE b ± S.E.	HETEROGENEITY FACTOR	DEGREES OF FREEDOM
		LD 50	LD 90	LD 99			
LAB	0.1	2.7 (3.2, 1.6)	4.0 (6.1, 3.3)	5.4 (14.5, 4.3)	7.58 ±2.03	<1	6
	0.2	4.3 (4.8, 3.8)	6.4 (8.1, 5.6)	9.0 (13.3, 7.3)	7.26 ±1.20	<1	12
FIELD	0.1	3.5 (4.0, 2.9)	5.2 (7.7, 4.4)	7.2 (14.3, 5.6)	7.42 ±1.46	<1	5
	0.2	4.0 -	7.7 -	13.3 -	4.44 ±2.50	5.19	10

TABLE 84. Susceptibility of diapausing larvae of Plodia interpunctella to phosphine at 15°C: II. LD values calculated as hours of exposure for the test at 0.2 mg/l.

STOCK	UNITS FOR RESULTS	LD VALUES WITH FIDUCIAL LIMITS IN PARENTHESIS			SLOPE b ±S.E.	HETERO-GENEITY FACTOR	DEGREES OF FREEDOM
		LD 50	LD 90	LD 99			
LAB	HOURS	26.5 (30.5, 22.6)	46.0 (62.4, 38.8)	71.8 (122, 55.1)	5.39 ±0.88	<1	12
	mg h/l with 0.155 mg/l	4.1 (4.7, 3.5)	7.1 (9.7, 6.0)	11.1 (18.9, 8.5)			
FIELL	HOURS	25.4 (29.0, 21.4)	41.7 (54.8, 35.6)	62.4 (110, 49.0)	5.96 ±0.97	<1	10
	mg h/l with 0.155 mg/l	3.9 (4.5, 3.3)	6.5 (8.5, 5.5)	9.7 (17.1, 7.6)			

TABLE 85. Susceptibility of diapausing larvae of *Aedes intermedius* to various concentrations of phosphine at 20 and 25°C, (LD values calculated as CT products in mg h/l).

TEMP. °C	STOCK	CONCN. mg/l	LD VALUES WITH FIDUCIAL LIMITS IN PARENTHESIS			SLOPE b ±S.E.	HETEROGENEITY FACTOR	DEGREES OF FREEDOM
			LD 50	LD 90	LD 99			
20	LAB.	0.04	1.3 (1.4, 1.2)	1.6 (1.8, 1.5)	1.9 (2.3, 1.7)	13.62 ±2.00	<1	9
		0.10	1.1 (1.3, 1.0)	1.7 (2.1, 1.5)	2.3 (3.5, 1.9)	7.34 ±1.24	<1	8
		0.20	1.7 (1.9, 1.5)	2.5 (3.1, 2.2)	3.3 (4.8, 2.7)	8.29 ±1.26	<1	13
	FIELD	0.04	1.4 (1.6, 1.3)	2.3 (2.7, 2.0)	3.4 (4.5, 2.8)	6.20 ±0.73	<1	13
		0.10	2.5 (2.8, 1.1)	3.9 (4.7, 3.5)	5.5 (7.9, 4.7)	6.85 ±1.18	<1	14
		0.20	2.9 (3.4, 2.5)	5.0 (6.5, 4.3)	7.7 (12.4, 6.0)	5.59 ±0.89	<1	10
25	LAB.	0.04	0.82 (0.92, 0.69)	1.1 (1.5, 0.95)	1.3 (2.5, 1.1)	11.04 ±2.31	<1	4
		0.10	1.2 (1.3, 0.89)	1.6 (2.5, 1.4)	2.1 (5.4, 1.7)	9.76 ±2.81	<1	7
	FIELD	0.10	1.3 (1.5, 1.0)	2.0 (2.4, 1.8)	3.0 (4.1, 2.5)	6.20 ±0.92	<1	15
		0.14	1.8 (2.0, 1.5)	2.4 (2.8, 2.2)	3.2 (4.0, 2.8)	9.08 ±1.33	<1	10

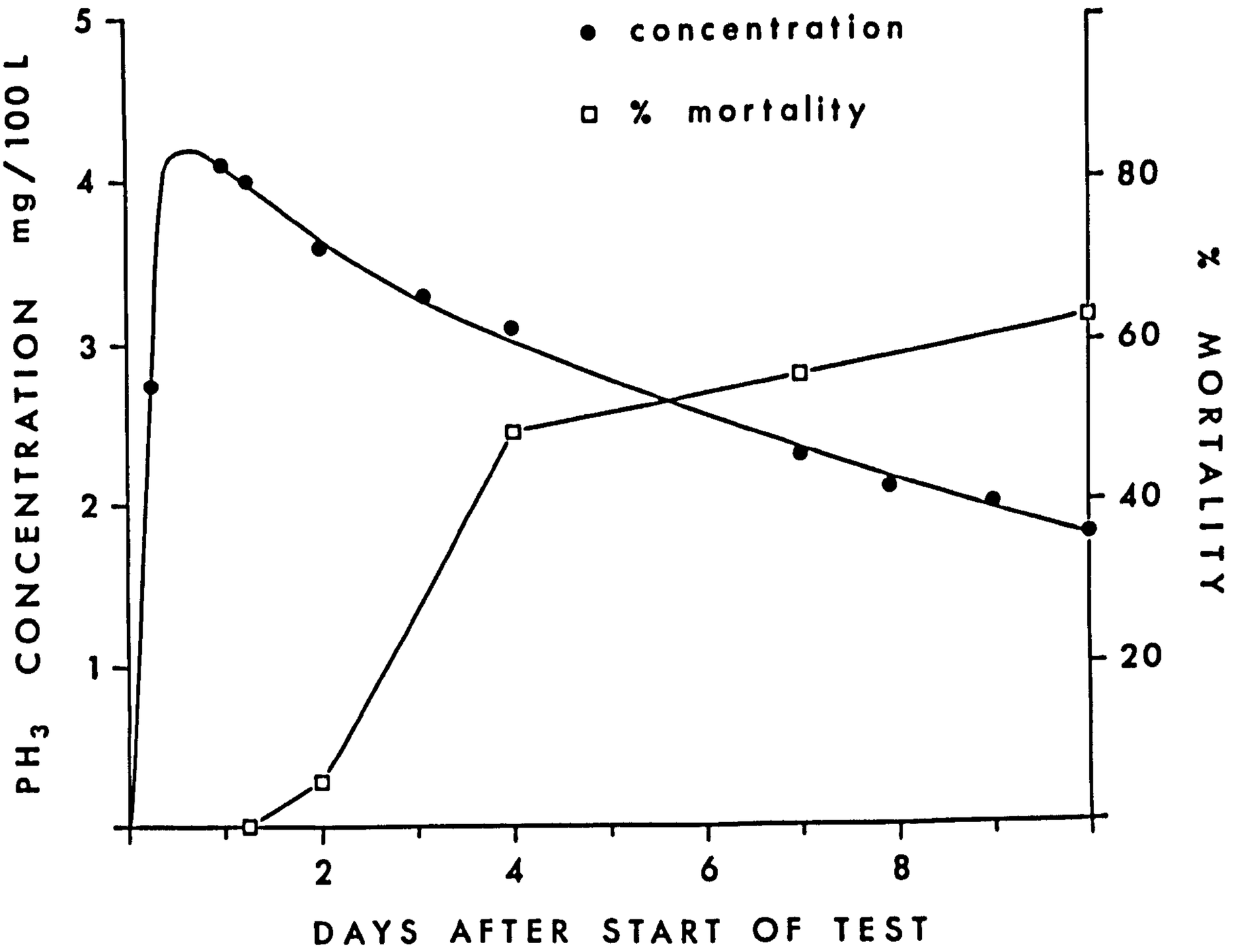
TABLE 86. Susceptibility of diapausing larvae of Ephestia elutella to various concentrations of phosphine at 10 and 15°C (LD values calculated in mg h/l).

TEMP. °C	STOCK	CONCN. mg/l	LD VALUES WITH FIDUCIAL LIMITS IN PARENTHESES			SLOPE b ± S.E.	HETEROG-ENEITY FACTOR	DEGREES OF FREEDOM
			LD 50	LD 90	LD 99			
10	LAB.	0.1	3.8 (4.8, 2.1)	5.5 (13.8, 4.4)	7.3 (47.8, 5.5)	8.19 ±2.28	<1	4
		0.2	4.1 (5.7, 1.8)	6.4 (16.5, 4.6)	9.2 (61.0, 6.4)	6.60 ±1.76	<1	4
	FIELD	0.1	6.1 (7.6, 2.4)	12.1 (39.8, 9.4)	21.1 (320, 13.6)	4.31 ±1.38	<1	8
		0.2	8.0 (10.0, 5.5)	15.4 (21.5, 12.4)	26.3 (55.4, 19.1)	4.51 ±0.87	<1	8
15	LAB.	0.1	3.0 (3.5, 2.2)	4.7 (7.2, 3.9)	6.8 (16.2, 5.2)	6.51 ±1.54	<1	7
		0.2	6.7 (9.4, 5.9)	10.9 (13.7, 9.5)	16.1 (24.5, 10.3)	6.07 ±0.94	<1	12
		0.4	7.0 (8.4, 5.7)	12.7 (17.0, 10.5)	20.5 (33.8, 15.6)	5.02 ±0.73	<1	8
	FIELD	0.1	7.7 (8.8, 6.6)	11.3 (16.7, 10.4)	18.0 (31.0, 13.9)	6.29 ±1.06	<1	9
		0.2	6.3 (9.5, 3.5)	13.1 (24.9, 10.6)	23.8 (103, 16.0)	4.05 ±1.08	<1	10
		0.4	7.4 (8.9, 5.2)	12.5 (16.7, 10.5)	19.3 (34.7, 15.0)	5.59 ±1.08	<1	7

TABLE 87. Susceptibility of diapausing larvae of Ephestia elutella to various concentrations of phosphine at 20 and 25°C (LD values calculated in mg h/l).

TEMP. °C	STOCK	CONCN. mg/l	LD VALUES WITH FIDUCIAL LIMITS IN PARENTHESIS			SLOPE b ± S.E.	HETEROG-ENEITY FACTOR	DEGREES OF FREEDOM
			LD 50	LD 90	LD 99			
20	LAB	0.03	2.4 (2.7, 2.2)	4.6 (5.5, 4.1)	7.8 (10.4, 6.4)	4.61 ±0.46	< 1	19
		0.05	2.9 (3.4, 2.2)	4.8 (6.2, 4.0)	7.2 (11.5, 5.7)	5.82 ±0.95	< 1	7
		0.10	2.4 (2.9, 1.9)	4.7 (6.2, 3.9)	8.2 (12.8, 6.3)	4.36 ±0.56	< 1	10
		0.20	3.9 (4.4, 3.5)	6.4 (7.6, 5.6)	9.5 (12.8, 7.9)	6.05 ±0.71	< 1	8
	FIELD	0.05	5.8 (6.3, 5.3)	8.1 (8.8, 7.3)	10.6 (12.7, 9.4)	8.88 ±1.04	< 1	15
		0.10	4.8 (5.8, 2.6)	8.9 (14.3, 7.3)	14.7 (48.0, 10.6)	4.73 ±1.20	< 1	7
		0.40	7.1 (8.0, 6.0)	11.9 (14.8, 10.4)	18.3 (27.3, 14.7)	5.64 ±0.85	< 1	12
25	LAB	0.04	2.7 (3.1, 2.3)	4.8 (6.1, 4.2)	7.9 (11.4, 6.3)	5.02 ±0.62	< 1	11
		0.14	2.8 (3.5, 1.9)	4.6 (7.3, 2.6)	6.8 (25.0, 5.2)	6.03 ±1.80	< 1	7
		0.25	3.8 (4.3, 3.3)	6.1 (7.1, 5.4)	8.8 (11.9, 7.4)	6.44 ±0.94	< 1	13
	FIELD	0.04	4.4 (5.0, 3.7)	23.0 (64.3, 14.7)	88.8 (607, 38.8)	1.78 ±0.93	< 1	11
		0.10	3.7 (4.5, 2.3)	6.1 (12.2, 5.0)	9.3 (38.1, 6.7)	5.75 ±1.52	< 1	6
		0.14	3.6 (4.3, 2.5)	5.5 (8.0, 4.6)	7.8 (16.0, 6.1)	6.90 ±1.45	< 1	5
		0.25	5.2 (5.7, 4.5)	7.5 (8.7, 6.8)	10.2 (13.3, 8.8)	7.85 ±1.12	< 1	11

Fig. 46. Threshold concentration of phosphine at 25°C for mortality of diapausing larvae of Ephestia elutella (field stock).


● concentration
□ % mortality
5
4
3
2
1
80
60
40
20
PH_3 CONCENTRATION mg/100 L
MORTALITY %
2
4
6
8
DAYS AFTER START OF TEST

level chosen was 0.1 mg/l against the field stock of E. elutella. As expected, tolerance was generally reduced with increased temperature (table 88), but at 15°C, tolerance was greater at the LD 50 level than at 10°C ($p = < 0.05$). The effect was not noticeable, however, at the LD 90 or 99 levels. At 30°C, the LD 50 was reduced to approximately one half, and the LD 99 to approximately one quarter, the result at 10°C.

Some of the results already presented (tables 81, 82, 86 and 87) have indicated that temperature affects the susceptibility of different stocks in different ways. Fig. 47 gives a comparison of the LD 50 and 99 values for all stocks at 10 to 30°C, based where possible on the averaged results of several tests at each temperature. Where there was difficulty in averaging results because of heterogeneity, the test showing the highest efficiency of fumigant action was used in the comparison. Two peculiarities were noted. Firstly, the laboratory stocks of E. elutella and P. interpunctella changed little in tolerance at the LD 50 level as temperature was increased from 20°C. Results at 30°C were not obtained for P. interpunctella, as diapause is terminated rapidly at this temperature. Secondly, two stocks were more tolerant at 15°C than at 10°C, and the other two, the laboratory stock of P. interpunctella, and the field stock of E. elutella, differed little in tolerance at these temperatures.

iv) Effect of method used to induce diapause

Batches of larvae of the laboratory stock of E. elutella were reared at 20°C under an 8-hour daylength, a 12-hour daylength, and under a daylength shortening from 12 hours by 3½ minutes per day. Samples of larvae that entered diapause under each light system were then fumigated at 20°C with phosphine at 0.1 mg/l (table 89). No significant differences in susceptibility were observed between samples of larvae from different batches, and no heterogeneity was observed when a probit analysis was performed on all fumigated samples together.

v) Effect of method used to terminate diapause

Batches of diapausing larvae of E. elutella (laboratory stock) tested at 20°C at about 0.03 mg/l, were placed under various conditions for the termination of diapause to observe the effect on survival. After correction for control mortality, the groups were subjected to probit analysis in various combinations (table 90). No differences between batches proved significant at the 5% level. No heterogeneity was observed when all results were combined and analysed together.

vi) Effect of length of time in diapause

Batches of diapausing larvae of the field stock of E. elutella held at 20°C under an 8-hour daylength for different lengths of time, were fumigated with 0.4 mg/l phosphine at 15°C (table 91). Variations in the levels of kill obtained within batches that had been in diapause about 1, 2 or 3 months were not significant, and the susceptibility of E. elutella in diapause was found to be fairly stable. No heterogeneity occurred in an analysis of results for all three age groups combined together.

B. Methyl Bromide

i) CT products required for complete kill

Diapausing larvae were highly tolerant to methyl bromide at low temperature. At higher temperatures, the susceptibility of diapausing larvae of P. interpunctella was greatly increased, while in E. elutella changes in susceptibility were slight, and diapausing larvae remained highly tolerant at 25 or even 30°C (table 92). As with phosphine, field stocks showed a higher tolerance than laboratory stocks.

ii) Effect of concentration and exposure period

With methyl bromide concentrations between 4.0 and 10.3 mg/l, there were no significant differences in the susceptibility of either stock of E. elutella

at the LD 90 level for any test temperature (tables 93 and 94). In P. interpunctella, some variation between results occurred at 20°C in both stocks. In the laboratory stock (table 95), larvae fumigated at 4.0 mg/l were significantly more tolerant at the LL 50 and 90 levels than larvae fumigated at 6.7 mg/l ($p = < 0.001$), or at 10.3 mg/l ($p = < 0.025$). In the field stock (table 96), larvae fumigated at 6.7 mg/l were significantly more susceptible at these LD levels ($p = 0.025$) than larvae fumigation at 4.0 or 10.3 mg/l. These results do not suggest that either concentration or length of exposure caused the observed variation. No significant heterogeneity was recorded in any test with either P. interpunctella or E. elutella.

iii) Effect of temperature

For each stock, the available results at each temperature were combined and analysed from a single probit regression line. No heterogeneity occurred in results for E. elutella (table 97). In the case of P. interpunctella (table 98), where differences in susceptibility between tests of different concentration at 20°C had been noted, a chi-squared test in the analysis of results for the laboratory stock, revealed heterogeneity at the 5% level. However, the calculated chi-squared value was not significant at the 2.5% level, and the heterogeneity factor was low. In the field stock, results at 20°C were combined without incurring heterogeneity at the 5% level.

In contrast with results for phosphine, laboratory and field stocks were affected similarly by temperature. In both stocks of E. elutella (table 97, fig. 48) the susceptibility of diapausing larvae at the LD 50 level varied only slightly between 10 and 25°C. At the LD 90 level, however, the increase in susceptibility at temperatures rising from 15 to 25°C was more marked, but larvae appeared more susceptible at 10 than at 15°C. In both stocks of P. interpunctella (table 98, fig. 48), susceptibility was more clearly related to temperature, maximum tolerance at the LL 50 and 90 levels occurring at 10°C.

TABLE 88. Effect of temperature on the susceptibility of diapausing larvae of Ephestia elutella (field stock) to phosphine at 0.1 mg/l. (LD values calculated in mg h/l).

TEMP. °C	LD VALUES WITH FIDUCIAL LIMITS IN PARENTHESIS			SLOPE b ±S.E.	HETEROGENEITY FACTOR	DEGREES OF FREEDOM
	LD 50	LD 90	LD 99			
10	6.1 (7.6, 2.4)	12.1 (39.8, 9.4)	21.1 (320, 13.6)	4.31 ±1.38	<1	8
15	7.7 (8.8, 6.6)	11.3 (16.7, 10.4)	18.0 (31.0, 13.9)	6.29 ±1.06	<1	9
20	4.8 (5.8, 2.6)	8.9 (14.3, 7.3)	14.7 (48.0, 10.6)	4.73 ±1.20	<1	7
25	3.7 (4.5, 2.3)	6.1 (12.2, 5.0)	9.3 (38.1, 6.7)	5.75 ±1.52	<1	6
30	3.1 (3.7, 2.5)	4.3 (5.9, 3.6)	5.7 (9.4, 4.5)	8.80 ±1.56	<1	5

Fig. 47. Temperature and susceptibility of diapausing larvae of A. Ephestia elutella and B. Plodia interpunctella to phosphine.

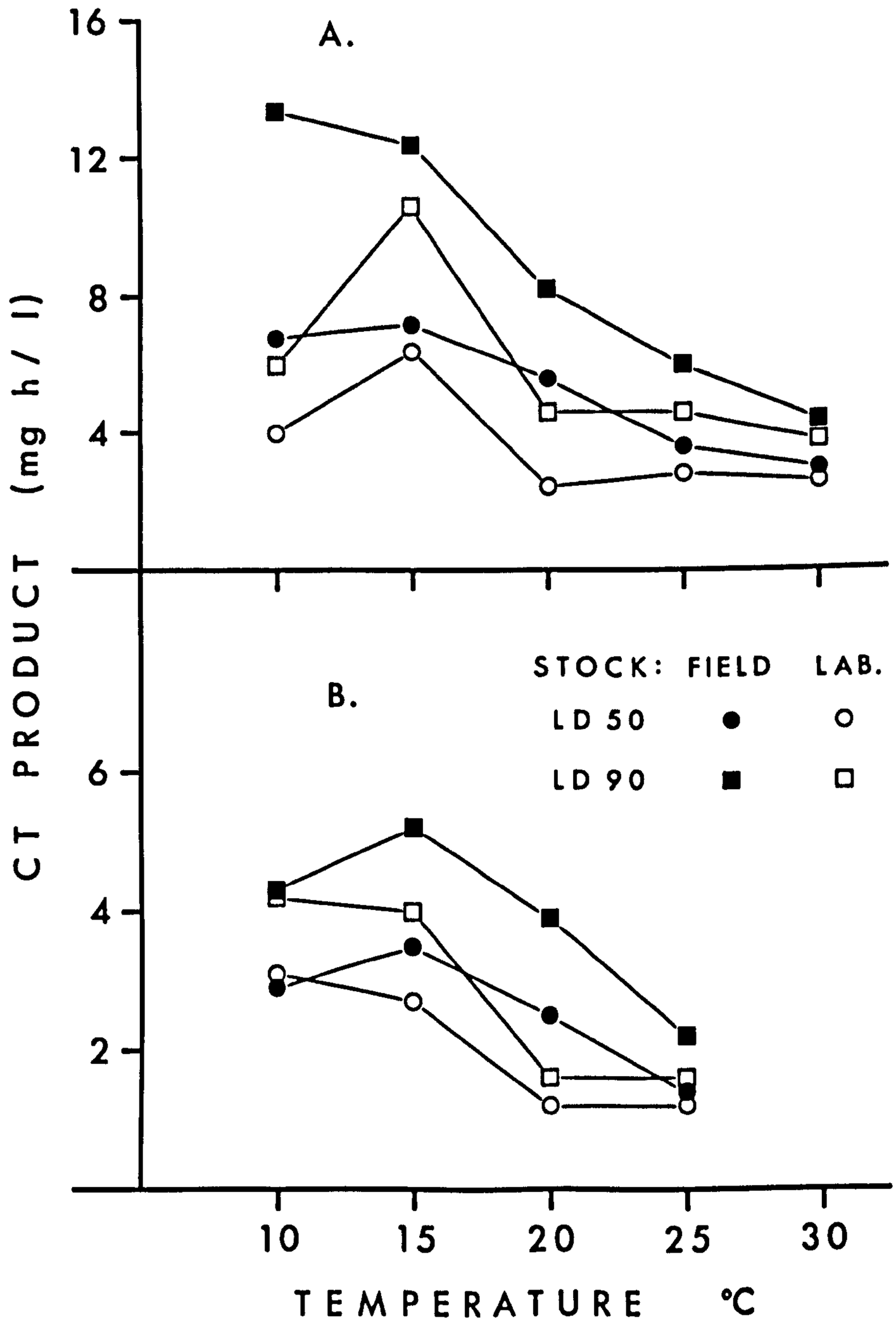

A.
B.
16
12
8
4
6
4
2
CT PRODUCT (mg h / l)
STOCK: FIELD LAB.
LD 50
LD 90
10
15
20
25
30
TEMPERATURE °C

TABLE 89. Effect of method of diapause induction on the susceptibility of diapausing larvae of Ephestia elutella (laboratory stock) to 0.1 mg/l phosphine at 20°C (LD values calculated in m.h/l).

LIGHT CONDITIONS FOR INDUCTION (HOURS PER DAY)	LL VALUES WITH FIDUCIAL LIMITS IN PARENTHESIS			H/F*	DEGREES OF FREEDOM
	LD 50	LD 90	LD 99		
8	2.3 (3.5, 0.7)	4.6 (14.8, 3.0)	8.1 (96.8, 4.8)	<1	4
12	2.5 (3.8, 1.1)	4.4 (13.6, 2.9)	7.1 (59.0, 4.4)	<1	3
12 (SHORTENING BY 3½ MIN./DAY)	2.4 (3.5, 1.3)	5.1 (12.3, 3.5)	9.3 (47.7, 5.6)	<1	4
ALL GROUPS	2.4 (2.9, 1.9)	4.7 (6.2, 3.9)	8.2 (12.8, 6.3)	<1	10

* H/F = Heterogeneity factor

TABLE 90. Effect of method of terminating diapause on the susceptibility of diapausing larvae of Ephestia elutella (laboratory stock) to 0.03 mg/l phosphine at 20°C (LD values calculated in mg h/l).

CONDITIONS FOR TERMINATION OF DIAPAUSE	LD VALUES WITH FIDUCIAL LIMITS IN PARENTHESIS			H/F*	DEGREES OF FREEDOM
	LD 50	LD 90	LD 99		
2 OR 4 WEEKS AT 2.5°C, THEN 20°C, 16 HR LIGHT	2.5 (3.2, 1.7)	4.9 (11.0, 3.7)	8.8 (37.9, 5.6)	<1	4
2 OR 4 WEEKS AT 10°C, THEN 20°C, 16 HR LIGHT	2.4 (3.2, 1.4)	4.5 (10.3, 3.4)	7.3 (39.5, 4.9)	<1	4
25°C, 16 HR LIGHT	2.6 (3.3, 2.1)	4.8 (8.0, 3.8)	7.8 (18.8, 5.4)	<1	4
10 OR 2.5°C FOR 2 WEEKS OR LESS, THEN 20°C, 16 HR LIGHT	2.2 (2.6, 1.7)	4.0 (5.5, 3.4)	6.5 (11.8, 4.9)	<1	7
10 OR 2.5°C FOR 4 WEEKS, THEN 20°C, 16 HR LIGHT	2.7 (3.5, 1.7)	5.4 (14.4, 3.9)	9.5 (59.0, 5.9)	<1	4
CHILLED OR UNCHILLED BATCHES ENDING UP AT 20°C, 16 HR LIGHT	2.4 (2.7, 2.0)	4.5 (5.8, 3.8)	7.6 (12.3, 5.9)	<1	13
ALL GROUPS	2.4 (2.7, 2.2)	4.6 (5.5, 4.1)	7.8 (10.4, 6.4)	<1	12

* H/F = Heterogeneity factor

TABLE 91. Effect of length of time in diapause on the susceptibility of diapausing larvae of Ephestia elutella (field stock) to 0.4 mg/l phosphine at 15°C. (LD values calculated in mg h/l).

WEEKS IN DIAPAUSE AT 20°C, 8 HR. LIGHT	LD VALUES WITH FIDUCIAL LIMITS IN PARENTHESIS			H/F*	DEGREES OF FREEDOM
	LD 50	LD 90	LD 99		
3 - 4	7.4 (8.9, 5.2)	12.5 (16.7, 10.5)	19.3 (34.7, 15.0)	<1	7
7 - 8	7.4 (9.1, 4.9)	11.4 (16.2, 9.5)	16.8 (32.4, 13.0)	<1	6
14 - 15	7.2 (9.0, 4.3)	12.2 (18.0, 9.8)	18.9 (43.1, 14.1)	<1	5
ALL GROUPS	7.3 (8.2, 6.3)	12.2 (14.2, 10.9)	18.6 (24.2, 15.8)	<1	22

* H/F = Heterogeneity factor

TABLE 92. Concentration-time products (mg h/l) of methyl bromide required for complete control of diapausing larvae at 10-30°C.

TEMPERATURE °C	Ephestia elutella		Plodia interpunctella	
	LAB STOCK	FIELD STOCK	LAB STOCK	FIELD STOCK
10	216	280	144	216
15	210	261	105	158
20	157	188	53	81
25	125	183	32	64
30	92	122	-	-

TABLE 93.

Effect of concentration on the susceptibility of diapausing larvae of the laboratory stock of Ephestia elutella to methyl bromide (LD values calculated in mg h/l).

TEMP. °C	CONCN. mg/l	LD VALUES WITH FIDUCIAL LIMITS IN PARENTHESIS			SLOPE b ± S.E.	H/F	DEGREES OF FREEDOM
		LD 50	LD 90	LD 99			
15	4.2	68.3 (80.9, 49.2)	126 (164, 108)	208 (369, 160)	4.81 ±0.91	<1	11
	7.0	79.7 (93.0, 63.2)	147 (188, 126)	243 (385, 191)	4.80 ±0.76	<1	13
20	6.7	70.0 (87.8, 34.6)	104 (151, 81.9)	144 (345, 113)	7.41 ±1.75	<1	4
	10.3	75.7 (85.3, 67.5)	118 (155, 102)	170 (264, 136)	6.61 ± 1.02	<1	11
25	4.0	75.6 (82.2, 67.7)	96.9 (112, 88.7)	119 (150, 105)	11.85 ±1.83	<1	7
	6.1*	60.5 (67.1, 53.2)	95.0 (110, 85.2)	137 (175, 117)	6.55 ±0.74	<1	11
	9.2	69.5 (79.2, 60.7)	95.2 (120, 83.0)	123 (176, 102)	9.37 ±1.50	<1	8

* Temperature fell to 23°C in first day of test.

TABLE 94. Effect of concentration on the susceptibility of *Aedes aegypti* larvae of the field stock of *Aedes aegypti* (Delhi) to methyl bromide (LD values calculated in mg h/l).

TEMP. °C	CONCN. mg/l	LD VALUES WITH FIDUCIAL LIMITS IN PARENTHESIS			SLOPE b ±S.E.	H/F	DEGREES OF FREEDOM
		LD 50	LD 90	LD 99			
15	4.2	93.3 (104, 81.2)	152 (190, 133)	227 (339, 183)	6.03 ±0.94	<1	16
	7.0	83.2 (99.9, 61.5)	163 (195, 141)	281 (408, 227)	4.40 ±0.67	<1	19
20	4.0	77.8 (95.9, 60.2)	114 (165, 93.3)	153 (287, 121)	7.91 ±1.62	<1	6
	6.7	80.4 (89.9, 68.8)	115 (134, 102)	153 (206, 132)	8.31 ±1.19	<1	8
	10.3	86.6 (96.9, 77.4)	127 (157, 112)	175 (243, 144)	6.21 ±1.06	<1	10
25	4.0	77.6 (85.5, 67.0)	109 (123, 99.8)	144 (180, 127)	8.63 ±1.29	<1	13
	9.2	74.9 (84.1, 66.2)	115 (134, 102)	164 (217, 137)	6.83 ±0.80	<1	8

TABLE 95. Effect of concentration on the susceptibility of dis[illegible] larvae of the laboratory stock of Plodia interpunctella to methyl bromide (LD values calculated in mg h/l).

TEMP. °C	CONCN. mg/l	LD VALUES WITH FIDUCIAL LIMITS IN PARENTHESIS			SLOPE b ±S.E.	H/F	DEGREES OF FREEDOM
		LD 50	LD 90	LD 99			
15	4.2	30.8 (42.6, 11.1)	67.6 (104, 51.3)	128 (429, 89.2)	3.76 ±0.99	<1	9
	7.0	31.5 (37.7, 22.7)	59.7 (77.9, 50.6)	100 (177, 77.3)	4.61 ±0.86	<1	13
20	4.0	29.6 (33.0, 24.5)	47.0 (57.0, 41.2)	66.1 (103, 54.4)	6.68 ±1.24	<1	10
	6.7	21.2 (24.2, 16.9)	31.7 (40.2, 27.8)	43.9 (70.5, 36.0)	7.39 ±1.52	<1	11
	10.3	23.8 (25.5, 14.1)	35.9 (45.7, 28.2)	50.1 (81.8, 30.7)	7.21 ±3.39	<1	5
25	4.0	16.3 (18.2, 12.5)	22.4 (34.1, 19.8)	29.0 (69.2, 23.7)	9.32 ±2.61	<1	8
	9.2	16.2 (18.3, 12.2)	21.1 (27.8, 18.6)	26.2 (46.8, 22.1)	11.17 ±2.78	<1	11

TABLE 96. Effect of concentration on the susceptibility of diapausing larvae of the field stock of Plodia interpunctella to methyl bromide (LD values calculated in mg h/l).

TEMP. °C	CONCN. mg/l	LD VALUES WITH FILUCIAL LIMITS IN PARENTHESIS			SLOPE b ±S.E.	H/T	DEGREES OF FREEDOM
		LD 50	LD 90	LD 99			
20	4.0	30.7 (33.8, 27.2)	50.6 (63.0, 44.4)	76.0 (113, 61.5)	7.98 ±0.86	<1	12
	6.7	24.7 (28.0, 20.2)	39.1 (48.0, 34.3)	56.8 (83.1, 46.8)	6.42 ±1.06	<1	11
	10.3	33.9 (39.1, 26.4)	54.8 (66.4, 47.7)	81.1 (120, 66.8)	6.15 ±1.06	<1	10
25	4.0	29.0 (33.1, 23.7)	48.8 (62.6, 40.7)	74.5 (114, 58.9)	5.67 ±0.90	<1	12
	9.2	24.7 (29.1, 20.8)	40.0 (50.0, 34.9)	59.3 (84.1, 48.6)	6.14 ±0.88	<1	15

TABLE 97. Effect of temperature on the susceptibility of diapausing larvae of laboratory (Lab.) and field stocks of *Aedes detritus* to methyl bromide (LD values calculated in mg h/l).

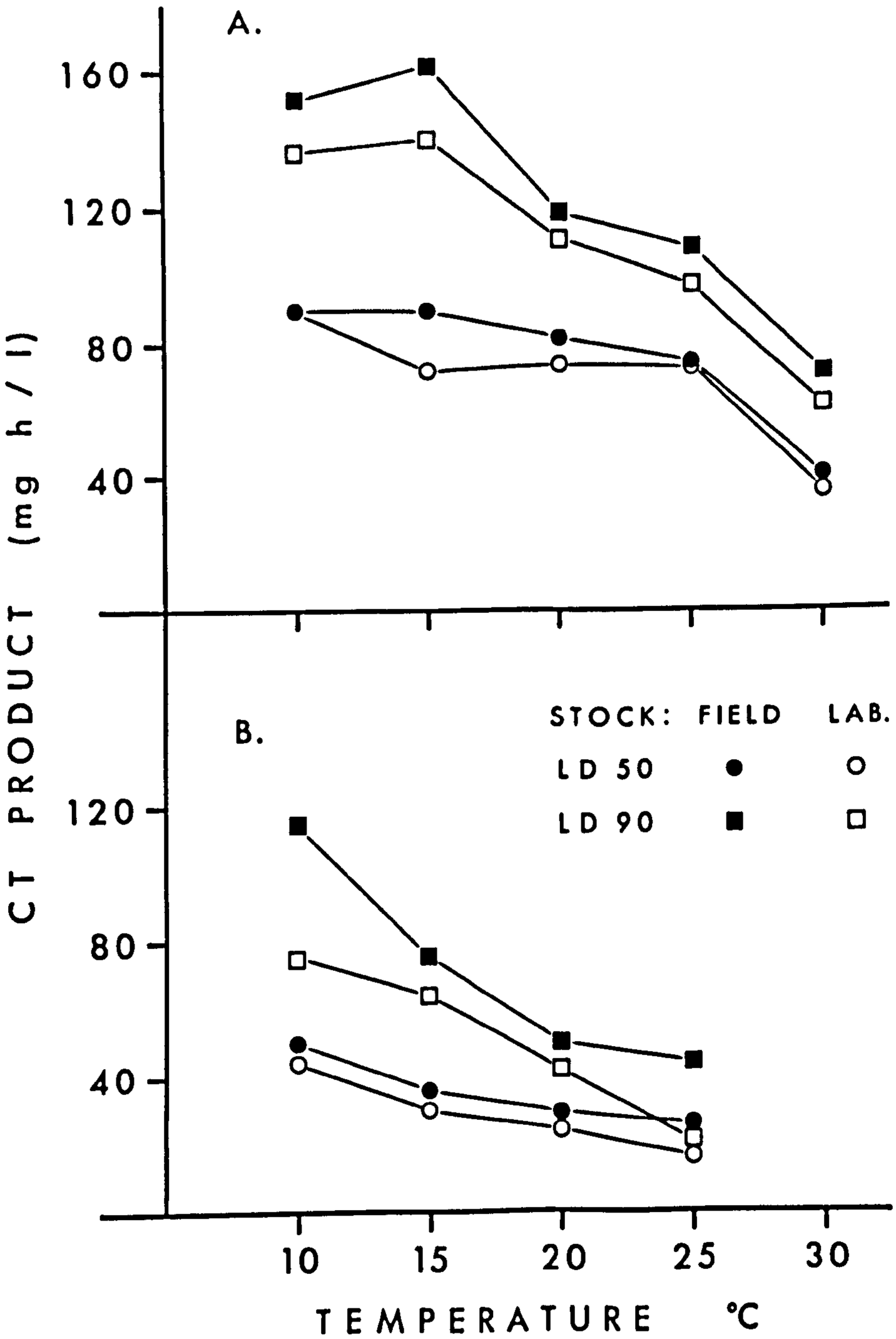
STOCK	TEMP. °C	LD VALUES WITH FIDUCIAL LIMITS IN PARENTHESES			SLOPE b ±S.E.	H/F	% OF PREPUPAE
		LD 50	LD 90	LD 99			
LAB	10	89.6 (108, 45.6)	137 (233, 114)	194 (699, 149)	6.93 ±1.89	<1	5
	15	71.9 (81.3, 60.2)	139 (162, 125)	239 (325, 198)	4.45 ±0.56	<1	26
	20	74.1 (80.7, 67.5)	111 (130, 99.3)	153 (201, 130)	7.38 ±0.95	<1	17
	25	72.8 (78.1, 67.0)	97.8 (107, 90.9)	124 (145, 112)	10.00 ±1.10	<1	16
	30	35.9 (44.0, 25.7)	61.6 (84.7, 50.3)	95.6 (173, 72.9)	5.48 ±0.92	<1	5
FIELD	10	89.6 (104, 69.0)	152 (198, 131)	234 (400, 184)	5.59 ±1.05	<1	10
	15	89.7 (98.3, 80.0)	162 (181, 147)	261 (320, 226)	5.02 ±0.47	<1	37
	20	81.7 (88.8, 77.5)	118 (131, 110)	158 (185, 141)	8.37 ±0.81	<1	28
	25	75.7 (81.3, 69.6)	110 (122, 102)	150 (177, 134)	7.83 ±0.78	<1	21
	30	40.8 (49.4, 29.8)	72.2 (92.0, 60.3)	115 (180, 90.6)	5.17 ±0.86	<1	10

TABLE 98. Effect of temperature on the susceptibility of diapausing larvae of laboratory (LAB) and field stocks of Ilodia interpunctella to methyl bromide (LD values calculated in mg h/l).

STOCK	TEMP. °C	LD VALUES WITH FIDUCIAL LIMITS IN PARENTHESIS			SLOPE b ± S.E.	H/F	DEGREES OF FREEDOM
		LD 50	LD 90	LD 99			
LAB	10	44.8 (52.5, 35.2)	74.4 (91.8, 64.0)	112 (165, 91.2)	5.83 ±0.96	<1	14
	15	29.3 (37.2, 23.5)	63.9 (80.2, 55.1)	114 (188, 91.7)	4.15 ±0.70	<1	21
	20	24.1 (27.5, 19.1)	41.8 (52.1, 36.8)	65.3 (106, 52.2)	5.39 ±1.04	1.60*	26
	25	16.3 (17.7, 14.1)	21.7 (26.6, 19.9)	27.5 (41.1, 23.7)	10.23 ±2.13	<1	18
FIELD	10	49.9 (60.7, 36.5)	114 (143, 96.9)	225 (360, 172)	3.56 ±0.53	<1	18
	15	36.6 (43.7, 27.9)	75.8 (99.2, 40.2)	137 (234, 104)	4.06 ±0.66	<1	18
	20	29.0 (32.2, 26.6)	49.5 (56.3, 44.2)	76.6 (97.0, 65.5)	5.52 ±0.59	<1	33
	25	25.8 (28.3, 23.0)	43.8 (51.4, 39.2)	67.3 (90.4, 56.1)	5.60 ±0.71	<1	24

* Heterogeneity significant at 5% level, but not 2.5% level.

Fig. 48. Temperature and susceptibility of diapausing larvae of A. Ephestia elutella and B. Plodia interpunctella to methyl bromide.


A.
160
120
80
40
PRODUCT (mg h / l)
B.
120
80
40
CT
STOCK: FIELD LAB.
LD 50
LD 90
10
15
20
25
30
TEMPERATURE °C

iv) Effect of method used to induce diapause

Larvae of P. interpunctella reared at 25°C in darkness with 10 insects per gram of food, were significantly more susceptible to methyl bromide ($p = < 0.025$) at all calculated LD levels, than larvae reared under an 8-hour daylength at 20°C with 2 larvae per gram of food (table 99).

Diapausing larvae of the laboratory stock of E. elutella reared under an 8-hour daylength at 20°C were compared with others reared under a daylength shortening by 3½ minutes per day from 12 hours, in a test with methyl bromide at 23-25°C (table 100). No significant difference in susceptibility was apparent, and a chi-squared test revealed no significant departure from linearity at the 5% level in an analysis of a regression line fitted to results of the two batches combined.

v) Effect of method used to terminate diapause

After fumigation with 4.0 mg per l methyl bromide at 30°C, diapausing larvae of the field stock of E. elutella were moved to a 16-hour daylength at 25°C, or to darkness at 30°C. Analysis of results (table 101) revealed no significant differences at any calculated LD level, and in an analysis of the two groups combined together as one, a chi-squared test revealed no significant heterogeneity at the 5% level.

vi) Effect of length of time in diapause

Batches of larvae of the laboratory stock of E. elutella that had been in diapause under short daylength at 20°C for about 3 weeks, and for about 12 weeks, were exposed to 6.7 mg/l methyl bromide at 20°C (table 102). Larvae that were in diapause for the longer period, required higher CT products for all LD levels; but differences were not significant at the 2.5% level, and an analysis of both groups combined revealed no heterogeneity at the 5% level.

C. Diapausing Larvae and Eggs Fumigated with a Mixture of Methyl Bromide and Phosphine.

Diapausing larvae and new-laid eggs of laboratory and field stocks of E. elutella and P. interpunctella were fumigated with about 2 mg/l methyl bromide, and a concentration of phosphine rising to about 0.4 mg/l, for exposure periods of 24 and 40 hours at 20°C (table 103). The CT product for methyl bromide in 24 hours was expected to achieve at least 99% kill of the egg stage of all stocks, about 90-95% kill of diapausing larvae of P. interpunctella, but little kill of diapausing larvae of E. elutella. The 24-hour CT product for phosphine was expected to achieve little kill of newly-laid eggs, substantial or complete kill of diapausing larvae of P. interpunctella little kill of diapausing larvae of the field stock of E. elutella, and probably about 50% kill of the laboratory stock. No stages of P. interpunctella were expected to survive the 40-hour CT product for methyl bromide, but most eggs of both species were expected to survive the CT product for phosphine.

With diapausing larvae of both stocks of E. elutella, about 50 - 60% mortality was expected from methyl bromide in this exposure, while the mortality expected from phosphine was about 90% for the field stock, and about 99% for the laboratory stock (table 103).

No survival occurred in P. interpunctella after the 24-hour exposure. Results for diapausing larvae of the field stock of E. elutella, of which each fumigant was expected to achieve only about 10 - 20% mortality after the 24-hour exposure, showed a marked additive effect of the mixture, as the level of kill obtained (74%) was equivalent to a CT product of 9.2 mg h/l for phosphine, or 98 mg h/l for methyl bromide, these values being approximately equal to the sum of CT products giving 10 and 20% kill. Similarly, the level of kill for the laboratory stock in this exposure (92%) resembled that for a CT product of about 9 mg h/l for phosphine or 120 mg h/l for methyl bromide, representing

TABLE 99. Effect of method of diapause induction on the susceptibility of diapausing larvae of Plodia interpunctella (field stock) to 4.0 m/l methyl bromide at 25°C. (LD values calculated in mg h/l).

METHOD FOR INDUCTION OF DIAPAUSE	LD VALUES WITH FIDUCIAL LIMITS IN PARENTHESIS			SLOPE b ±S.E.	H/F	DEGREES OF FREEDOM
	LD 50	LD 90	LD 99			
25°, DARK, 10 larvae/g food	22.8 (25.6, 18.4)	31.4 (44.3, 27.7)	40.8 (79.9, 33.5)	9.22 ±1.91	<1	4
20°C, 8HR LIGHT - 16HR DARK, 2 larvae/g food	29.0 (33.1, 23.7)	48.8 (62.6, 40.7)	74.5 (114, 58.9)	5.67 ±0.86	<1	12
p values for difference at each LD level	< 0.025	<0.025	<0.05			

TABLE 100. Effect of method of diapause induction on the susceptibility of diapausing larvae of Ephestia elutella (laboratory stock) to 6.1 mg/l methyl bromide at 23° - 25°C. (LD values calculated in mg h/l).

METHOD OF DIAPAUSE INDUCTION	LD VALUES WITH FIDUCIAL LIMITS IN PARENTHESIS			HETERO-GENEITY FACTOR	DEGREES OF FREEDOM
	LD 50	LD 90	LD 99		
20°C, 8 HR LIGHT/DAY	57.0 (74.5, 28.8)	92.7 (199, 71.2)	138 (674, 97.4)	<1	4
20°C, 12 HR LIGHT SHORTENING BY 3½ MIN./DAY	62.9 (75.0, 49.2)	96.8 (131, 80.7)	138 (232, 108)	<1	5
BOTH GROUPS	60.5 (67.1, 53.2)	95.0 (110, 85.2)	137 (175, 117)	<1	11

TABLE 101. Effect of method of terminating diapause on the susceptibility of diapausing larvae of Ephestia elutella (field stock) to 4.0 mg/l methyl bromide at 30°C. (LD values calculated in mg h/l).

CONDITIONS FOR TERMINATION OF DIAPAUSE	LD VALUES WITH FIDUCIAL LIMITS IN PARENTHESIS			HETEROGENEITY FACTOR	DEGREES OF FREEDOM
	LD 50	LD 90	LD 99		
30°C DARK	39.8 (55.7, 24.4)	71.3 (160, 49.9)	115 (504, 68.2)	<1	4
25°C 16 HR LIGHT	42.3 (57.9, 26.1)	73.7 (161, 54.5)	116 (475, 77.3)	<1	4
BOTH GROUPS	40.8 (49.4, 29.8)	72.2 (92.0, 60.3)	115 (180, 90.6)	<1	10

TABLE 102. Effect of length of time in diapause on the susceptibility of diapausing larvae of Ephestia elutella (laboratory stock) to 6.7 mg/l methyl bromide at 20°C (LD values calculated in mg h/l).

WEEKS IN DIAPAUSE AT 20°C, 8 HR LIGHT	LD VALUES WITH FIDUCIAL LIMITS IN PARENTHESIS			HETEROG-ENEITY FACTOR	DEGREES OF FREEDOM
	LD 50	LD 90	LD 99		
3	70.0 (87.8, 34.6)	104 (151, 81.9)	144 (345, 113)	<1	4
12	92.8 (105, 46.0)	124 (173, 110)	156 (440, 133)	<1	7
BOTH GROUPS	82.0 (92.2, 65.4)	118 (137, 107)	160 (219, 138)	<1	13

TABLE 103. Susceptibility to a mixture of methyl bromide and phosphine of batches of 100 newly-laid eggs and 50 diapausing larvae, of Ephestia elutella and Plodia interpunctella.

HOURS FUMIGATED (AT 20°C)	CT PRODUCTS mg h/l MeBr	CT PRODUCTS mg h/l PH_3	STAGE	% MORTAL-ITIES	E. elutella LAB. STOCK	E. elutella FIELD STOCK	P. interpunctella LAB. STOCK	P. interpunctella FIELD STOCK
0	0	0	EGGS	CONTROL	16	12	10	7
			DIAPAUSING LARVAE	CONTROL	6	2	0	2
24	51	5.6	EGGS	EXPECTED FOR MeBr	100	99+	100	100
				EXPECTED FOR PH_3	0	0	0	0
				OBSERVED	100	100	100	100
			DIAPAUSING LARVAE	EXPECTED FOR MeBr	10	10	95	90
				EXPECTED FOR PH_3	50	20	99	70
				OBSERVED	92	74	100	100
40	84	12.5	EGGS	EXPECTED FOR MeBr	100	100	100	100
				EXPECTED FOR PH_3	10-20	10-20	10-20	10-20
				OBSERVED	100	100	100	100
			DIAPAUSING LARVAE	EXPECTED FOR MeBr	60	50	100	99
				EXPECTED FOR PH_3	99	90	100	100
				OBSERVED	100	100	100	100

for each fumigant a sum of the doses required for 10 and 50% mortality. Here evidence for an additive effect was obtained when no insects survived the 40-hour exposure, because larvae of the field stock of E. elutella able to survive a dose of phosphine giving 90% kill, were therefore unable to survive an added dose of methyl bromide which would normally kill little more than half of the population.

D. Observations on Adults Developing From Fumigated Diapausing Larvae

After fumigation of diapausing larvae with either methyl bromide or phosphine, adults subsequently produced occasionally had difficulty in freeing themselves from the pupal case, and sometimes wings were incompletely expanded. The longevity of such adults was normal. Examination of mouthparts and genitalia boiled in caustic potash and cleared in glacial acetic acid, revealed no structural deformities.

For investigations on fertility, batches of 4 females emerging after fumigation of larvae in diapause were weighed and were placed over food together with 4 males from the same fumigated batch. The number of progeny was noted (table 104). Comparison with control batches revealed that, when kill after fumigation with methyl bromide exceeded 40 - 50%, the fertility of survivors of normal weight was significantly depressed ($p \approx 0.02$-0.05). After fumigation with phosphine, the fecundity of adults surviving fumigation was not significantly different from control samples. Both fumigants were, however, similar in hastening the termination of diapause (see Biological Results Section 4H, figs. 42 and 43). After fumigation, subsequent development of diapausing insects to the next stage was, in general, hastened. Normally, a delay in development is expected after fumigation.

5. EGGS AND PUPAE IN DIAPAUSE

Diapausing pupae of Pieris brassicae and diapausing eggs of Bombyx mori proved

highly tolerant to both phosphine and methyl bromide, particularly at low temperatures.

A. Pupae of Plodia brassicae

i) Phosphine

Pupae were fumigated with a concentration of about 0.05 mg/l at 20°C, and about 0.8 mg/l at 10°C (table 105). Non-diapausing pupae succumbed to a CT product of 4.7 mg h/l at 20°C, while diapausing pupae were quite tolerant, mortality in the batch exposed to a CT product of 10.8 mg h/l being no higher than in the control. At 10°C, three out of eighty diapausing pupae survived a CT product of 138 mg h/l in an 8-day exposure.

ii) Methyl bromide

Pupae were exposed to 10.3 mg/l methyl bromide at 20°C, and 9.2 mg/l at 25°C (table 106). Diapausing pupae were very tolerant to methyl bromide. At 20°C, a CT product of 239 mg h/l failed to achieve 50% control of diapausing pupae, while at 25°C, a CT product of 403 mg h/l was required for 100% kill. Non-diapausing pupae succumbed to a CT product of 60.5 mg h/l at 20°C.

B. Eggs of the Univoltine Race of Bombyx mori

i) Phosphine

Samples from four batches of eggs of Bombyx mori were fumigated at 25°C with a stable concentration of about 0.5 mg/l phosphine for 1, 2, 3 and 7 days (table 107). Two batches were comprised of diapausing eggs. One batch had been in diapause for about 7 weeks, and the other had fulfilled the requirements for the breaking of diapause by being held at 1°C for 6 months. The other two groups comprised eggs aged 1-2 days and eggs aged 3-5 days, which had been treated with 6N hydrochloric acid in their first day to prevent the onset of diapause. All batches showed survival after the 3-day exposure, but % hatch was greatest in the younger age group of eggs treated for the avoidance

of diapause, mortality being less than 10%. No eggs survived the 7-day exposure.

At 10°C, diapausing eggs were apparently unaffected by a CT product of 73.7 mg h/l over 16 days. Hatch in batches of about 300 eggs was about 70% in the controls and exposures of 4, 8 and 16 days.

ii) Methyl bromide

Eggs treated for the avoidance of diapause with 6N hydrochloric acid, and eggs that had been in diapause for about 9 weeks, were exposed in batches of 300 to CT products of 135, 179, 222, 280 and 343 mg h/l in a test with 9.2 mg/l methyl bromide at 25°C. Hatch was recorded only in the batch of diapausing eggs exposed to the CT product of 135 mg h/l (34%, compared with 84% in the control).

Batches of 300 diapausing eggs fumigated at 10°C with 4.8 mg/l methyl bromide for 24, 32 and 48 hours (CT products 110, 144 and 216 mg h/l) gave 91, 77 and 95% hatch, compared with 88% in the control. Like phosphine at 10°C, methyl bromide was ineffective against eggs in diapause.

TABLE 104. Number of progeny from groups of four pairs of Ephestia elutella selected after emergence from batches of fumigated and unfumigated diapausing larvae.

FUMIGANT	CONTROL			FUMIGATED		
	% NATURAL MORTALITY BEFORE EMERGENCE	AV. WT. OF 4 ♀♂s mg	NO. OF PROGENY	% MORTALITY BEFORE EMERGENCE	AV. WT. OF 4 ♀♂s mg	NO. OF PROGENY
METHYL BROMIDE	12	44.4	149	66	38.5	22
	0	38.6	126	52	45.3	149
	4	48.7	173	44	48.9	164
	0	40.9	151	59	42.7	113
	6	43.8	91	75	40.8	17
	7	43.0	222	82	43.6	27
	0	39.9	59	79	50.1	53
	9	45.1	105	90	39.0	96
	5	42.4	84	85	38.7	32
	5	46.0	197	63	42.5	128
Totals:	432.8		1357		430.1	801
Mean and S.E.			135.7 ± 51.9			80.1 ± 56.4
PHOSPHINE	6	45.3	114	43	43.8	82
	0	44.8	156	59	41.6	137
	5	40.6	97	73	41.4	119
	9	48.0	252	84	42.7	99
	4	45.8	198	59	48.8	327
	0	45.4	126	77	52.3	286
	6	42.7	182	86	44.4	206
	10	41.3	203	65	44.8	145
	8	46.5	231	67	40.2	53
	4	43.1	79	91	49.8	264
Totals:	443.5		1638		449.8	1718
			163.8 ± 58.8			171.8 ± 93.1

TABLE 105. Percentage mortalities of pupae of Pieris brassicae after fumigation with phosphine (2 replicates of 40 pupae per exposure; held at 25°C, 16 hours light for emergence.)

TEMP. (°C)	EXPOSURE (HOURS)	CT PRODUCT (mg h/l)	DIAPAUSING PUPAE	NON-DIAPAUSING PUPAE
10	0	0	22.5 17.5	-
	48	15.3	32.5 10.0	-
	96	55.0	30.0 30.0	-
	192	138	97.5 95.0	-
20	0	0	27.5 17.5	7.5 2.5
	24	0.9	15.0 25.0	5.0 12.5
	30	1.3	25.0 12.5	10.0 5.0
	48	2.3	7.5 20.0	35.0 55.0
	96	4.7	22.5 47.5	100 100
	168	7.9	42.5 27.5	100 100
	240	10.8	30.0 7.5	-

TABLE 106. Percentage mortalities of pupae of Picris brassicae after fumigation with methyl bromide. (2 replicates of 40 pupae per exposure; held at 25°C under a 16-hour daylength after emergence)

TEMP. (°C)	EXPOSURE (HOURS)	CT PRODUCT (mg h/l)	DIAPAUSING PUPAE	NON-DIAPAUSING PUPAE
20	0	0	35.0 20.0	2.5 2.5
	3	30.5	-	45.0 37.5
	4	40.5	17.5 25.0	92.5 95.0
	6	60.5	15.0 32.5	100 100
	8	80.4	15.0 22.5	100 100
	16	157	32.5 30.0	100 100
	24	239	65.0 50.0	-
25	0	0	7.5 12.5	-
	8	72.1	15.0 2.5	-
	16	138	5.0 10.0	-
	24	210	22.5 40.0	-
	32	276	90.0 92.5	-
	48	403	100 100	-

TABLE 107. Percentage mortalities of eggs of the univoltine race of Bombyx mori after fumigation with phosphine at 25°C (300 eggs per treatment, hatching observed at 25°C).

EXPOSURE (HOURS)	CT PRODUCT mg h/l	UNCHILLED EGGS, IN DIAPAUSE 7 WEEKS	DIAPAUSING EGGS CHILLED FOR 6 MONTHS	HCl-TREATED EGGS AGED 1-2 DAYS AT 25°C	HCl-TREATED EGGS AGED 3-5 DAYS AT 25°C
0	0	23	10	2	11
24	12.5	18	11	2	15
48	24.6	27	36	3	21
72	36.5	63	80	7	30
168	82.0	100	100	100	100

DISCUSSION

DEVELOPMENTAL ECOLOGY

Of the four species investigated two occur predominantly in temperate zones, while the other two, although better established in warmer climates, are more cosmopolitan in their distribution. This is reflected in the ranges of temperature over which development occurs in each species. Thus Ephestia elutella and E. kuehniella breed successfully at 15°C, but are adversely affected at 30°C, while E. cautella and Plodia interpunctella breed successfully at 30°C, but will not complete development at 15°C. In E. elutella and E. kuehniella development proceeded from egg to adult at 30°C, but infertility prevented the establishment of a second generation. Similar results for E. kuehniella were reported by Brindley (1930). Norris (1933) noticed infertility in males of E. kuehniella reared at 27°C. Females mated with younger males bore little or no sperm in the receptaculum seminalis. The number of unsuccessful pairings was increased with high rearing densities, probably as a result of increased temperature. Raichoudhury (1936) found that tubules in the testes of males emerging at 27°C contained immature spermatozoa, and few mature sperm bundles were present. Older males recovered potency to some extent, but at high temperatures, sperm was motile for a shorter period of time. Further work (Raichoudhury and Jacobs, 1937) showed that high temperatures also affected the fertility of the female, as eggs layed by females aged over two days at 30°C, failed to hatch. Similar data are not available for E. elutella but the present findings indicate that this species is able to tolerate slightly higher temperatures than E. kuehniella.

Eggs of E. kuehniella and E. elutella are less tolerant to cold than fully grown larvae. At 10°C, eggs of E. kuehniella survived nearly twice as long as those of E. elutella. King (1934) obtained 80% survival of E. kuehniella eggs exposed at a temperature ranging between 0 and 3.3°C for 1 week, but

hatch was reduced to 1.6% after an exposure of 6 weeks at this temperature. Payne (1934) stated that larvae were quite tolerant to cold while eggs were more susceptible. She quoted lower development limits of 8 and 10°C for two strains of E. kuehniella. Howe (1965) quoted a lower limit of 10°C for a population increase to occur in both E. kuehniella and E. elutella. However, E. elutella is aided by a cold-tolerant diapause stage to overwinter in this country (Richards and Waloff, 1946; Waloff, 1949) while E. kuehniella is not. As a result E. elutella is more successful in unheated warehouses, whereas E. kuehniella is more abundant in mills and factories where some heat is available throughout the year. Nevertheless Solomon and Adamson (1955), investigating the ability of stored product pest species to overwinter in this country, found E. kuehniella to be one of the most cold hardy, and predicted that larvae would be able to survive in unheated premises.

In P. interpunctella, newly laid eggs were more cold susceptible than all other developmental stages, succumbing to a 19-day exposure at 15°C or a 10-day exposure at 10°C. Similarly, Tsuji (1963) observed that eggs kept at 15 - 16°C from laying all failed to hatch. In contrast, larvae hatched at higher temperatures are able to grow and pupate at 15°C (Tzanakakis, (1959). Cline (1970) found a 48-hour exposure at 2.4°C sufficient to kill all eggs laid at 27°C and aged less than 4.5 hours at the start of the exposure, while older eggs required up to 8 days for 100% kill. He further observed a reduced survival among larvae hatching from eggs exposed to cold.

Howe (1965) thought a minimum temperature of 18°C to be necessary for populations of P. interpunctella to increase. Like E. elutella, P interpunctella overwinters in this country as a diapausing larva (Potter, 1935; Williams, 1964), and the rather high threshold for development has not prevented the build up of large populations during the summer season. The optimum range for development is, however, about 28 - 32°C (Howe, 1965).

Unlike P. interpunctella, the development of E. cautella at lower temperatures is not limited only by the egg stage. Burges (1956) found that eggs laid at 25°C and moved to low temperatures after 0-3 days, hatched at 13°C. Later work (Burges and Haskins, 1965) showed that the lowest temperature at which larvae completed development was 15.5°C, and mortality during the larval and pupal stages was greater than at higher temperatures. Howe (1965) gave 17°C as the lower limit for a population increase of E. cautella. In this country, it can become established in heated premises, and breeds in warehouses during the summer, but mortality in winter is very heavy and warehouse populations are maintained only by the continual arrival of imports (Burges, 1956). The upper developmental limit for E. cautella is about 36°C (Burges and Haskins, 1965).

Differences are often noticed in the development times quoted for each species by different authors. Compared with the available literature, the current results indicate fairly rapid development in all species. Often, differences are related to the suitability of the food medium, or the occurrence of a resting stage in the life cycle (Waloff, 1948; Williams, 1964). Such differences, however, should apply only to the larval stage, and large discrepancies in the duration of the egg or pupal stages cannot be explained in this manner.

There are also considerable variations in the lower developmental limits quoted for different stocks of the same species. Eggs of the field stocks of E. kuehniella and P. interpunctella proved more tolerant to cold than those of the laboratory stocks. Continuous rearing in the laboratory at high temperatures may reduce the cold tolerance of laboratory stocks, and cold tolerance in wild stocks seems to be related to the locality where the population overwinters (Sømme, 1965). It is often difficult to pick out the stage in which the coordination of vital chemical reactions is the most likely

to be disturbed at low temperatures, because mortality may be delayed until development has proceeded to the next stage. Eggs will often hatch over a greater temperature range than is tolerated by larvae, but larvae hatching from eggs after an exposure to cold may not complete development (Howe, 1967). Development at high or low temperature may be completed to the adult stage in which infertility prevents the establishment of a second generation. Often, the susceptibility of pupae is responsible for infertility in adults.

Most of the work so far discussed has been conducted with fixed ambient temperatures, and care must be taken in relating such results to natural environments where daily fluctuations of temperature occur. Evidence is that with fluctuating temperatures, the higher temperature rather than the mean is important in determining the lower development limit of insects, and populations can develop with a mean temperature below the threshold determined by life cycle experiments at constant temperatures (Howe, 1967).

Humidity, particularly at extreme temperatures, is another important factor that influences insect development. In the present tests, field stocks were much more tolerant to low RH than laboratory stocks (table 6). The optimum range of constant humidities for E. cautella lies between 70 and 80% RH, but development will proceed at 50 and 90% RH at all but extreme temperatures (Burges and Haskins, 1965). At 20% RH, however, development is completed only at 25 - 30°C, and at 20°C, a minimum RH of about 40% is required. The other species are more tolerant to low RH. In the present work, E. elutella and E. kuehniella completed development at about 20 - 25% RH at 15°C. Fraenkel and Blewett (1944) found larvae of E. kuehniella able to complete development at 1% RH at 25°C. More food was consumed than at higher humidities. Tzanakakis (1959) found that P. interpunctella completed development readily at 41% RH at 35°C.

Insects in the storage environment produce metabolic heat which increases the temperature of their surroundings. Flanders (1933) recorded temperature increases from 6 days after hatching in cultures of Sitotroga cerealella on white corn at 29°C. Rapid heat production continued until cessation of feeding, at which point temperature rises of up to 3.5°C were recorded. Howe (1962) recorded sharp peaks of temperature in grain infested with Sitophilus granarius, which correspond to the feeding period of last instar larvae. Tsuji (1963) noted temperature rises of 2 - 5°C in cultures of P. interpunctella larvae set up with 400 eggs per 30 g rice bran. Wishart (1942) observed temperature rises of up to 5.7°C in cultures of E. kuehniella. Various numbers of larvae were reared at 23°C on 120 g whole wheat flour in shallow cardboard boxes measuring 15 x 15 cm. Wishart noted two temperature peaks separated by about 5 days, and, as in the lower density cultures of the present experiments, observed a rather gradual temperature rise followed by a more rapid fall at pupation. The two peaks obtained in cultures of E. kuehniella (figs. 15, 19, and 24), and in one or two cultures of E. elutella (fig. 26), probably represent the periods just before tha last larval and pupal moults. Wishart discussed the earlier results of Norris (1933) and Raichoudhury (1936), which showed the tendency of males reared at 27 or 30°C to be sterile. He deduced that rearing at lower temperatures at high population densities may also induce sterility because of the heat produced by crowding. It is clear, however, that rearing temperatures described by Norris and Raichoudhury referred only to the conditions at which cultures were placed, and temperatures within cultures were not measured.

Adults produced in cultures of E. kuehniella set up with over 4,000 insects (fig. 24) in which temperatures rose from 25°C to between 30 and 32.3°C for the last two weeks of larval development, were successfully used to set up fresh cultures. It is true that lower temperatures were present at the bottom

of the 7 cm deep food layer, but most larvae were observed to develop and pupate near the surface. The two weeks at which temperatures were highest were nearly over at the start of pupation, and, as no infertility was evident in adults produced, it may be concluded that larvae of E. kuehniella are quite tolerant to heat, and that infertility is only likely to result when pupae are exposed to high temperature.

The rapid temperature changes that occur within developing cultures cause changes in humidity. However, in cultures of E. cautella, the effect was less marked than in other species. Although set up with a similar number of eggs, fewer moths were produced and food more than 2 or 3 cm below the surface was commonly not utilized. Glycerol was present in the rearing food as a mould depressant, moisture content stabilizer, and additional nutrient. Its hygroscopic properties have long been used to control humidity (Grover and Nicol, 1940; Durbin, 1965). In new cultures of all species, glycerol was responsible for a lowering of humidity at the food surface. As food was consumed and water released, the effect of glycerol disappeared before emergence in all species other than E. cautella. Even after emergence, however, cultures of E. cautella still showed a low humidity at the food surface, probably because of glycerol in unutilized food.

Temperature rise in insect cultures is correlated with population density. In E. cautella, the accumulation of heat in cultures with 1,000 - 4,000 developing larvae was greater than in the other species. This was surprising as survival to the adult stage was lower and presumably less larvae were present to cause the large increase. Also, less food was consumed because of crowding in the surface layer.

Experiments conducted on food masses of similar weight and volume but of

different shape, revealed higher percentage survivals to the adult stage, and slightly faster development, in broader and shallower food layers. The effect was more marked in E. cautella than in other species. Varying the depth of food while not changing the area of the food surface had little effect on the number of moths produced. Takahashi (1959a) and Smith (1969) obtained similar results with E. cautella and E. kuehniella respectively, but the results of Wool (1969) with Tribolium castaneum were less well defined.

In all species, percentage survival to the adult stage in similar cultures was reduced as the number of eggs added was increased. The effect, although slight, was noticed at quite low culture densities. Takahashi (1953; 1956; 1956a) obtained similar results with E. cautella on rice bran, and noted a correlation between the rearing density, the head width of the adult moth, and the length of time for development. Moths produced at higher population densities were smaller, less fecund, and of shorter longevity, and the overall time for development was longer. Takahashi proposed that development was lengthened by the destruction of the first pupae by larvae which had not finished feeding. Also, development was lengthened, and survival was lowered, by rearing in food contaminated with faecal pellets (Takahashi, 1957).

In preparing for fumigation tests on all stages (Fumigation Results, Section 1), it was noticed that only 100 - 140 moths were obtained from about 200 newly hatched larvae added in three equal portions at weekly intervals, but when the 200 larvae were added in a single batch, 150 - 170 moths were obtained. Takahashi (1959) obtained similar results adding batches at 9 or 10-day intervals and comparing yields with single batches. Once again, the destruction of pupae by younger larvae appeared to be responsible for the discrepancy.

Takahashi (1955; 1961) also investigated the effect of high population density

on the movement of E. cautella larvae in cultures. Young larvae were observed to crowd into the upper layers of food, and often large numbers wandered up from the food surface. Only from the third instar onwards did some larvae start to move into the deeper layers of the culture. The inference of this and other work showing the importance of the area of the food surface in larval development, is that moth larvae tend to occur near the surface of bulk grain or other tightly packed media. Here, they would experience changes in temperature and light, and seasonal regulation of the life cycle would occur. The data of Waloff and Richards (1946) supports this view by showing that most larvae of E. elutella occurred in the top 30 cm of bulk grain with peak density at about 11 cm. Furthermore, larvae placed half way down long towers of grain worked their way up to the surface in three or four weeks.

ECOLOGY OF DIAPAUSE

Induction of Diapause

Diapause occurs in E. elutella and P. interpunctella (Richards and Waloff, 1946; Waloff and Richards, 1946; Zacher, 1950; Michelbacher, 1953), and there is some evidence of a slight tendency towards diapause in E. cautella and E. kuehniella. Zacher (1950) reported a facultative diapause in E. cautella but in fact a resting stage has never been demonstrated in this species. However, a wandering stage occurs in the mature larvae which can be prolonged for a number of weeks before a pupation site is chosen. Some seasonal variation may occur in E. kuehniella, which has been reported to require longer to complete development during winter than in summer, even though the same temperature is chosen for rearing test samples (von Gierke, 1932; Wigglesworth, 1972). Currently, attempts to induce a resting stage in a wild stock of E. cautella have been unsuccessful.

In E. elutella, diapause is brought about by low temperatures or high-starch

foodstuffs (Waloff, 1948), and by short photoperiods (Strümpel, 1964). Of the two stocks considered here, one had been reared in the laboratory at 25°C for a number of years while the other was obtained from a London granary in July, 1969. Under short daylength, most larvae of the laboratory stock entered diapause at 15 or 20°C, but very few did so at 25°C. Larvae of the field stock entered diapause in short daylength or darkness at 25 and 30°C. Laboratory rearing for some years had apparently selected in favour of those individuals developing without diapause at 25°C. The change in this stock can be regarded as a lowering of the minimum temperature for development without diapause in short daylength, and a lowering of the temperature at which larvae enter diapause regardless of photoperiod. A laboratory stock of E. elutella with similar properties was described by Waloff (1948). The incidence of diapause has been reduced by laboratory breeding in various Noctuid moths of the genus Heliothis (Benschoter, 1970) the Codlin moth Laspeyresia (Carpocapsa) pomonella (L) (Wildbolz and Riggenbach, 1969), the Oriental fruit moth Grapholitha molesta (Busck) (Glass, 1970), in the Shield-bugs Aelia acuminata (L) and A. rostrata Boh. (Hodek and Hŏnek, 1970; Hŏnek, 1972), and in P. interpunctella (Tsuji, 1959a; 1960). Currently, a stock developing without diapause under short daylength at 20°C has been isolated from the laboratory stock of E. elutella. It is clear that for studies on diapause, care must be taken to obtain fresh stocks from natural conditions.

In the field stock of E. elutella, diapause occurred under short daylength or in darkness at 25 or 30°C, at 15°C, all larvae entered diapause regardless of photoperiod, and at 20°C most entered diapause, even under long daylength. These results indicate that in cooler situations, larvae at the right stage of development may enter diapause at any time of year.

The critical photoperiod for E. elutella at 25°C was about 14 hours, transition from 0 to over 95% diapausing larvae occurring between photoperiods of 15 and

13 hours respectively. Continual darkness was effective in producing diapause in only about half of each batch of larvae exposed. Strümpel (1964) obtained a very similar result with a stock of E. elutella in Hamburg, although a higher proportion of larvae entered diapause in darkness at 25°C. He identified the sensitive phase as the first few days of the last larval instar. After this time, less than 10% of larvae could be stimulated to enter diapause under short photoperiod. However, Strümpel stated that the stimulus for diapause was cancelled by 16-hour photoperiods at any time during the feeding period of the last instar. In the present experiments, diapause occurred in all larvae held for 18 days or less at 25°C before transferring to an 8-hour daylength at 20°C. Some larvae entered diapause at 20°C after remaining under long daylength at 25°C for up to 28 days, but no diapause occurred in a sample held for 31 days under those conditions (table 18). Emergence in cultures of the field stock of E. elutella occurs predominantly over the range of 40 - 58 days and the pupal stage is completed in 13 - 14 days (table 5). Allowing about 8 - 12 days for the last larval instar, the estimated time for the last larval moult is about 18 - 32 days after egg laying, and this coincides with the range over which the percentage of larvae entering diapause declines. However, in contrast with Strümpel's (1964) observations, when larvae reared for various lengths of time under a 13-hour daylength at 25°C were moved to a 16-hour daylength, there were indications that the stimulus for diapause was reversible only for a short period of time. A more or less normal emergence pattern was seen in a sample transferred to the 16-hour daylength after 28 days, but the sample moved after 31 days gave a very different emergence pattern (fig. 27). This sort of emergence pattern is typical of emergence after the termination of diapause under long daylength (fig. 29), indicating that most larvae had entered diapause, and were subsequently stimulated to pupate.

The critical photoperiod was not accurately determined at 20°C as most larvae of the field stock entered diapause regardless of the light conditions. However, the change from 100% to about 80% diapausing larvae occurred between 12 and 16-hour photoperiods with an intermediate result for 14 hours. In the laboratory stock, a change from about 80% to less than 7% larvae in diapause occurred over the same range of photoperiods. These results indicate that there was no increase in critical photoperiod when the temperature was lowered from 25 to 20°C. Danilevskii (1961) described an increase in critical photoperiod of 1½ hours for each 5°C lowering of temperature in the Colorado beetle Leptinotarsa decemlineata Say, the Noctuid moth Acronycta rumicis L., and the Cotton-boll worm Chloridea obsoleta F. He regarded Pieris brassicae as an exception because the critical photoperiod changed little between 12 and 26°C, but in the present study, E. elutella and P. interpunctella behaved in a similar manner to P. brassicae.

In some species, diapause is controlled by progressively shortening or lengthening photoperiods below or above the critical photoperiod, rather than by a particular number of short daylengths (Corbet, 1954; Tauber & Tauber, 1970). In the present work, only progressively shortening daylengths were investigated with E. elutella, and no diapause occurred in the field stock at 25°C (table 18), and very little in the laboratory stock at 20°C (table 17), when photoperiods were gradually decreased to the critical level by the end of larval development.

The sensitivity of E. elutella to light signals is great. A 16-hour photoperiod of intensity 1 lux at the surface of glass tubes containing larvae, alternated with total darkness, was at least as effective as a 16-hour photoperiod at 1000 lux in preventing the incidence of diapause. However, an 8-hour photoperiod at 1 lux did not stimulate many more larvae of the

laboratory stock to enter diapause than holding in total darkness. Danilevskii (1961) stated that the lower limit of light intensity is dependent on the contrast present in the alternation of light and dark phases. He found that for full effect in Acronycta rumicis, light intensity during the darker phase needed to be less than 2 - 3 lux. When no light was present during the dark period, the effect of a short photoperiod started to decline when intensity fell to 5 lux. These and the present results indicate that a difference exists in the threshold light intensities for the identification of long and short daylength signals. Short photoperiods of low light intensity elicit the response expected in continual darkness where a proportion of insects may continue development, whereas long photoperiods of similar intensity can be fully effective in avoiding diapause.

As a further investigation, a 16-hour photoperiod of intensity 1 lux was arranged to include an 8-hour photoperiod at 400 lux. Thus the photoperiod included 4 hours at 1 lux, followed by 8 hours at 400 lux, and finally another 4 hours at 1 lux. This photoperiod gave the same results as 16-hour photoperiods of constant intensity 1 or 400 lux. The long low intensity photoperiod completely overrode any response to the short high intensity photoperiod, indicating that the change from darkness to dim light was more important than the change from dim light to bright light. Further evidence for the importance of low intensity light in avoiding diapause was obtained in work arising as an offshoot of this project (Bell and Walker, 1973). A background intensity of 0.3 lux during the dark phase caused up to 40% of larvae of the field stock of E. elutella under short photoperiods to complete development without diapause. Light in this work was supplied from 40-W bulbs and passed through "daylight-blue" acetate paper to produce a more natural spectrum.

Very high sensitivity to light has been found in several species. De Wilde

and Bonga (1958) noticed that Leptinotarsa decemlineata responded to photoperiods of 0.1 lux. A similar threshold was found for the Silkmoth Bombyx mori (Kogure, 1933), and for the Codlin moth Laspeyresia pomonella (Wildbolz and Riggenbach, 1969). Even more sensitive are the larvae of the midge Metriocnemus knabi Coq, which reacted to intensities of 0.0025 foot-candles (0.025 lux) (Paris and Jenner, 1959).

Danilevskii (1961) observed that forms with very high sensitivity to light live in relatively inaccessible environments. The storage environment is certainly one of this type. Hence moonlight, which hardly ever exceeds 0.5 lux, and late twilight, are not expected to be of significance in the photoperiodic reaction.

Low relative humidity (RH) encouraged the incidence of diapause in larvae of E. elutella held in darkness at 30°C. Although little work had been done on the influence of RH on the photoperiodic induction of diapause, the water content of food plants can influence reaction to photoperiod in certain species. Geyspits (1960) demonstrated this in the mite Tetranychus urticae Koch.

Rearing at high population density did not increase the proportion of larvae entering diapause in darkness in the laboratory stock at 20°C or the field stock at 25°C. It was noticed in these experiments that the laboratory stock was able to survive better at high density than the field stock. This added ability was probably a result of selection by prolonged laboratory rearing in crowded cultures.

In Plodia interpunctella, diapause can be induced by short photoperiod (Danilevskii, 1956; 1961), low temperature (Tsuji, 1958; Tzanakakis, 1959),

high population density (Tsuji, 1959), and by diet (Williams, 1964). In both laboratory and field stocks, a very high proportion of insects entered diapause when samples were reared at 20°C, or dropped to 20°C from 25°C during the first 15 days after egg laying, when the photoperiod was 12 hours or less (tables 20 and 21). Tsuji (1958; 1963) obtained diapause in all larvae dropped to 20 or 22°C from 30°C 10 or 12 days after egg laying, but after 16 days at 30°C, larvae often failed to enter diapause. He discovered that for 100% diapause, larvae needed to be at the lower temperature for the whole of the last two instars. He also found that all larvae reared from egg laying in darkness at 20 or 22°C entered diapause while larvae reared at 25°C did not. Tzanakakis (1959) found that temperatures above 25°C in the egg stage followed by temperatures of 20°C or below for at least the latter half of larval development greatly increased the proportion of larvae of his Berkeley stock entering diapause. Sardesai (1968), testing a strain that had been reared mainly in darkness for about 10 years, induced diapause in only 4 - 20% of larvae reared under short photoperiods or in darkness at 20°C. Prevett (1971) found diapause present in a South African strain and absent in a Nigerian strain of P. interpunctella. The South African strain entered diapause in response to a temperature drop from 30 to 20°C 1 or 2 weeks after egg laying.

There is obviously considerable variation in the incidence and control of diapause in different stocks and strains. In the present investigation, the laboratory stock of P. interpunctella resembled the original stock of Tsuji in that diapause occurred in darkness at 20°C with or without a temperature drop. Also consonant with Tsuji's (1963) results, was the action of a 16-hour photoperiod at 20°C in preventing the incidence of diapause. The field stock, differed from the stocks of Tsuji and Tzanakakis in entering diapause under short photoperiods, darkness or continual light at 25°C. Williams (1964) obtained diapause at 25°C in larvae reared on American yellow corn.

Some larvae, of the field stock of P. interpunctella entered diapause when samples kept for 10 days at 30°C were dropped to a 16-hour daylength at 20°C. Very few larvae entered diapause in samples dropped to these conditions from 25°C, illustrating the greater effectiveness of a large temperature drop in inducing diapause. Tsuji (1963) found a 16-hour daylength at 20°C fully effective in avoiding diapause in samples dropped from 30°C. Tzanakakis (1959) observed only a general inhibitory effect of all photoperiods on the induction of diapause at 20°C.

The critical photoperiod for the field stock was about 13 hours at 20 or 25°C. Tsuji (1963) found that the principle sensitive phase to daylength in P. interpunctella occurred during the 3rd and 4th of the 5 larval instars, but did not estimate the critical photoperiod. Bell and Walker (1973) estimated the sensitive phase to occur around 8 days after hatching at 25°C, and obtained a value of 13¼ hours for the critical photoperiod.

Diapause in P. interpunctella was not stimulated by progressively shortening photoperiods, as at 20°C very few larvae delayed pupating under photoperiods shortening from 14.5 to 13 hours during development.

A 16-hour photoperiod at an intensity of 1 lux at the surface of tubes containing insects proved as effective in limiting the number of larvae entering diapause at 20°C as long photoperiods at about 800 lux. Thus P. interpunctella was similar to E. elutella in being highly sensitive to light. However, P. interpunctella responded more readily to high population density than E. elutella (table 22). Under conditions successful in producing diapause in a small proportion of larvae exposed, increased population density caused more larvae to enter diapause.

Tsuji (1959) managed to isolate a density-dependent diapause in P. interpunctella at 30°C. He deduced from further experiments (Tsuji, 1963) that the signal to enter diapause in response to density was received during the crowding together of newly hatched larvae. In the present experiments, all larvae were hatched together in a dish before adding in different numbers to tubes holding about 1 - 1½ g food. The different proportions of larvae entering diapause in tubes with different numbers of insects can therefore be explained only by the effect of population pressure later in development.

Exposure to natural autumn conditions was highly successful in inducing diapause in samples of both E. elutella and P. interpunctella. For E. elutella the results discussed above indicate that the main causative factor for the autumn induction of diapause in unheated premises in Britain is temperature. Short daylength is of course an additional factor, but it would assume paramount importance only when autumn temperatures were high. For P. interpunctella, photoperiod is a more important factor as it can override the effect of falling temperatures if the drop is not too great.

Termination of Diapause

In both P. interpunctella and E. elutella, the duration of diapause was dependent on the temperature and daylength at which samples were held, and sometimes was also dependent on the method in which diapause had been induced. Exposure to long daylength, increases in temperature, periods at low temperature, or sublethal doses of fumigants, all hastened the termination of diapause. The duration of diapause in E. elutella was not influenced by humidity at 15°C. Under all conditions tested, diapause in E. elutella was of longer duration than in P. interpunctella, and in both species was generally longer in the field stock than in the laboratory stock.

Diapause is commonly terminated by increased temperature after exposure to cold, and the termination of diapause by photoperiod is relatively unusual (Lees, 1955; Danilevskii, 1961; Beck, 1968). The latter may be a result of infrequent examination of this factor since the importance of photoperiod in the termination of diapause is being demonstrated in an increasing number of cases. Beck (1968) lists 19 species responding in this way, but his list was not intended to be comprehensive. Lepidoptera more recently shown to respond to long daylength during diapause termination include the swallowtail butterfly Papilio polyxenes F (Oliver, 1969), the tortrice Lobesia botrana Shiff (Rochrich, 1969), the wild silkworm Actias luna (L) (Wright, 1970), the Tasar silkworm Antheraea mylitta Drury (Jolly et al., 1971), and the webworm Chrysoteuchia topiaria (Zeller) (Kamm, 1973).

Strümpel (1964) found that short daylengths were necessary for the maintenance of diapause in E. elutella, but no detailed account has yet appeared on the role of photoperiod in the termination of diapause in P. interpunctella.

At 25°C, the effect of a 16-hour photoperiod was to reduce the mean duration of diapause in both E. elutella and P. interpunctella to about one quarter of its value at other photoperiods. Even diapause induced in P. interpunctella partly by high population density was effectively terminated by 16-hour photoperiods at 25°C. Exposure to 16-hour photoperiods on only 4 consecutive days at this temperature terminated diapause in over half the larvae in a batch reared in darkness at 20°C (table 31).

In both species, long photoperiod was less effective in terminating diapause at 20°C. Larvae of P. interpunctella were actually stimulated to enter diapause under a 16-hour daylength at 20°C by a 10°C temperature drop from 30°C, and delayed pupation under this daylength more than in batches lowered to 20°C from 25°C.

At 30°C, which is near the upper developmental limit for E. elutella, diapause in both species was terminated in darkness much more rapidly than at 25°C, and the enhancement in rate of termination obtained with long photoperiods was accordingly reduced. Thus the temperature range over which long photoperiods are potentially the most powerful agency for diapause termination is quite narrow..

The effect of a temperature rise during diapause was investigated in both species. In E. elutella, results at first indicated that a temperature rise from 20 to 25°C had itself little influence on diapause termination. In the field stock of P. interpunctella, however, diapause development proceeded faster at 25°C in samples experiencing a temperature rise. For tests on temperature rises, E. elutella was reared under an 8-hour daylength at 20°C, while P. interpunctella was reared in darkness. Investigations on the method of inducing diapause and the subsequent duration, revealed that rearing at 20°C in darkness resulted in less delay of pupation in both species under a 16-hour daylength at 25°C, than rearing at 20°C under an 8-hour daylength. However, batches of P. interpunctella raised to darkness at 25°C from an 8-hour daylength at 20°C, showed a significant decrease in the mean time for pupation when compared with samples reared in darkness at 25°C, thus confirming the positive effect of a temperature rise in terminating diapause in this species. With E. elutella, a temperature rise helps the termination of diapause if larvae are reared in darkness at the lower temperature. Although rearing in darkness increases the readiness of larvae to terminate diapause in response to high temperatures or long daylength, it should be noted that, in both species, the duration of diapause at a particular temperature is longer in darkness than under any light system.

Diapause development (Andrewartha and Birch, 1954) in E. elutella is favoured

by lower temperatures. In the field stock, the duration of diapause was shorter at 15°C than at 20°C under the same photoperiod. Woodroffe (1951) obtained a similar result with Hofmannophila pseudospretella. A further indication of the advantage of low temperature in terminating diapause was that field stock larvae exposed for 10 weeks at 5°C, pupated more readily under a 16-hour daylength at 25°C than larvae held at 20°C. In addition, emergence of the two sexes was better synchronised after the cold exposure (table 41). In the laboratory stock, a 4-week exposure to 10°C favoured diapause termination under a 16-hour daylength at 20°C more than 4 weeks at 2.5°C, indicating that the optimum temperature for diapause development was higher than expected. Two-week exposures at 2.5°C or 10°C only very slightly increased pupation rate at 20°C. Waloff (1949) observed a similar slight acceleration in pupation rate at 25°C after exposing diapausing larvae collected from a warehouse in autumn for 2 weeks at -2°C.

In P. interpunctella, the duration of diapause was not assessed at 15°C, but as in E. elutella, low temperatures favoured diapause development in at least some individuals. Six and ten-week exposures at 10°C increased the rate of pupation under a 16-hour daylength at 25°C. However, the potent action of long photoperiods at 25°C in terminating diapause masked the contribution of the exposure to 10°C. The effect was seen better in samples returned from 10°C to darkness at 20 or 25°C. These completed pupation in a much shorter time than control samples, even when the 10 weeks spent at 10°C was added on. Similar results for the duration of diapause in darkness at 20°C after a cold exposure were obtained by Tsuji (1963). Larvae were exposed to 8 - 10°C for 30 and 60 days, and diapause was terminated at 20°C in about 50 - 125 days and 45 - 75 days respectively.

Not only did the 10-week exposure to 10°C speed diapause development, but also

it lowered the temperature at which long photoperiods were highly efficient in ending diapause. All larvae returned to a 16-hour daylength at 20°C, which is not normally very effective in stimulating pupation, pupated within 25 days.

Tzanakakis (1959) concluded that a 14-week exposure at 10°C was no more effective than a 14-week exposure at 20°C in terminating diapause at 25°C, although the synchronisation of pupation was a little better after the exposure at the lower temperature. The present results indicate that synchronisation after a 6-week or longer exposure to temperatures below the range for complete development is very strongly improved. Individuals which in unchilled samples would terminate diapause after relatively short exposures at 20°C, are held back at the low temperatures, while individuals apparently in a more intense diapause are actively stimulated to speed up diapause development so that they are ready to pupate when temperature is increased. Exposure to 15°C for 6 weeks was less effective in synchronising pupation times. In the laboratory stock, exposure to 15°C for 6 or 10 weeks may even have increased the duration of diapause at 25°C. Such an effect could be explained as complications arising from the nearness of this temperature to the developmental threshold.

The mechanism by which photoperiod terminates diapause in E. elutella and P. interpunctella seems to be independent of the action of chilling, although cold exposures lower the range of temperatures over which photoperiod is effective. Some other species for which photoperiod has been cited as an agent in the termination of diapause will readily continue morphological development after exposure to cold, the effect of photoperiod no longer being significant. The effect of photoperiod is limited in this way in the Saturnid Actias luna (Wright, 1970), but not in the Oak silkworm Antheraea pernyi Guer (Williams and Adkisson, 1964), Lobesia botrana (Roehrich, 1969), Papilio polyxenes (Oliver, 1969) or Chrysoteuchia topiaria (Kamm, 1973).

Strümpel (1969) regarded the induction and termination of diapause in E. elutella as being under the control of photoperiod alone, and described diapause in this species as an oligopause, after the definition of Müller (see Thiele, 1973). However, the present results indicate that low temperatures can initiate diapause, and that chilling reduces the duration of diapause even under long daylength at high temperature. Müller's definition of oligopause would not apply to this situation. Furthermore, the duration of diapause under long photoperiods is dependent on temperature, being over four times longer at 20°C than at 25°C or 30°C.

A minimum temperature exists for sensitivity to photoperiod in diapause termination, and, as in P. interpunctella, a period at low temperature may lower the minimum temperature required for long photoperiods to operate efficiently. A second minimum temperature exists, near the upper developmental limit, for the rapid termination of diapause by high temperature. As with the response to long photoperiods, the second minimum is lowered after exposure to low temperature. This is clearly indicated in the overwintering experiments with E. elutella when samples were removed from an outbuilding at monthly intervals from December to March. In the first year, when samples induced to enter diapause in the laboratory under an 8-hour daylength at 20°C were maintained at 5 - 10°C above ambient in an outbuilding from October, the batch moved in December to darkness at 25°C in the laboratory pupated much more slowly than the batch moved to a 16-hour daylength. Batches moved to darkness at 25°C in January, February and March showed a progressive reduction of about 50 days in the mean time required for pupation. The batch moved in March terminated diapause as rapidly as the batch moved to a 16-hour daylength.

In the second winter, larvae of both laboratory and field stocks were reared

under natural conditions from mid September in an unheated outbuilding. Samples moved to darkness at 25°C in January pupated much faster than samples transferred in December, or than samples moved in January or February the previous year. The lower temperatures encountered this year had evidently enhanced development during diapause. Results for February and March batches were virtually identical, indicating that by this time diapause development at low temperature had been completed. Samples transferred to darkness at 30°C showed little change in pupation rate from January onwards. Waloff (1949) obtained a similar result for samples of E. clutella larvae removed from a warehouse between September 1945 and February 1946. Batches moved in January or later pupated within 60 days at 25°C, indicating the completion of a phase of diapause development. The biggest change in the duration of diapause at 25°C occurred between batches moved in early and mid December. Waloff weighed larvae at intervals during diapause, and concluded that pupation was stimulated by high temperatures after a weight-loss of about 35%. The present results indicate the importance of low temperatures, however, because batches required longer exposures in the slightly heated outbuilding than in the unheated one for rapid pupation at 25°C.

In the heated outbuilding, where temperatures oscillated around 14°C during the winter months, results for the field stock of E. clutella resemble those obtained by Oliver (1969) for the butterfly Papilio polyxenes, in which the duration of diapause at high temperature was inversely proportional to the length of the chilling period. However, most species require a certain minimum period of low temperature before morphogenesis is resumed at higher temperatures (Lees, 1955), and transition from 0 - 100% response may occur with a quite short addition to the minimum period. This situation has been demonstrated in Bombyx mori, an increase from about 12% to 82% termination of diapause in batches of eggs occurring when exposure to 5°C was increased from

40 to 60 days (Muroga, 1951; Lees, 1955). The sudden shortening of the duration of diapause at 25°C between samples of E. elutella moved from the unheated outbuilding in December and in January, supports the idea of a minimum exposure period to low temperature for reactivation in most individuals, and the dependence of the subsequent duration of diapause on the length of a cold exposure may be limited to certain temperatures in the upper part of the range for diapause development. The mean pupation time for the sample removed from the slightly heated outbuilding in February was to some extent artificial because two groups could be separated in the emergence, one of which had presumably completed a phase of diapause development, while the other one had not (fig. 35, table 42).

The emergence of male and female moths in the unheated building was observed during summer after overwintering. Long periods at low temperature improve the synchronisation of emergence, and yet the summer emergence of female moths of E. elutella was rather protracted. This was not observed in samples moved to 25°C in March. The pupation and emergence of E. elutella in the slightly heated outbuilding was even more protracted, showing that lower winter temperatures were at least partly effective in synchronising the termination of diapause. Synchronisation seems to rely on periods at low temperature being followed by a substantial and sustained temperature rise. In both outbuildings, temperatures rarely exceeded 25°C during the summer of 1972.

As diapausing stages of Pieris brassicae and Bombyx mori were to be included for comparative purposes in toxicity tests, some experiments were conducted on the termination of diapause in these species. In darkness, the duration of diapause in pupae of P. brassicae was approximately 250 - 300 days at 10 and 20°C. Diapause was of short duration under a 16-hour daylength at 25°C, and was further halved after a 16-week exposure at 5 - 10°C. An eleven-week

exposure to 5 - 10°C had no effect on the duration of diapause at 25°C. The large difference in the rate of emergence in samples chilled 11 and 16 weeks illustrates the need for a minimum exposure to cold before morphogenesis is readily resumed.

Diapausing eggs of B. mori held at 10°C for 52 weeks all failed to hatch, while at 20°C, diapause lasted about 200 days. After 15 weeks at 10°C, hatch at 25°C occurred within 25 days. After 25 weeks at 10°C, or 12 weeks at 7.5°C, hatch occurred within 18 days at 25°C. These results agree with the data of Muroga (Lees, 1955) which showed 7°C to be the optimum for diapause development in B. mori.

Diapausing stages of E. elutella and P. brassicae which survived fumigation, resumed morphogenesis sooner than control samples placed under similar conditions. In this respect, diapausing stages differed from non-diapausing stages which normally show a delay in their subsequent development after fumigation. The premature termination of diapause after fumigation is a typical response to stress. Many examples of breaking diapause by imposing various adverse conditions are to be found in the literature. Tzanakakis (1959) terminated diapause in P. interpunctella by wounding larvae with a red-hot needle, or by repeatedly destroying the 'hibernaculum' spun by larvae after cessation of feeding. Diapause in the univoltine race of B. mori can be averted by immersing 24-hour-old eggs in 6N hydrochloric acid for 4 - 6 minutes at 44 - 46°C (Nayar and Fraenkel, 1963).

The Physiology of Diapause Development

The data now available on the factors influencing the duration and termination of diapause in E. elutella and P. interpunctella give much information on the control of development during diapause. Firstly, it is apparent that not one

but several environmental factors have the potential ability to stimulate the resumption of morphogenesis, but for maximum efficiency, the actions of all factors need to be combined in a carefully integrated system. Secondly, development during diapause can be divided into various phases, each phase being closely linked to a different environmental factor. Thirdly, the end point of diapause needs to be considered carefully, as it is difficult to divide diapause development from post-diapause development.

Within a population, there is much variation in the reaction of individuals in diapause to a given set of experimental conditions. If an important diapause-terminating factor is absent, the overall time for diapause development is lengthened, and the synchronisation of emergence is reduced. In E. elutella and P. interpunctella, the efficiency of some environmental factors in terminating diapause is increased after exposure to others. In the natural situation, where a period of low temperature and short daylength in winter is followed by long daylength and warmer conditions in spring and summer, the stimuli for diapause development are provided in a set order, and are highly efficient in producing a synchronised post-diapause emergence. With artificial sets of conditions, it is possible to break down the contribution to diapause development of each of the factors characteristic of the period from autumn to spring.

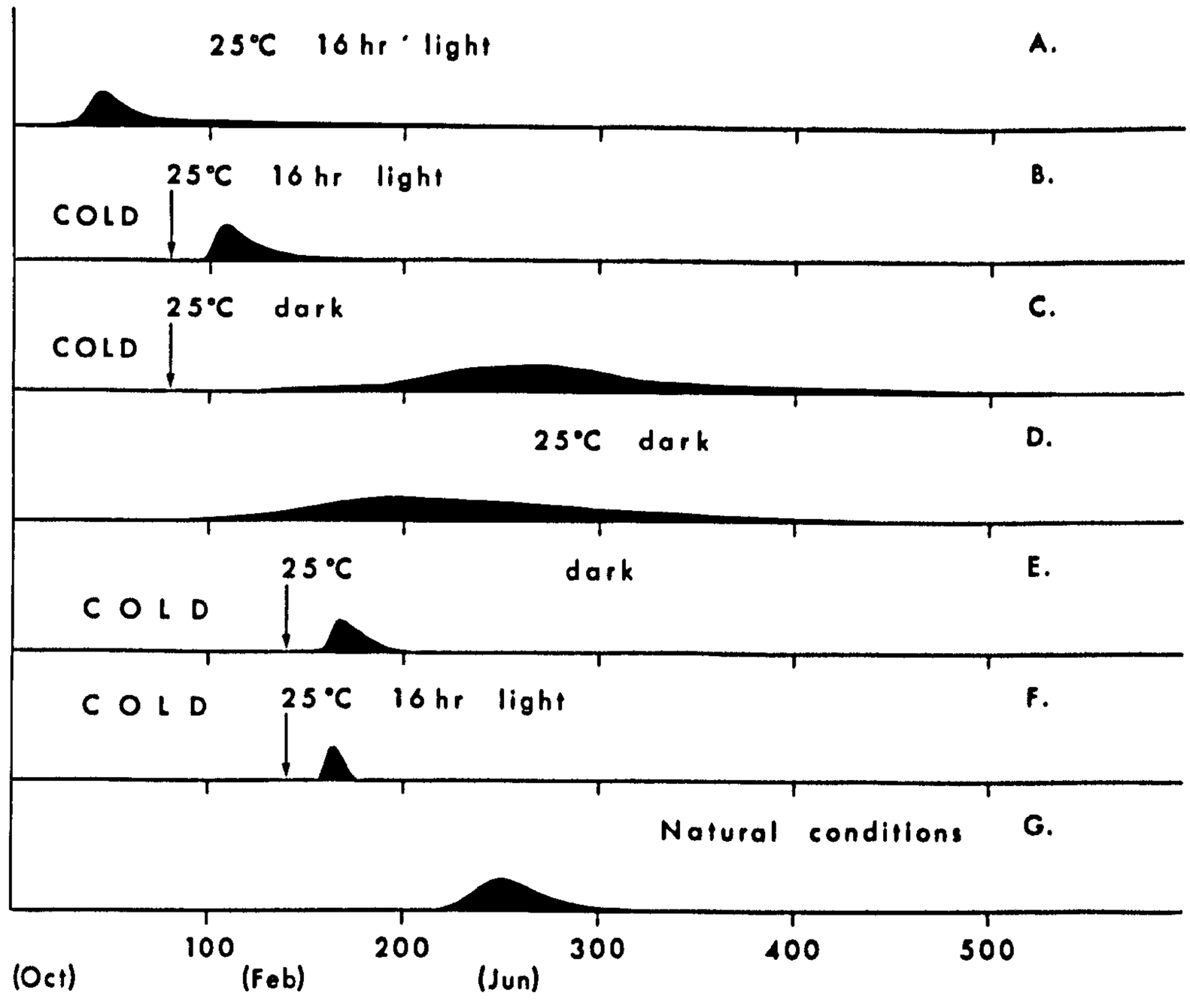
Fig. 49 presents schematic patterns of pupation in batches of the field stock of E. elutella under various sets of conditions. In each model, the scale has been adjusted to depict the range of pupation times rather than the absolute number of insects pupating at a given time. Whether or not a requirement of low temperature represents the first phase of diapause is not certain, as a primary phase may first be completed at higher temperatures, but it is apparent that further phases of diapause development under the

control of long daylength and high temperature occur after the phase at low temperature. After 2 or 3 months at low temperature, pupation is highly synchronised in long daylength at 25°C, much more so than in the absence of a cold period (models A and B in fig. 49). Nevertheless it is clear that this cold exposure does not itself terminate diapause, because batches raised to darkness at 25°C (model C) show little synchronisation of pupation, and do not give very different results from batches placed in darkness at 25°C without any exposure to cold at all (model D). Extending the exposure to cold by about 2 months can reduce the need for long daylength to synchronise pupation, and high temperature alone is sufficient (model E). However, the effect of long daylength in further synchronising pupation is still clearly apparent (model F).

Long daylength is only efficient at higher temperatures. As seen most clearly in the results with P. interpunctella (table 38), exposure to low temperature can decrease the temperature needed for long daylength to be effective. Nevertheless, the rather wider than expected range of pupation times for E. elutella during a cool summer after overwintering in unheated premises (model G) was probably caused by temperatures still being too low for the efficient action of the long-day stimulus in the last phase of diapause development. The ranges shown in models F and G differ too widely to be explained simply by slower post-diapause development at the lower temperature.

It has been commonly been supposed that diapause development can be clearly divided from the periods of quiescence or morphogenesis which follow. In E. elutella, however, it is difficult to divide the last phase of diapause development from the period which may be regarded as post-diapause development, during which the larvae develops towards the pupal stage, the arrival at which is often the first clear indication that diapause has ended. The normal period at 25°C between the cessation of feeding and the completion of the pupal moult

Fig. 49. Models showing the distribution of pupation times in various batches of the field stock of Ephestia elutella to illustrate the contribution of chilling periods, long daylength and high temperature to diapause development.


25°C 16 hr ' light
A.
25°C 16 hr light
COLD
B.
25°C dark
COLD
C.
25°C dark
D.
25°C
dark
COLD
E.
COLD
25°C 16 hr light
F.
Natural conditions
G.
100
200
300
400
500
(Oct)
(Feb)
(Jun)
DAYS FROM ENTRY INTO DIAPAUSE

in non-diapausing insects is about 2 - 7 days. However, even in batches which had overwintered until March, no pupae were obtained at 25°C within this period. In batches returning to long daylength at 25°C after chilling, 2 or 3 weeks elapsed before the first larvae pupated, and even in the most highly synchronised batches, some individuals required over 5 weeks for pupation (table 44).

Although the process of diapause termination may have been triggered, physiologically insects remain in diapause until the enzymes and hormones maintaining diapause are removed and factors appear to stimulate the resumption of morphogenesis. The period of chemical change within the insect may be estimated by subtracting from pupation times the normal developmental period between the mature larval and pupal stages at the temperature concerned. Thus in the overwintering batch returning to long daylength at 25°C in February, which was used to construct model F, the mean period is 20 $\pm$ 5 days if 4 days are allowed for non-diapause development. The elimination of factors maintaining diapause may comprise the whole or part of the later phases of diapause development, and it is this process that in E. elutella is primarily under the control of warm long-day conditions. Strümpel (1964) observed the link between the resumption of neurosecretory activity and long daylength in diapausing larvae of E. elutella, but discounted the contribution of periods at low temperature to diapause development. The present work has identified two discrete phases of diapause development, the first favoured by low temperature, and the second by long daylength and high temperature. Both phases can stimulate morphogenesis independently, but only at the expense of synchronisation in the post-diapause emergence.

TOXICITY OF PHOSPHINE AND METHYL BROMIDE

Tolerance to Phosphine During the Egg Stage.

Ephestia elutella, E. kuehniella, E. cautella, and Plodia interpunctella proved highly tolerant to phosphine during part of the egg stage, but were otherwise quite susceptible. Similar results were obtained with laboratory and field stocks of the same species. The length of the tolerant period was increased as temperature decreased, and occupied the first 30 - 40% of the period for egg development. In exposures longer than the tolerant phase, complete kill of eggs of all species was obtained with very low concentration - time (CT) products. The tolerant phase in E. elutella lasted 2 days at 25°C, 4 days at 20°C, and 8 days at 15°C. In E. kuehniella and E. cautella, it lasted up to 2 days at 25°C, over 3 days at 20°C, and over 6 days at 15°C. In P. interpunctella, eggs were tolerant for 2 days at 25°C, 4 days at 20°C, but at 15°C, although fairly tolerant in exposures up to 7 days, eggs were adversely affected by the low temperature, and were less tolerant than at higher temperatures.

A tolerant period to phosphine during the egg stage is not restricted to the above four Pyralid species. In tests prior to the present project, eggs of the Greater wax-moth Galleria mellonella (L) showed high tolerance to phosphine during early development (Bell and Glanville, 1970). At 25°C, eggs of G. mellonella required about 10 - 12 days for complete development, and the tolerant phase lasted 4 - 7 days. Few other species have been investigated, but Vincent and Lindgren (1972a) testing 0 - 1 day-old eggs of P. interpunctella and Ephestia (Cadra)figulilella with phosphine in 24-hour exposures at 26.7°C, did not observe high tolerance in the latter species, although the LC 95 value calculated from hatch of eggs of P. interpunctella was 1.9 mg/l. The present results suggest that LD values are of little use, as the amount of survival after short exposures is proportional to the number of very young eggs in the

fumigated sample, even within age groups of 0 - 24 hours, rather than to the concentration (tables 49, 60, 61 and 62). There are indications that both laboratory and field stocks of P. interpunctella are more tolerant to phosphine than Vincent and Lindgren's stock, as, at 25°C, survivals from a 24-hour exposure at about 2.6 mg/l (table 62), were 33% and 25% respectively.

Baskaran and Mookherjee (1971) fumigated 0 - 1 day-old eggs of E. cautella in 24-hour exposures at 29 ± 1°C. From their quoted regression equations, LD 50 and 90 values were about 0.13 and 0.20 mg/l respectively. Again, the present laboratory stock was more tolerant, 10 - 24% survival to the adult stage being recorded from concentrations of 0.34 - 0.39 mg/l at 30°C (table 62). In contrast Muthu (1973), assessing mortality by failure to hatch, calculated an LD 99 for eggs of E. cautella of 28 mg/l in a 24-hour exposure at 26.7°C, while the LD 50 was only 1.2 mg/l. Eggs of the Rice moth Corcyra cephalonica were even more tolerant, the calculated LD 99 being 47 mg phosphine/l. The present field stock of E. cautella showed a 52% survival after a 24-hour exposure at about 2.6 mg/l (table 62), but higher concentrations were not tested. The findings of Muthu indicate that during the tolerant phase some eggs may be virtually immune to phosphine.

The relationship known as Haber's rule, whereby the level of kill is proportional to the product of concentration and time, completely breaks down with phosphine. Insects at different stages of development differ widely in tolerance, and development continues during the actual exposure (Reynolds et al., 1967; Howe, 1973). High tolerance results when exposure periods are too short to allow insects to develop from a tolerant stage to one of greater susceptibility. Increased temperature speeds development and so shortens the exposure period required for effective control.

The dependence of mortality on temperature and exposure period was shown by subjecting 0 - 1 day-old eggs to various exposures at 15 - 30°C with CT product held constant. In these experiments, the level of kill at a particular exposure was regulated primarily by temperature (tables 56 and 57). At each temperature, more kill was obtained in longer exposures, and the fact that concentrations were lower in longer exposures was of little consequence until a threshold level was approached in very long exposures at 15°C. This level represented the point at which the concentration was no longer fully effective against the susceptible phase later in the development of the egg. When concentration rather than exposure period was held constant (tables 58 and 59), survival in the longer exposures at 15°C was largely eliminated, but otherwise the pattern of survival was similar to tests with CT product held constant. Both series of tests shared 24-hour exposures at 25 and 30°C, with a concentration of about 0.4 mg/l and a CT product of about 9 - 10 mg h/l. CT products in the series with concentration held constant were of course much higher for all other exposure periods, and consequently more kill was obtained, but again the relative unimportance of concentration was illustrated by the failure of all but the longest exposures at each temperature to achieve 100% control.

Laboratory and field stocks were quite similar in tolerance during the egg stage. The field stock of P. interpunctella was more tolerant to cold than the laboratory stock, and survived better at 15°C. On the whole, the eggs of field stocks were a little more tolerant in later development than those of laboratory stocks, and more survival occurred in long exposures at low concentrations.

The results discussed so far have indicated the position and length of the period during which eggs are tolerant to phosphine at a range of temperatures.

However, the kill obtained after short exposures was not always proportional to concentration, possibly because different levels of susceptibility occur during the tolerant phase, and variation in the exact age of eggs in different groups 0 - 24 hours old may be critical. Howe (1973) found that eggs of Sitophilus granarius aged 2 - 3 or 3 - 4 days at 25°C were highly tolerant to phosphine at a concentration of 4 mg/l for 8 hours. He found that the tolerance of eggs under gas decreased sharply at age 5 days. He also found that the age of the most tolerant group of eggs at the start of fumigation decreased as concentration was decreased and exposure periods were correspondingly increased, and postulated a short phase of relatively high susceptibility early in egg development, probably coinciding with the formation of the blastula.

To find whether or not moth eggs were less tolerant for a short period early in development, batches of 0 - 1 day-old eggs were fumigated with phosphine in exposures including the initial evolution of gas from the solid formulation, and in exposures of similar duration started when the concentration had risen to a maximum level, and was beginning to fall. As no consistent differences were observed in results from the two types of exposure (table 63), it was concluded that a susceptible phase early in development was absent, and that the level of survival was determined by the developmental range within the test sample, and the concentration towards the end of the exposure rather than the gas level at the start of the test.

Tolerance early in the development of the egg has also been observed in many beetle pests of stored products, although the tolerant period is sometimes very short. Lindgren and Vincent (1966) observed that tolerance to 16-hour exposures at 26.7°C was higher in younger eggs of Tribolium confusum. Qureshi et al. (1965) found in the Cadelle Tenebroides mauritanicus (L), that eggs at

25°C, 70% RH were tolerant to phosphine during the first 4 days of development. Barker (1969) found 0 - 24 hour-old eggs of the Rust-red grain beetle Cryptolestes ferrugineus (Stephens) highly tolerant to phosphine in 24-hour exposures at 24°C. Vincent and Lindgren (1972) obtained some survival of 0 - 1 day-old eggs of 3 Dermestids, Trogoderma glabrum (Herbst), T. sternale Jayne, and T. variabile Ballion, exposed to 6 mg phosphine/l in 24-hour exposures at 21.1 ± 1°C. Recent work by Miss B.D. Hole at the Pest Infestation Control Laboratory has demonstrated a tolerant period early in the egg stage of the Lesser grain borer Rhizopertha dominica (F).

High tolerance to phosphine in the egg stage can be regarded as a common phenomenon, and is undoubtedly linked with metabolism during embryology. Phosphine is toxic only in the presence of oxygen (Bond et al., 1967) and stages which can survive without oxygen for a period of time are likely to be tolerant. Oxygen consumption during the egg stage varies with the species under consideration (Wigglesworth, 1972) and at the present time too few data exist to correlate low oxygen consumption and high tolerance to phosphine during the egg stage.

As eggs of Bombyx mori were known to enter diapause very early in development, before segmentation of the embryo (Lees, 1955), a comparison of the toxicity of phosphine to non-diapausing, diapausing, and post-diapausing eggs was included in this study. Surprisingly, diapausing eggs were the least tolerant to phosphine at 25°C, although many eggs in all three groups survived 0.5 mg/l phosphine for 3 days, and all were killed by a 7-day exposure. In this species, therefore, eggs remaining at an early stage of development were by no means immune to phosphine, except in exposures of 2 days or less (table 107). The results with eggs of B. mori may indicate that periods of tolerance to phosphine in moth eggs can be controlled by factors other than simple progressive

development. The toxicity of phosphine has been shown to be proportional to rate of uptake (Bond et al., 1969) and, in addition to having high natural tolerance, younger eggs may be unable to take up phosphine quickly enough to be killed. Much additional work is needed on the rate of uptake of phosphine in eggs and other tolerant stages for such hypotheses to be explored.

Toxicity of Methyl Bromide to Eggs

Apart from diapausing eggs of B. mori, high tolerance during the egg stage was not observed in tests with methyl bromide. Different age groups of Pyralid species did not differ widely in tolerance, and variation between laboratory and field stocks was small. In each age group, a 10°C decrease in temperature from 30 or 25°C approximately doubled tolerance to methyl bromide.

In order to check that the CT relationship known as Haber's rule was operating in tests with methyl bromide, three different concentration levels were tested against three age groups of eggs of E. elutella and P. interpunctella (laboratory stocks) at 15°C. The regression coefficients for mortality on concentration, b_1, and mortality on time, b_2, were found to be similar in each age group, and so logarithmic concentration and time components could be added for conversion of dosages to CT products. However, of the six values for $b_1 - b_2$ (table 66), five were negative, and the single positive value was very small. The possibility that the time component of the CT product was slightly more effective than the concentration component, cannot therefore be excluded. Significant heterogeneity occurring in some of the analyses of combined results for each age group, however, was probably caused by variation within batches, rather than by a failure of Haber's rule, as age ranges of 1 day at 25°C are wide enough to include eggs at quite different stages of development It should nevertheless be noted that the concentration range tested, 4.2 - 10.8 mg/l, was a small one, and these conclusions cannot be applied to concentrations

outside these limits.

Howe and Hole (1966) observed differences between results at 3, 6 and 9 mg/l in tests with methyl bromide on all stages of Sitophilus granarius at 25°C, better results being obtained at the higher concentrations, and hence the shorter exposures. These results indicated that a concentration of 3 mg/l was below the most efficient range for effective control of S. granarius with methyl bromide rather than a generally greater importance of concentration in the CT product. Estes (1965) described an increase in CT product for the LD 50 value against Tribolium confusum as time of exposure was increased, but once again the effect was largely explained by the inclusion within the dose range of a concentration of less than 2 mg/l, which was below the lethal threshold for some members of the test sample. Concentrations of 2 mg/l or below were stated by Brown (1959) to be too low for effective control of a range of beetle pests of stored products. More recent work (Howe and Hole, 1966; Bell and Glanville, 1973) has indicated that this threshold may be higher for some species.

In addition to the lower limit for concentration, there is a minimum for the length of exposure (Knight, 1925). In the present tests with methyl bromide, exposures of 1 hour were apparently above this minimum.

Fumigation of eggs with methyl bromide reveal much smaller variations in susceptibility between different age groups than encountered with phosphine. Also, quite short exposures are effective in producing a kill, and the CT relationship holds fairly well. Results obtained in the current tests differ from those obtained by other workers chiefly because survival has been assessed on the basis of adult emergence. Mostafa et al. (1972) used hatch to calculate LC 50 values of 10.8 - 15.4 mg h/l, and LC 95 values of

25 - 38 mg h/l, for different age groups of eggs of E. kuehniella in 5, 6 and 7-hour exposures at 26°C. Concentrations were, however, determined from the amount of fumigant weighed into 30-l glass bottles, and no samples were taken to assess loss of gas through leakage or sorption. At all exposures, eggs were progressively less tolerant as they aged. In the present work with both laboratory and field stocks, eggs were more tolerant at age 2 - 3 days (at 25°C) than at 0 - 1 or 1 - 2 days, and eggs of all ages required lower CT products for control than in the tests conducted in Egypt.

Phillips et al. (1959) obtained some hatch of E. elutella eggs aged 1 - 2 days at 24°C after a CT product of about 35 mg h/l in an 8-hour exposure. Again, a lower tolerance is shown in the present results based on adult emergence.

Hatch and Adult Emergence After Fumigation of Eggs

Hatch counts were compared with the subsequent adult emergence in tests with methyl bromide and phosphine. The greater the level of kill, the more counts based on hatch underestimated the total mortality. When hatch was reduced to 20% or below, very few or no survivors were recorded at the adult stage. Mortality after hatching occurred predominantly during the first larval instar. Survivors of egg fumigations reaching the adult stage were of normal fertility.

The hatch of eggs was delayed after sublethal doses of phosphine or methyl bromide. With phosphine, the delay was equal to at least half of the period spent under gas. However, development was not greatly slowed during fumigation, because the levels of kill in batches of eggs exposed to phosphine at different ages, are similar if batches are removed from the chamber when reaching a particular age (table 53). The delay probably represents the period required to repair damage suffered under gas. A similar delay, but linked with the level of kill rather than the length of the exposure, was

observed in tests with methyl bromide.

Toxicity of Methyl Bromide and Phosphine to Feeding Larvae and Pupae

Little work has been done on the toxicity of fumigants to later developmental stages of storage moths. The present experiments showed feeding larvae and pupae of all four species to be susceptible to both phosphine and methyl bromide. Shepard and Buzicky (1939) obtained an LD 50 of 5 mg/l in a 5-hour exposure to methyl bromide at 25°C for last instar larvae of P. interpunctella, when mortality was assessed 5 days after fumigation. Harein and Press (1966) found that a dosage of about 4 mg/l was required for 50% kill of second instar larvae of P. interpunctella in 24-hour exposures at 26.7°C. Mortality was assessed 14 days after fumigation, and a dosage of 6 - 7 mg/l was required for 95% kill. Taken at face value, these results imply that second instar larvae are much more tolerant than mature larvae, which is highly unlikely. Fumigations were however conducted in stoppered glass bottles, and gas losses after dosing were unknown.

Phillips et al. (1959) found all stages of E. elutella to show low tolerance to methyl bromide at 24°C, no survival occurring after 24 hours at about 2 mg/l or 12 hours at about 4 mg/l. With phosphine, larvae of E. cautella (Baskaran and Mookherjee, 1971) and P. interpunctella (Lindgren and Vincent, 1966; Vincent and Lindgren, 1972a) showed little tolerance, LD 50 values being less than 1 mg h/l in 24-hour exposures at 26 - 30°C. Pupae of P. interpunctella were a little more tolerant than larvae while adults were more susceptible (Vincent and Lindgren, 1972a).

In the present tests, phosphine was more efficient in longer exposures against pupae, whose tolerance decreased with age after 3 or 4 days of development. The change in tolerance during the pupal stage was not, however, so marked as

in the egg. Pupae aged between 0 and 3 days at 25°C were also more tolerant than older pupae to methyl bromide. In tests on pupae, mortality was far more dependent on temperature with phosphine than with methyl bromide.

Adults emerging from fumigated pupae usually showed lowered fertility. Abnormal weight losses at adult emergence were demonstrated after fumigation of pupae with phosphine, and some adults survived for only a few hours. The results underline the importance of investigating fumigation survivors in evaluating the effectiveness of a fumigant.

Tolerance of Diapausing Stages

Larvae are much more tolerant to fumigation when in diapause. Reynolds (1961) testing diapausing larvae of P. interpunctella with methyl bromide at 25°C, obtained a 16% survival after a CT product of 100 mg h/l, and considered that for 99.9% kill, a CT product of 200 - 300 mg h/l could be required. Results were based on larval mortality and not on survival to the adult stage. Sardesai (1968; 1972) found larvae of P. interpunctella in diapause to be only 1.8 times as tolerant to methyl bromide as other larvae at the LD 50 level. Basing results on mortality counts after 5 days, an LC 95 of 58 mg h/l was obtained with 4-hour exposures at 26.7°C.

In the present tests, diapausing larvae of E. elutella were more tolerant to both methyl bromide and phosphine than those of P. interpunctella, and diapausing larvae of field stocks were more tolerant than laboratory stocks.

Diapausing larvae of both stocks of P. interpunctella and E. elutella were quite susceptible to phosphine, but were nevertheless more tolerant than other stages in all exposure periods long enough to span the tolerant phase in eggs. As metabolism during diapause is relatively stable, large changes in

susceptibility would not be expected during long exposures. Larvae of E. elutella that had been in diapause for 3 or 4 weeks, or 2 or 3 months, were not significantly different in susceptibility to either phosphine or methyl bromide. Nevertheless, phosphine was more effective in longer exposures, except in one test when the concentration fell below 0.03 mg/l, and survival increased considerably (fig. 46). The possibility that factors other than development are responsible for the efficiency of longer exposures has already been mentioned in relation to eggs. Diapausing stages, like eggs in early development, are likely to be tolerant to periods of anoxia, and the rate of uptake of phosphine may be slow. Recent work at the Pest Infestation Control Laboratory by K.A.Mills has shown that, in addition to the moth species, at low temperatures diapausing larvae of Trogoderma granarium are more tolerant to phosphine than other stages in the life cycle.

Because of the variation of the CT product required for a particular level of kill according to the concentration of phosphine and exposure period tested, the LD 50 for diapausing larvae of E. elutella has since been observed over a wide range of concentrations and exposures. The results of the first series of tests at concentrations between 0.02 and 1.4 mg/l at 20°C (Bell and Glanville, 1973), indicated that up to 0.27 mg/l, CT products could be used to express dosage because the LD 50 increased quite slowly with increasing concentration. When higher concentrations were considered, the CT product for the LD 50 increased sharply, and the level of mortality became dependent only on the length of the exposure period. Mortality was also dependent on exposure time rather than concentration in the test on P. interpunctella with 0.2 mg/l at 15°C (table 84).

When concentration was held constant in tests with phosphine against diapausing larvae of the field stock of E. elutella, temperature greatly influenced

tolerance, and between 15 and 30°C, a 10°C rise approximately halved the CT product required for a particular level of kill. The laboratory stocks of both E. elutella and P. interpunctella changed little in tolerance between 20 and 25 or 30°C (fig. 47). This is almost certainly a peculiarity caused by laboratory rearing for many years at constant temperature. The laboratory stock of E. elutella, a species highly tolerant to cold in nature, was also much more tolerant to phosphine at 15°C than at 10°C. Surprisingly, the field stock of P. interpunctella to some extent gave a similar result, but at 10°C, only one test was performed. The wide difference in the responses of laboratory and field stocks to phosphine at various temperatures illustrates most clearly the need for results of toxicity tests conducted on laboratory stocks alone to be treated with caution. However, differences are not likely to be revealed by testing laboratory and field stocks at the laboratory rearing temperature.

In tests with methyl bromide, in which exposure periods are generally shorter than in tests with phosphine, differences in the pattern of tolerance at various temperatures between laboratory and field stocks, were less obvious. At concentrations between 4.0 and 10.3 mg/l, the CT product required for a particular level of kill was not greater in longer exposures than in shorter ones. Diapausing larvae of E. elutella proved more highly tolerant to methyl bromide than any other insect commonly infesting stored products, with the possible exception of diapausing larvae of T. granarium (Reynolds, 1956).

Temperature did not influence tolerance to such a great extent as in tests with phosphine or in tests with methyl bromide against eggs. At the LD 50 level, both stocks, but particularly the laboratory stock, changed little in tolerance between 15 and 25°C, but at higher LD levels (table 97), tolerance reduced sharply when temperature was increased from 15 to 20°C.

Such results indicate a very large variation in the response of individuals to temperature. At 10 and 15°C, in each stock results were similar at each LD level.

Diapausing larvae of P. interpunctella were more sensitive to temperature change at the LD 50 level and, at higher LD levels, those of the field stock, in contrast to the results with phosphine, were far more tolerant at 10°C than at 15°C (fig. 48, table 98).

In E. elutella, neither the method of diapause induction, nor the method of diapause termination, was demonstrated to affect tolerance to methyl bromide or phosphine. In P. interpunctella, a difference was demonstrated between the tolerance of diapausing larvae prepared under an 8-hour daylength, and others prepared at high rearing densities, when fumigated with 4 mg/l methyl bromide at 25°C (table 99).

Action of a Fumigant Mixture

Because it was apparent that diapausing larvae were tolerant to methyl bromide but not to phosphine, while eggs were tolerant to phosphine but not to methyl bromide, a fumigant mixture was tested against both stages together. The fact that different stages are tolerant to each fumigant suggests that phosphine and methyl bromide act in different ways. This view is supported by the work of Bang (1966) who observed that phosphine increased and methyl bromide decreased the uptake of oxygen. However, results with the fumigant mixture showed that the two fumigants had a strong additive effect, indicating a common mode of action. The mixture was not observed to be synergistic, although if the fumigants did act independently, each may have behaved synergistically by sensitising the centres acted on by the other, thus increasing the level of kill to resemble that for an additive rather than

an independent mode of action. However, the most likely explanation for the results is that the two poisons are in fact additive, each having many modes of action, most of which are to some extent shared.

Other Experiments on Diapause and Fumigation

Experiments on adults of normal appearance after fumigation of diapausing larvae showed that, with methyl bromide, fertility was slightly lowered, while no effect was demonstrated with phosphine. Diapausing larvae surviving fumigation are thus much less likely to produce infertile adults than are pupae, and the level of the adult emergence may be taken as a realistic guide to the success of a fumigation. The termination of diapause was hastened by fumigation, and therefore survivors from diapausing larvae differed from other stages in emerging earlier rather than later than control samples. It may be inferred that unsuccessful fumigations may not only fail to kill pests, but also may speed the increase of an infestation if the temperature is favourable for moth development, by hastening and synchronising the emergence of a diapausing population.

To further assess the effect of diapause on fumigation, tests were performed on diapausing pupae of Pieris brassicae and on diapausing eggs of Bombyx mori. Both stages proved tolerant to methyl bromide and phosphine, indicating that tolerance to fumigation when in diapause was not restricted to larvae. At 10°C, both stages survived long exposures to phosphine, and hatch of diapausing eggs of B. mori was not lowered after exposure to methyl bromide at 4.8 mg/l for 48 hours. Pupae of P. brassicae were not tested with methyl bromide at 10°C, but were much more tolerant than eggs of B. mori at 25°C. A CT product of 403 mg/l was required for complete control, whereas pupae which did not enter diapause succumbed to a CT product of 60.5 mg h/l at 20°C. Currently, diapausing pupae of P. brassicae are the most tolerant of insect stages tested

with methyl bromide, and are rivalled only by the hypopi of certain mites (Brown, 1959).

METHODS OF CONTROL

Diapause

The importance of diapause in increasing tolerance to fumigation has been clearly indicated. Much information has been gathered on the factors controlling diapause in stored product moths, and possibilities for using some of these factors to the insect's disadvantage can be explored. The manipulation of photoperiod to reduce overwintering populations has already been proposed for some crop pests (Hayes et al., 1970; Sullivan et al., 1970). The storage environment offers further possibilities for the control of diapause. In heated premises, diapause induced by shortening daylength in the autumn can be avoided or terminated if long-day conditions provided by artificial light can penetrate to situations where moths breed. It is true that populations may then build up at a faster rate than usual, but there is a much better chance of a successful fumigation with methyl bromide in the absence of diapause. The alternative possibility in heated premises of encouraging diapause at all times of year by artificial short daylength, and hence reducing population increase, is limited by the difficulty of completely shielding the environment from natural daylight, which even at very low intensity will encourage development without diapause during summer. Furthermore, there is a tendency for populations to isolate a non-diapausing strain if kept continually at temperatures high enough for breeding.

In unheated premises, it would be difficult to prevent the autumn induction of diapause and render the population cold-susceptible, because falling temperatures bring about diapause, even in long daylength. The cost of heating large premises would prove prohibitive, and even if diapause was

successfully avoided by a long period at high temperature and long daylength, winter conditions would not normally be severe enough to prevent survival of larvae feeding in the food bulk, and fumigation would still be necessary. Heating for a few weeks would be of much greater value in the spring, when a highly synchronised emergence could be produced after the long exposure of diapausing larvae to winter conditions. Fumigation with methyl bromide should then be very successful against pupae, adults, and eggs, a CT product of 50 mg h/l being adequate at 25°C or above, and 80 mg h/l at lower temperatures.

An alternative method for controlling the post diapause emergence is the use of dichlorvos sprays or vapours (Green et al., 1966; Press and Childs, 1966; Schulten and Kuyken, 1966). An oil mist containing dichlorvos sprayed nightly has been shown to give good control of adult E. elutella, and had the advantage of avoiding contact with warehouse staff (Green et al., 1968).

Fumigation

The present work has provided much data on the toxicity of methyl bromide and phosphine to moth species, but information on other fumigants is still lacking. Of the two fumigants considered, phosphine is effective against all stages in long exposures at high temperatures, while methyl bromide is effective against all stages other than those in diapause, which require CT products too high for the safety of the treated commodity. A mixture of these two fumigants would be highly effective, but is not at present an economic proposition for large-scale use because chemical properties and modes of application differ widely.

An attempt has been made in table 108 to give fresh guidance on the treatment levels of phosphine and methyl bromide required for control of various groups of insect pests of stored products based on the present work on moths, and on

work done by others at the Pest Infestation Control Laboratory on beetles. Dosages for methyl bromide are expressed as CT products, while for phosphine they are expressed as days of exposure at 0.2 mg/l. With methyl bromide, the CT product listed is the calculated result in free space after sorption and leakage have taken their toll of the dose originally applied. It should be noted that CT products of over 200 mg h/l are not always practical, as at these levels some commodities are liable to tainting or residue problems. Furthermore, any periods when the free-space concentration is 3 mg/l or below may not be effective, and it is not known to what extent temperature affects this level. The exposures listed for phosphine should not require adjustment unless the free-space concentration falls below 0.1 mg/l during the first 8 days, or 0.05 mg/l thereafter.

TABLE 108. Dosages recommended for complete control of different groups of insect pests of stored products by fumigation with phosphine or methyl bromide.

GROUP NO. INSECTS (ALL STAGES INCLUDING THOSE IN DIAPAUSE)

1 Pyralid moths other than Ephestia elutella
2 Beetles other than Trogoderma granarium and (phosphine only) Sitophilus spp.
3 All moths tested
4 All beetles tested
5 All moths and beetles tested

TEMP °C	GROUP No.	PHOSPHINE (days at 0.2 mg/l)	METHYL BROMIDE (CT products : mg h/l)
25 and Above	1	3	50
	3	3	125
	4*	8	180
	5	8	180
15 - 20	1	9	70
	2	14	180
	3	10	300
	4	21	200/
	5	21	300
10 - 15	1	16	80
	2	16	200
	3	16	300
	4	21	300/
	5	21	300

* Data not available for diapausing larvae of Trogoderma granarium.

/ Tolerance of larvae of T. granarium can be further increased by starvation or by provision of crevices (Reynolds, 1956).

SUMMARY

1. Studies on the developmental limits of 4 stored product moths showed that newly laid eggs of P. interpunctella were more susceptible to low temperatures than the three Ephestia species. In all species, field stocks were much more tolerant to low RH than laboratory stocks.

2. The percentage yield of adults from cultures set up with eggs was determined by the area of the food surface rather than by the food volume or mass. Yield in cultures of P. interpunctella, E. kuehniella and E. elutella was markedly lowered with 8 larvae/cm^2, and in cultures of E. cautella, with 4 larvae/cm^2.

3. Temperature rises of several ^{o}C were demonstrated in dense cultures of all species, and were correlated with the number of larvae hatching from eggs added.

4. Diapause was induced in larvae of E. elutella by rearing at 15 or 20°C, or in short daylength or darkness at 25 or 30°C. The laboratory stock, which had been reared for many generations at 25°C, did not enter diapause at 25°C, and required short photoperiods to enter diapause at 20°C.

5. Diapause was induced in larvae of P. interpunctella by rearing in darkness or short photoperiods at 20 or 25°C, or by dropping larvae from 25 or 30°C to 20°C during development. Rearing at high densities encouraged the induction of diapause. As in E. elutella, the laboratory stock of P. interpunctella did not enter diapause at 25°C.

6. Larvae of both E. elutella and P. interpunctella avoided diapause when reared in long photoperiods less than 1 lux in intensity, indicating a very high sensitivity to light.

7. Long daylength, high temperatures and periods at low temperature were important in terminating diapause in P. interpunctella and E. elutella. Two phases of diapause development were distinguished, the first favoured by low temperature and the second by long daylength and high temperature. One of the effects of a cold exposure was to lower the minimum temperature for the termination of diapause by long daylength.

8. In P. brassicae, the termination of diapause under a 16-hour daylength at 25°C, was hastened by 16 weeks, but not by 11 weeks, at 5 - 10°C.

9. Fumigation hastened the termination of diapause in E. elutella and P. brassicae. In contrast, non-diapausing stages show a delay in development after fumigation.

10. Eggs of P. interpunctella, E. cautella, E. kuehniella and E. elutella were highly tolerant to phosphine for the first 30 - 40% of the developmental period. Other stages were quite susceptible, although diapausing larvae of E. elutella were more tolerant than feeding larvae or pupae.

11. Phosphine was more efficient against all stages in longer exposures, and the lower the temperature the longer was the exposure period required for effective control.

12. For phosphine to be effective against diapausing larvae of the field stock of E. elutella at 25°C, a minimum concentration of 0.03 mg/l was required.

13. Diapausing larvae were highly tolerant to methyl bromide, while other stages proved quite susceptible. Diapausing larvae of E. elutella were more tolerant than those of P. interpunctella, and diapausing larvae of field stocks were more tolerant than those of laboratory stocks.

14. With both fumigants, the difference in susceptibility between diapausing larvae of laboratory and field stocks was less apparent at some temperatures than others. Rearing under constant conditions had altered the effect of temperature on susceptibility in laboratory stocks, underlining the importance of using wild stocks in toxicity studies.

15. Haber's rule was verified for methyl bromide over a restricted range of concentrations. Tests were conducted on eggs at 15°C.

16. With larvae of E. elutella, the method of diapause induction or termination, or the length of time samples had been held in diapause before fumigation, did not significantly affect susceptibility to fumigants.

17. In P. interpunctella, diapausing larvae reared under high population pressure were more susceptible than larvae reared at low density.

18. Diapausing eggs of B. mori and diapausing pupae of P. brassicae were highly tolerant to phosphine and methyl bromide. Diapausing pupae of P. brassicae have the highest tolerance to methyl bromide of all insect stages so far tested.

19. Practical recommendations for fumigant dosages have been prepared for various groups of insect pests, illustrating the need for fumigation temperatures to be high for effective control. At 15°C or below, CT products of 300 mg h/l are required for complete kill of diapausing larvae with methyl bromide, while with phosphine at 10°C, a 16-day exposure is needed for complete control of moth eggs.

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