

BIOLOGICAL CONTROL
OF
THE GLASSHOUSE MEALYBUG
USING
PARASITIC HYMENOPTERA

BY

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ABSTRACT

A combination of laboratory and glasshouse studies was used to evaluate the potential of three hymenopteran parasitoids, Anagyrus pseudococci (Girault), Leptomastix dactylopii Howard and Leptomastidea abnormis (Girault) as biological control agents for the mealybug Planococcus citri (Risso).

Laboratory investigations using a series of constant temperatures in the range 18-35°C, revealed a profound effect of temperature on the reproductive performance of the parasitoids and their host. The developmental period of both was found to decrease with rising temperature. Oviposition by the parasitoids increased up to 30°C and declined thereafter, whilst the number of eggs laid by the mealybug was severely reduced at 30°C. The searching efficiency of the parasitoids increased with rising temperature within the range studied. Two distinct phases of searching behaviour were identified. In the absence of host cues the area covered by the parasitoids increased with rising temperature, but within host patches searching was found to be independent of temperature. Adult longevity was found to decrease at high temperature for fed, virgin individuals, with the survival curve changing in shape from type II at lower temperatures to type III at 30°C.

A temperature driven model of development times, based on best fit, high order, polynomial regression lines was used to predict development of the pest and its parasitoids under fluctuating conditions. Predictions were tested against actual developmental periods observed at different times of year in the glasshouse and found to be in good agreement.

Host plant appeared to be a critical factor in determining the success of the parasitoids in suppressing mealybug populations in the glasshouse.

L. abnormis was found to be established and contributing to control of the mealybug on three out of five sample plants, with parasitism up to 60% during the summer and autumn. None of the parasitoids appeared to be able to prevent mealybug populations from building up to damaging levels on plants of the genera Passiflora and Aristolochia. Possible reasons for this are discussed.

L. abnormis appears to be better adapted to temperatures favourable to the mealybug and its success over the other two parasitoid species in the glasshouse is examined within this context.

ODE TO A MEALYBUG

P. citri i.e. mealybug,
Resembles most a waxy rug,
The reason, you will see, no doubt,
It is to keep insecticides out.
But Anagyrus pseudococci
Mounts P. citri like a jockey,
Oviposits in her tummy
And turns the rug into a mummy.

Gillian M. Beattie 1984

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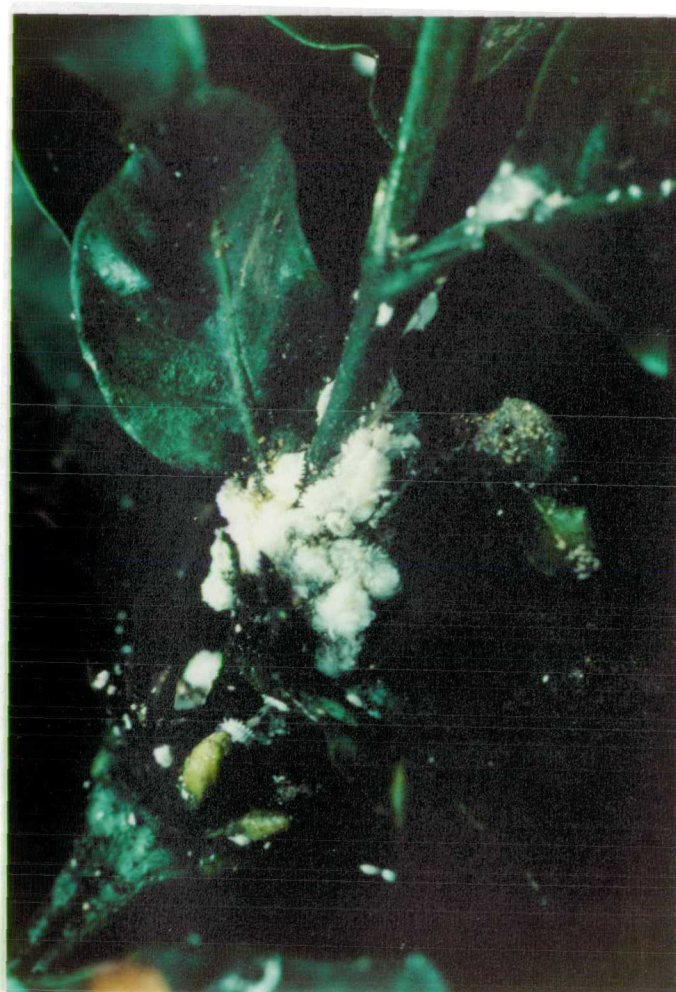


PLATE 1. The glasshouse mealybug, Planococcus citri (Risso), on shoot of Coffea arabica.

GENERAL INTRODUCTION

Planococcus citri (Risso) [Homoptera: Pseudococcidae] is a mealybug of tropical and sub-tropical origin, which has become established as a pest in glasshouses and areas of protected cultivation throughout the temperate regions (see plate 1). It attacks a wide range of ornamental plants, causing damage by sucking plant sap and by soiling the foliage with the honeydew which it secretes. This honeydew provides an excellent growth medium for sooty moulds, which cover affected areas of the plant with a grey/black deposit. Ornamental plants are grown for their attractive flowers, fruit or foliage and their high commercial value is dependant on their appearance. The damage caused by mealybugs can seriously detract from the aesthetic (and thus the economic) value of the plant. Hence the need for control (Pritchard, 1949).

Chemical control has been standard practice in the field, but is largely unsatisfactory. Mealybugs are protected by a waxy coat and tend to congregate in inaccessible parts of the plant, which are difficult to reach with sprays (Dean, Hart and Ingle, 1971, French and Reeve, 1979, Meyerdirk, French, Chandler and Hart, 1981). Insecticides induce phytotoxic responses in many ornamental plants and also cannot be used in areas where the public have free access. Alternative control measures are therefore desirable. In glasshouses the mealybug finds itself in a favourable environment, freed from the restraints which would normally be imposed upon its population growth by natural enemies. It is therefore an excellent candidate for classical biological control, using introduced predators and/or parasitoids. The protected environments necessary for the propagation and maintenance of ornamentals are also favourable for natural enemy introductions and thus biological control is an obvious possibility (Copland, 1983).

Investigations into the biological control of P. citri at Wye college began in 1977, with the introduction of the predator Cryptolaemus montrouzieri (Mulsant). This coccinellid was released into the College conservatory, which contains a number of tropical crop and ornamental plants subject to mealybug attack. The predator was successful in reducing heavy infestations of the pest, but was ineffective at low host density. Despite the presence of alternative food sources (eg. scale insects), its populations tended to dwindle, allowing the pest to resurge. Similar results have been reported by other workers using C. montrouzieri (Whitcomb, 1940; Panis and Brun, 1971), but there is evidence to suggest that when the predator is used in combination with the parasitoid Leptomastix dactylopii, excellent control of P. citri can be achieved (Doutt, 1952). Parasitoids tend to be better adapted to maintaining their populations at low host densities than are predators, due to their reduced prey requirements, host specificity and high mobility (Debach, 1964).

The aim of this study was to evaluate the potential of three hymenopterous parasitoids Anagyrus pseudococci, Leptomastidea abnormis and Leptomastix dactylopii as biological control agents for P. citri, using a combination of laboratory studies and glasshouse trials. This approach has been recommended for the investigation of natural enemies for use in glasshouses, although there is considerable dispute as to the relative importance of pre-introduction research and trial-and-error releases (van Lenteren, 1980).

The objective of the glasshouse trials was to monitor the establishment of the parasitoids, their effects on mealybug populations and their ability to stabilise and enhance control provided by C. montrouzieri.

Previous use of C. montrouzieri has revealed a distinct seasonality in its ability to control P. citri and it has been suggested that it is ineffective at temperatures below 70°F (21°C) (Whitcomb, 1940). As all the parasitoids are tropical or sub-tropical species, it is likely that temperature will also be a major constraint on their efficiency. The aim of the laboratory studies was to determine the influence of temperature on the biology of the parasitoids and their host, in order to build up an understanding of the influence of temperature on their population dynamics. The intention was to use the results of this research to reveal ways in which the glasshouse temperature regime could be set to favour population growth of the parasitoids over that of their host. Energy conservation is now a major consideration in protected cultivations and the aim was to improve the performance of the natural enemies, without increasing energy consumption and heating costs to the grower. Such manipulation of the glasshouse environment may prove a useful tool in assisting biological control in protected cultivation.

SECTION 1: REVIEW OF THE LITERATURE

SECTION I: REVIEW OF THE LITERATURE

1.1 BIOLOGICAL CONTROL

1.1.1 Theory and scope

Biological control has been defined by DeBach (1964) as : "... the action of parasites, predators and pathogens in maintaining another organism's density at a lower average than would occur in their absence". It is a natural, ecological phenomenon - biotic agents regulating the numbers of plants and animals to prevent the normal tendency of populations to increase in an exponential fashion. When applied successfully by man to a pest control problem, it can provide a relatively permanent, harmonious and economical solution (van den Bosch and Messenger, 1973). There are several ways in which biological control can be applied, but they fall into two major categories

- 1) Biological control by augmentation - the activities of endemic or introduced natural enemies may be enhanced by conservation practices (for example, manipulating environmental factors), or by augmenting natural enemy populations, through culture and release of natural enemies (Rabb, Stinner and van den Bosch, 1976).
- 2) "Classical" biological control - through the importation, release and establishment of exotic natural enemies (van den Bosch and Messenger, 1973).

In principle, biological control can be applied to any organism and some organic products. Viruses, bacteria and their toxins, fungi and other microbial pathogens, nematodes, snails, insects, mites and some vertebrates have all been used in biological control programmes. Either singly or in combination, these control agents have been used to tackle problems

involving pest insects, mites, snails, vertebrates, plants of many sorts and even cattle dung! (Wilson and Huffaker, 1976).

Cherret, Ford, Herbert and Probert (1971) include autosterilisation within their discussion of biological control, but generally autosterilisation should be classed along with genetic manipulation, the use of pheromones, breeding for resistance and cultural practices, as a distinct group of biologically based control strategies (van den Bosch and Messenger, 1973 , Price-Jones and Solomon, 1974; Jacobson, 1975; Wilson and Huffaker, 1976). Much of the biological control attempted to date has dealt with insects (DeBach, 1964) and the term is often used in its most narrow sense to describe the use of entomophagous insects in pest control.

The first successful example of this type of biological control was the control of the cottony cushion scale, Icerya purchasi Mask., by the vedalia beetle, Rodolia cardinalis (Mulsant) in California. The success of this coccinellid in saving the Californian citrus industry and subsequently bringing about control of the same pest in 25 countries around the world, serves to show how effective this type of biological control can be.

Reviews of other successful examples of classical biological control abound in the literature and need not be repeated here (Sweetman, 1935; Clausen, 1951; 1958; Franz, 1961, Guerrero, 1969, DeBach, Rosen and Kennett, 1971, van den Bosch and Messenger, 1973; DeBach, 1974; Laing and Hamai, 1976, Samways, 1981; Caltagirone, 1981)

The discussion as to how successful biological control has been and will be, is a continuing and sometimes heated one (Clausen, 1951, 1958, Taylor, 1955; Turnbull and Chant, 1961; DeBach, 1964, Douth, 1967, Huffaker, Messenger and DeBach, 1971, Price-Jones and Solomon, 1974, van den Bosch, Messenger and Gutierrez, 1982). It has by no means been resolved, but

figures for the rate of success in bio-control projects of 16% total success and 58% partial success are impressive (Hall, Ehler and Bisabri-Ershadi, 1980). The figure for the rate of success in turning a candidate chemical into a commercially used product is 0.02% (DeBach, 1974, van Lenteren, 1980). Considering that expenditure on biocontrol research to date has been only about one-thousandth of that on pesticide research and usage (Wilson, 1974), the prospects for biological control appear good given adequate funding.

Very few attempts have been made to estimate the financial benefits of biocontrol and indeed this is extremely difficult, due to the large number of variables involved (Samways, 1981). However, cost analysis of biological control of the Comstock mealybug showed that successful biological control was also an economic success (Ervin, Moffitt and Meyerdirk, 1983). When economic estimates have been made, the figures have always been substantial. For example, the control of the armyworm Mythimna separata (Walker) in New Zealand by a parasitic wasp, led to savings estimated to be in excess of U.S.\$10,000 000 during 1974-1975 (Mohyuddin and Shah, 1977). A cost-benefit analysis of the biocontrol of rhodesgrass mealybug done by Dean and co-workers in 1979, estimated benefits of U.S.\$177,000 000 annually to the Texas economy (Caltagirone, 1981), and savings from major biological control programmes in California over a 36 year period (up to 1959) were thought to be over U.S.\$110,000 000 (DeBach, 1964). A fairly extensive analysis of the costs and benefits of operations undertaken by the Commonwealth Institute for Biological Control demonstrates an economic return in excess of 330% on annual expenditure up to 1973, and indicates that "there is little reason for the hesitancy with which decisions to engage in biological control are approached" (Huffaker, Simmonds and Laing, 1976).

Even more difficult to assess on a financial basis, is the importance of endemic natural enemies in biological control. It is virtually impossible to put a figure on the value of this type of naturally occurring biological control, as it is basically preventative. Nonetheless, many authors argue that it is much undervalued, and that conservation and augmentation of natural enemies is a practical method of pest control in many cases (Hagen, van den Bosch and Dahlsten, 1971; Rabb, 1971, MacPhee and MacLellan, 1971, van den Bosch and Messenger, 1973, DeBach, 1974, Wilson, 1974, Ehler and van den Bosch, 1974, van den Bosch, 1975, Rabb et al., 1976, Wilson and Huffaker, 1976, Shumakov, 1977, Starler and Ridgway, 1977; Knipling, 1977, Ehler and Miller, 1978). Either method of applying biological control avoids the pesticide induced problems of environmental pollution, pollinator mortality, phytotoxicity, pest resurgence, secondary pest outbreaks, pesticide resistance, and threats to human health. The long term benefits in preventing these cannot be measured in purely economic terms (van den Bosch et al., 1982).

Biological control has the disadvantage of being slow in producing results, whereas chemicals act quickly and so tend to be used in solving immediate problems (Samways, 1981). However, using insecticides as an emergency measure in this way has "...resulted in no final solution to pest problems, rather repeated applications have been necessary in order to maintain control. Such repetition of treatment may indeed serve in time to intensify the problem.... through the development of pesticide resistance... and the reduction in natural enemy populations..., the emergency thus becomes self-perpetuating" (DeBach, 1951). In the long term the use of chemicals in this way will not be possible, unless the use of biological control spreads sufficiently to release insects from the selection pressure to develop pesticide resistance. The lesson to be learnt from this is not that the use of chemicals should be stopped

entirely, but that it should be restricted to essential cases. The use of selective chemicals or carefully timed application in these instances would help minimize the drawbacks (Ripper, 1944; Pickett, 1949; Ripper, Greenslade and Lickerish, 1949; Ripper, Greenslade and Hartley, 1951). This realisation provided the initial basis for the development of integrated control. From a rather simplistic beginning, integrated pest management (I.P.M.) has now evolved into a system that incorporates the use of all suitable, compatible techniques in order to manipulate pest populations so that they are prevented from causing economic injury (Smith and van den Bosch, 1967). As a practical strategy it is still in its infancy, as its application requires a thorough understanding of the ecology of the agro-ecosystem in which it is to be used. The indications are that this type of understanding will be the basis of successful pest control in the future.

There seems little doubt that (in the words of DeBach, 1974) "... the empirical and unilateral use of chemicals to attempt to hammer pests into submission by repeated costly blows, is increasingly failing to provide a solution." This being the case, biological control as a component of integrated pest management, is going to be increasingly important in the future (DeBach, 1964, Doutt, 1967, Cherrett et al., 1971; van den Bosch and Messenger, 1973).

1.1.2 Biological control in glasshouses

The controlled and protected environment of the glasshouse is designed specifically to provide optimum conditions for plant growth. In doing so it also provides an ideal situation for pests to exploit their hosts. The factors which contribute to this (even, monocultural stands; equable environment; physical protection; provision of over-wintering sites; confinement and prevention of dispersal) also favour the use of biological control in glasshouses (McLeod, 1939; Wyatt, 1974). Speyer (1927) was the first to exploit this potential, when he used the parasitoid Encarsia formosa Gahan to control the whitefly Trialeurodes vaporariorum (Westwood). The methods used at this time were rather haphazard and with the arrival of effective synthetic insecticides in the 1940's, interest in biological control declined and chemical control became the norm (Hussey and Bravenboer, 1971; Greathead, 1976). However, the ecological isolation of the glasshouse environment provides problems in the use of chemicals, due to the selection pressure induced by repeated pesticide applications on a captive population (Hussey and Scopes, 1977). The red spider mite Tetranychus urticae Koch soon responded to this pressure, becoming resistant to a number of acaricides, and it was the need to control this pest which led to renewed interest in biocontrol in the late 1950's (Hussey and Bravenboer, 1971, Vet, van Lenteren and Woets, 1980, van Lenteren, Ramakers and Woets, 1980).

The success of the programme against T. urticae using the predatory mite Phytoseiulus persimilis Athias-Henriot, developed by the Glasshouse Crops Research Institute (G.C.R.I.), the susceptibility of this predator to chemicals, and the rediscovery of E. formosa to control whitefly, encouraged the search for biological control practices for other major pests (Vet et al., 1980). The search intensified as the reduced

insecticide pressure, associated with the increase in the use of biological control, allowed previously minor pests to build up.

In the U.S.A. successful control of mealybugs on ornamental plants was achieved using a combination of predators and parasitoids (Doutt, 1951; 1952). Some success was also reported against several species of mealybug in France (Panis and Brun, 1971). These examples are discussed in more detail in section 1.3.3.

In the U.S.S.R., experiments on biological control under glass have been widespread. As well as success with P. persimilis, the lacewing Chrysopa carnea Stephens has been used in an integrated programme against aphids. A Cecidomyid, Aphidoletes aphidimyza (Rondani) commercially controlled aphids in 230,000 m² of hothouse area (Beglyarov and Smetnik, 1977).

In Finland, biological control is reported to be widely popular, the use of P. persimilis, E. formosa, and A. aphidimyza (against spider mite, whitefly and aphids respectively), being the normal practice in glasshouse vegetable culture. On ornamental crops integration with chemical techniques has been necessary (Markkula and Tiittanen, 1980).

Greathead, (1976) reviewed attempts at biological control throughout Europe, reporting trials against aphids, thrips, Tomato moth Lacanobia oleracea^(L), and Chrysanthemum leaf-miner Phytomyza syngenesiae^{Hardy}. The results were often encouraging, though none of these could be controlled on a commercial scale. However, a programme integrating the use of P. persimilis, E. formosa, and the aphid parasitoid Aphelinus flavipes^(Forst) with selective chemicals has been developed for the pest complex of cucumbers. A similar system for control of pests on year-round Chrysanthemums also exists, giving growers a practical alternative to routine pesticide

applications (Hussey and Bravenboer, 1971; Wyatt, 1974).

The use of biological control in glasshouses gradually increased, and as a focus for research and development in the field, the I.O.B.C. (The International Organisation for Biological Control of Noxious Animals and Plants) was set up in 1971. By 1979 eleven countries were using biological control in glasshouses, but commercial use of parasitoids and predators other than P. persimilis and E. formosa was limited. Despite the fact that the total greenhouse area under biological control was still increasing, concern was growing amongst workers in the field that application was not more widespread. In 1978 less than 2000 ha out of an estimated total of 80,000 to 90,000 ha was under biological control. Discounting glasshouse area unsuitable for biological control, there remained 20,000 ha where biocontrol was feasible, but not practised (van Lenteren et al., 1980). This was probably due to problems with production and distribution of natural enemies, improper use of chemicals, and the complexity of integrated chemical and biological techniques. Suggested solutions to these problems generally comprise better "extension services" to growers and increased research effort (Hussey and Scopes, 1977; Tauber and Helgesen, 1978; van Lenteren et al., 1980).

Further implementation of biological control schemes may depend ultimately on the availability and expense of alternatives. The increasing problems of pest resistance and the soaring costs of developing new insecticides, may tip the balance in favour of biocontrol more quickly than the research efforts of workers in the field (Hussey and Bravenboer, 1971).

The widespread acceptance and use of biological and integrated control will also require modification of existing political and social philosophy.

"However, consensus and economic systems continue to evolve and there seems

to be a distinctly discernable trend toward the recognition of the essentiality of managing critical resources, including agroecosystems and forest lands, from a more holistic viewpoint. If this trend continues, much existing knowledge, as well as that to be generated, will be used more effectively in pest population management systems" (Rabb et al., 1976).

"The decision to produce and release natural enemies is only the beginning of a long and ever changing road to the implementation of rational pest control" (Hussey and Scopes, 1977).

1.2 THE EFFECTS OF TEMPERATURE ON INSECT BIOLOGY

Biological processes are dependent on a complex of physical and chemical phenomena, each of which is greatly influenced by temperature. As each chemical reaction and physical change is affected to a different degree, the relationship between temperature and insect biology has a complicated physiological basis (Huffaker, 1944).

The importance of temperature in affecting the activities of organisms has been recognised for over 2 centuries, and attempting to quantify its effects has occupied biologists throughout this period. Studies on the temperature relations of insects have intensified with the increase in research on population ecology. Temperature is a fundamental driving variable in the population dynamics of insects, and knowledge of its effects on the birth rate and death rate of the population is central to the understanding of population ecology. The birth rate and death rate of the population are themselves dependent on a number of biological characteristics (Kitching, 1977).

1.2.1 The temperature/development relationship

There is a definite relationship between the time taken for an insect to develop and the temperature at which development occurs. Generally development is slower at low temperature and quicker at higher temperature, but the relationship is different for each species of insect and each stage of growth (Sanderson, 1910).

Development is adversely affected by temperature extremes. Above optimum conditions mortality rapidly approaches 100%, and the same is true as temperature nears the lower threshold for development. Within the

favourable range however, temperature greatly affects the speed of development without producing differential mortality and thus this relatively narrow range is generally used in defining the relationship.

It has now become routine procedure to examine the temperature/development relationship for any species of economic importance. This is usually done by observation of the duration of readily identifiable stadia of the life history at a number of constant temperatures. The developmental period or (more usually) its reciprocal, the developmental rate, may then be plotted against temperature. The former gives a generally J-shaped curve, whilst the latter is more or less S-shaped. Since the end of the 19th century numerous attempts have been made to describe the relationship using mathematical equations. Many formulae have been put forward, some with a theoretical basis and others which are purely empirical, but none have met with universal acceptance (Davidson, 1944; Andrewartha and Birch, 1954).

An in-depth analysis of the derivation and application of all the mathematical descriptions of the effects of temperature on development will not be attempted here. The volume of literature on the subject is enormous and the early work in the field has been reviewed by Wigglesworth, (1939), Davidson, (1944), Huffaker, (1944), and Andrewartha and Birch, (1954). Instead attention will be centred on attempts to understand and predict development under field conditions. This has invariably been done by relating the results of constant temperature studies to the fluctuating conditions of the field. The validity of this has been disputed, as several workers found that the speed of development appeared to be accelerated or retarded by alternating temperatures (Huffaker, 1944; Messenger, 1964a). It is now generally accepted that this can be explained purely as a result of comparing the mean developmental period of a fluctuating regime with that of a constant temperature corresponding to the

mean of the fluctuating regime. This implies a linear relationship between temperature and development rate, which is not the case (Pradhan, 1945). Providing that account is taken of the curvature of the graph, there is no firm evidence to indicate that the developmental rate at fluctuating temperatures is not closely equivalent to that at corresponding constant temperatures (Andrewartha and Birch, 1954; Howe, 1967).

The problem then arises as to which of the many mathematical descriptions of the temperature development relationship to apply. The most widely used is the thermal summation or day-degree method. This developed from earlier botanical techniques relating the environmental temperature to development and growth, and is based on a linear relationship between temperature and the rate of development (Kitching, 1977). A linear regression equation of the form $y = a + bT$ is fitted, where y is the rate of development at temperature T , and a and b are constants. When the function connecting developmental rate with temperature is extrapolated back until it intercepts the x-axis, the temperature at which the developmental rate = 0 can be identified. This is the "lower threshold" (T_l), which may be calculated from $T_l = -a/b$. The total amount of heat required to complete development is regarded as a thermal constant (K), and may be calculated from the reciprocal of the slope of the regression line, $1/b$ (Campbell, Fraser, Gilbert, Gutierrez and Mackauer, 1974, Rabasse and Shalaby, 1980).

Once T_l and K have been defined from constant temperature data, the developmental period under any fluctuating regime may be determined by a simple summation procedure. Unit time degrees (day-degrees or hour-degrees) above T_l are accumulated until the value of K is reached, at which point development is complete. This approach has been used successfully in a number of simple models (Apple, 1952; Morris and Fulton, 1970, Lee, Barr, Gage and Kharkar, 1976, Hughes and Sands, 1979, Butts and McEwan, 1981;

Goodenough, Hartsack and King, 1983). However the method has been much criticised as it has several inherent faults. Firstly the assumed linear relationship is known to hold as an approximation for the median portion of the temperature range only (Campbell et al., 1974; Syrett and Penman, 1981). Towards the temperature extremes the relationship deviates from the linear, and estimates therefore become inaccurate. Secondly, the lower threshold, on which the thermal summation is based, is a purely theoretical point determined by extrapolation of the linear portion of the relationship into a region where the relationship is not linear. It therefore has very little biological meaning (Andrewartha and Birch, 1954).

In an attempt to improve on this method, an algorithm was developed using a sigmoid function with the relationship inverted when the temperature is above optimum (Stinner, Gutierrez and Butler, 1974). This can be written as:

$$R_t = C / (1 + e^{k_1 + k_2 t'})$$

Where R_t = rate of development at temperature t .

C = (maximum developmental rate) $\times (e^{k_1 + k_2 t_{opt}})$, ie. the asymptote.

t_{opt} = temperature at which the maximum developmental rate occurs.

k_1, k_2 = empirical constants.

$t' = t$, for $t < t_{opt}$.

$t' = 2.t_{opt} - t$, for $t > t_{opt}$.

The assumed symmetry about the optimum is not correct, but the authors claim that the resultant errors are negligible. Predicted development times for larvae and pupae of Trichoplusia ni under two variable temperature regimes yielded results with errors of up to 35.4% using

thermal summation, but only 6% using the sigmoid function. This algorithm has also been used in other simulations (Ables, Shepard and Holman, 1976; Berry, Foerster and Ilken, 1976; Whalon and Smilowitz, 1979, Allsopp, 1981). In some cases the improvement in accuracy of simulations over the thermal summation method has been small or negligible, and it is questionable whether the use of this more complex model is necessary under normal field conditions (Kitching, 1977, Whalon and Smilowitz, 1979, Allsopp, 1981).

Other non-linear descriptions of the temperature development relationship have been attempted more frequently, as the widespread use of computers has reduced the problems associated with complicated calculations. For example, polynomial regression analysis can be used to select the best fitting curve to a given set of temperature development data. Successively higher order polynomials can be fitted until no significant improvement in F-value results. This was found useful in describing temperature dependent development of the MacDaniel spider mite (Tanigoshi, Hoyte, Brown and Logan, 1975), and Diglyphus intermedius Girault (Patel and Schuster, 1983). The method of matched asymptotic expansions was used to develop an analytical model describing a sigmoidal curve, but without the symmetry about the optimum found in Stinner's algorithm. Again the authors claimed excellent results (Logan, Wollkind, Hoyte and Tanigoshi, 1976).

With the increase in the sophistication of population dynamics models made possible by computer use, inaccuracy in other areas can also be reduced. Until very recently the developmental period of a population of insects at a particular temperature was almost always represented by a mean and standard deviation. This is not strictly justifiable as the distribution of emergence times is not normal, but shows a distinct skew towards longer developmental periods (Eubank, Atmar and Ellington, 1973, Howe, 1967;

Sharpe, Curry, DeMichele and Cole, 1977)). Several models have now been developed which include a function to account for the asymmetrical distribution of development times (Stinner, Butler, Bachelier and Tuttle, 1975; Sharpe et al., 1977; Wagner, Wu, Sharpe and Coulson, 1984). These can then be incorporated into population dynamics models, making them more realistic and potentially more accurate (Barfield, Sharpe and Bottrell, 1977; Regniere, 1982). However, the poikilotherm model of Sharpe et al., (1977) was not found to give any great improvement in accuracy over day degree models, when predicting development of Trichogramma pretiosum (Goodenough et al., 1983).

The benefits of the increased accuracy of these models over simpler methods are seldom great, as other factors then become limiting. When predicting or simulating development under fluctuating conditions there is a problem in accurately monitoring temperature. It is almost impossible to monitor the microclimate directly surrounding an insect in the field, and this is almost certain to be slightly different from the conditions in the macroclimate (Bursell, 1964).

The method used to monitor the macroclimate can also affect the accuracy of predictions. The errors induced by using daily maximum/minimum figures to represent fluctuating conditions are likely to far outweigh any improvement which may be gained by using a more accurate representation of the temperature/development relationship. However, where accurate hourly readings of the temperature are available, the increased accuracy of the more complex formulae may result in better simulations (Bursell, 1964, Howe, 1967).

1.2.2 Temperature/oviposition

Like development, oviposition is adversely affected by temperature extremes, and the limits to the favourable range are often of the same order, although there is great variation from species to species (Bursell, 1964). Definition of the relationship between temperature and oviposition is complicated by the fact that egg laying has distinct behavioural and physiological components. These include egg production and the ability to search for and locate suitable oviposition sites. Both of these are affected by temperature, but may also be strongly influenced by other factors. For example, egg production will depend on humidity, the availability of food, crowding and the age and size of the female (Andrewartha and Birch, 1954; Coombs and Woodroffe, 1983).

Generally the rate of oviposition(i.e. the number of eggs laid per female per day) reaches a maximum at a relatively high temperature, near to the upper limit for egg laying. Oviposition rate tends to fall steeply above this maximum, but more gradually below it. The position of the optimum will differ from species to species (Bursell, 1964, Ables and Shepard, 1976).

Temperature can also affect the pattern of egg laying, with a higher proportion of the eggs being laid sooner at higher temperatures (Siddiqui, Barlow and Randolph, 1973, Ragusa, 1974; Browning and Oatman, 1981; Herbert, 1981; Allsopp, 1981). The preovipositional period is also reduced with increasing temperature (Siddiqui et al., 1973; Barfield, Bottrell and Smith, 1977).

The age specific fecundity is the parameter which is generally used in relating the influence of temperature on oviposition to population growth

for a given insect. This is determined by observation of the number of eggs laid during each day of the insect's life. The net reproduction rate (R_0) may then be calculated as the total number of individuals per female per generation (Messenger, 1964b).

No attempts appear to have been made to describe the relationship between oviposition rate and temperature with mathematical equations, as has been done with development. Several workers have found that alternating temperatures seem to stimulate insect fecundity (Messenger, 1964a; Barfield, Bottrell and Smith, 1977), and thus it may be invalid to estimate oviposition rates in the field directly from constant temperature data.

1.2.3 Temperature/longevity

The lethal effects of temperature on insects have been much investigated (see Andrewartha and Birch, 1954; Bursell, 1964), and the upper and lower temperature limits defined for many species. Temperatures which are not lethal nonetheless affect the subsequent life expectancy, and as with development and oviposition, there is a definite relationship between longevity and temperature within the favourable range. In general, longevity decreases with increasing temperature, but data is often extremely variable and no more than a trend may be apparent (Barfield, Bottrell and Smith, 1977).

Longevity may also be influenced by other factors such as humidity, food, mating and in some cases egg laying. These factors must be kept constant when determining the effects of temperature on longevity. Few attempts have been made to show the relationship between mean longevity and temperature, and those that have are based on so few points that little can be said other than that the relationship is an inverse one (Bursell, 1964,

Raney, Coles, Eikenbary, Morrison and Starks, 1971). Force and Messenger (1964) show the relationship as a gentle curve.

Longevity data is often represented as a "survivorship curve", with the proportion surviving plotted against time. A time to 50% mortality (LT_{50}) may then be determined and the significance of differences in this figure tested at different temperatures (Siddiqui et al., 1973). The LT_{50} is a measure of average life span, but gives no further information about the shape of the survival curve. The same is true of other measures of mean longevity. However, two insects with exactly the same mean longevity, may have very differently shaped survival curves. Thus important information is lost when longevity is represented by the mean or LT_{50} .

The Weibull distribution has also been used to represent survival data (Allsopp, 1981). This frequency distribution has commonly been used in engineering as a time to failure model, but has also been found to fit existing ecological concepts of survivorship. The advantage of the Weibull distribution as a description of longevity data, is that it summarises all the information contained in an age specific survival curve, using 2 parameters, b and c . These represent the scale and shape of the curve, respectively and give all the important information about the number of individuals dying over a given period of time. This is something that the other methods described above fail to do. Furthermore, methods exist for estimating confidence intervals around the parameter values (Thoman, Bain and Antle, 1969), and standard statistical techniques are available for comparing b and c values determined under different conditions (Thoman and Bain, 1969). Previously, comparison of survival data was an extremely complex process, and one seldom performed (Pinder, Wiener and Smith, 1978). The Weibull parameters could thus be used as a convenient method for comparing the effects of temperature on longevity. Allsopp (1981) found

that the value of the scale parameter b decreased with increasing temperature, but no consistent effect of temperature on the shape parameter c can be identified from his data. Much of the advantage in using this technique was lost, however, as differences in the values of the parameters at different temperatures were not tested for significance.

1.2.4 Temperature/searching

The ability of an insect to succeed in a particular environment is largely dependant on its ability to exploit the available resources. To do this it must be able to search for and locate these resources (Kitching, 1977). A great deal of work has been done on the physical and chemical stimuli which attract insects to their hosts (be they plant or animal), or "arrest" them in the vicinity of hosts (Thorsteinson, 1960; Waage, 1978). The behavioural responses of entomophagous insects to host density have also been widely studied and incorporated into theoretical models of foraging strategy (Hassell, 1982). However, relatively little research has been done on the effects of temperature on insect searching behaviour.

The most obvious way in which temperature affects searching behaviour is through its influence on the general activity level of insects. Several authors have shown correlations between insect activity in the field and environmental temperature (Andrewartha and Birch, 1954; Willmer, 1983). However, field observations of this type are often difficult to interpret due to the influence of other factors, such as rainfall and windspeed. However, observations of activity levels at controlled temperatures in the laboratory have shown that activity increases with rising temperature up to a point, but drops markedly if temperatures exceed this optimum (Biever, 1972, Kitching, 1977). The lower threshold for activity tends to be considerably higher than that for reproductive development, but insects may

continue to be highly active at temperatures very near the upper lethal limit. For example, the optimum constant temperature for tsetse fly activity was found to be around 25°C, with less activity occurring both above and below this temperature. When the temperature was gradually increased by one degree every 10 minutes, the proportion of time spent active remained relatively constant as the temperature rose from 25°C to 30°C and increased between 30°C and 39°C, but above 40°C high levels of activity were only sustained for short periods (Barrass, 1970).

Several other factors also influence the locomotory activity of insects. The effects of relative humidity and diurnal periodicity appear to be secondary to that of temperature, whereas light has a pronounced effect, with darkness inhibiting activity at any temperature (Barrass, 1970, Barbosa and Frongillo, 1977).

Searching behaviour has probably been most studied in the insect parasitoids, and several components of the host finding process identified. These can be classified as host habitat location, host location; host acceptance and host suitability (Doutt, 1959). Host habitat location may be "directed" in response to chemical or visual stimuli, or the result of extensive random search. In either case a high level of activity is required. Host location, on the other hand, generally results from reduced locomotory activity in response to specific stimuli and increased klinotaxis if those stimuli are subsequently removed (Wylie, 1958; Vinson, 1976, Waage, 1978). Three basic parasitoid responses have been defined, which are important in host location behaviour. These are the functional response to host density, the aggregative response to host distribution and the interference effect in response to parasitoid density (Cook and Hubbard, 1977). Very little work has been done on the effects of temperature on these aspects of searching behaviour. However, it has been

shown that the functional response of the parasitoid Praon exsoletum to its aphid host is influenced by temperature. The maximum number of hosts attacked was low at temperature extremes and the shape of the functional response curve different, resulting in a faster effective searching rate at medial temperatures. These changes were due to the effect of temperature on three aspects of behaviour. The first was a shift in the way in which time was apportioned between various activities. The second, a change in the proportion of successful ovipositional strikes and the third a total inhibition of ovipositional responses (Messenger, 1968).

1.3 THE PEST

Planococcus citri (Risso). The citrus, common or glasshouse mealybug.

1.3.1 Pest status and damage

Planococcus citri is an important pest with a world-wide distribution, being found outdoors in the tropics and sub-tropics and in greenhouses throughout the temperate regions (Quayle, 1938). Its enormously wide host range makes it a problem in many branches of agriculture and horticulture. It was rated as the most serious mealybug pest of Citrus in southern California in 1915, and ranked sixth most damaging pest overall (Clausen, 1915). In the late 1970's it was still a serious pest of citrus in Texas, infesting an estimated 1200 - 1600 hectares of grapefruit (French and Reeve, 1979). It is very damaging in Citrus plantations throughout the mediterranean basin (Panis, 1979) and Berlinger and Gol'berg (1978) mention heavy infestations on Citrus in Israel. In Australia it is a major pest of commercially grown Passiflora edulis (Murray, 1978) and in the West Indies, West Africa, Papua and New Guinea and Ceylon it causes serious damage to Cocoa, Theobroma cacao, particularly when involved in the transmission of Cocoa viruses (Entwistle, 1972).

P. citri has been known as a serious greenhouse pest for well over a century (Smith, 1917, Pritchard 1949). Steiner and Elliott (1983) name it as the most common species found in indoor ornamental plantings.

Mealybugs cause direct and indirect damage to their host plants by sucking sap (Steiner and Elliott, 1983). The direct damage takes the form of distortion, stunting and yellowing of foliage, sometimes followed by defoliation. If flowers or fruit are present these often drop. The

indirect damage caused is due to the copious quantities of honeydew produced by these insects, which coats leaves, stems, flowers and fruit and serves as a growth medium for sooty moulds (Pritchard, 1949). Reports of the damage done to Citrus are given by Clausen, (1915); Smith and Armitage, (1931), and Panis, (1979). On ornamental plants the cosmetic damage due to unsightly egg masses and sooty moulds is enough to render P. citri a pest (Pritchard, 1949). (See plate 2).

1.3.2 Biology and life cycle

Mealybugs (Homoptera: Pseudococcidae) are members of the scale insect family (Coccoidea), but have no hard, shell-like, outer covering and unlike the true scales, they retain the power of locomotion as final instar nymphs and adults.

The adult female is oval, flattened, soft bodied and covered with white, mealy, wax filaments, which give the insect its common name (Smith and Armitage, 1931). These filaments can be used to distinguish between species, as they vary in length and form (Hussey, Read and Hesling, 1969). P. citri has 18 pairs of relatively short filaments around the body margin, and a pair of anal filaments which are not much longer than the others. The body is 2.5-4.0 mm in length, by 2.0-3.0 mm in width, and the antennae are 8-segmented (Avidov and Harpaz, 1969). The adult male is a small delicate two-winged insect with two long anal filaments. It does not feed and its sole purpose is to fertilise the female.



PLATE 2. Passiflora sp. heavily infested with Planococcus citri.

(Note clumps of white, cottony egg mass).

The eggs are straw-yellow and laid within an ovisac, composed of a white, cottony mass of wax fibres. There is some disagreement about the average size of the eggs, but measurements of length and width range from 0.25-0.40 mm and 0.09-0.2 mm respectively (Sutherland, 1932; Al-Ali, 1969; Avidov and Harpaz, 1969). The eggs hatch to produce very active first instar nymphs known as "crawlers". This is the dispersive phase of the life cycle and the young crawlers actively seek out new feeding sites. The sexes are similar at this stage, resembling the adult female in shape and form, but lacking the waxy coat, being yellow in colour (Smith and Armitage, 1931). The second instar is slightly larger, darker in colour and less active (Avidov and Harpaz, 1969). Males may be distinguished from females at this stage when examined under the microscope, as the males have no mouthparts (Sutherland, 1932). After the second moult the sexes become more easily distinguishable. The male becomes a dark brown pre-pupa, which rapidly forms the pupa within a white cottony cocoon, whilst the female changes little in shape, but starts to secrete its waxy coat and settles down to feed. This 3rd instar female has only 7 antennal segments whereas the adult has 8 (Sutherland, 1932, Avidov and Harpaz, 1969).

The sexes are produced in approximately equal numbers, although sex ratio varies with temperature and the age of the female on mating (Nelson-Rees, 1960). The adult male soon emerges from its cocoon and starts to search for females (Entwistle, 1972; Nelson-Rees, 1960). Although it does not feed, and appears fragile it shows quite remarkable sexual activity, mating with up to 23 females consecutively. On average the male will copulate with 9 different females (James, 1937). Nelson-Rees (1959) has observed two males mating simultaneously with one female!

After mating the female begins to produce an ovisac. As the eggs are laid and the ovisac grows, the female shrinks and finally dies. Unfertilised

females do not produce ovisacs, but can continue to live and feed for up to eight months as virgins (James, 1937). This finding dispelled earlier confusion over the ability of female P. citri to reproduce by parthenogenesis (Myers, 1932, Gray, 1954).

The biology of P. citri has mainly been studied under uncontrolled or fluctuating temperatures, resulting in very variable reports of the number of eggs laid and the length of the developmental period for each stage and for the whole life cycle. Study of egg production at constant temperature showed that the average number of eggs laid per female increased from 217.89 at 15.55°C to 342.1 at 26.67°C, with a maximum of 706 at the higher temperature (Whitcomb, 1940). Attempts to quantify the effects of a series of constant temperatures on the incubation period for eggs and the developmental period of the nymphal stages, showed that both decrease with rising temperature. Relative humidity was also reported to have an effect on egg development, but not on the development of nymphs. The incubation period of the eggs decreased with rising relative humidity, up to 70% r.h. At this optimum relative humidity, egg hatching occurred after 3.9 ± 0.09 days at 33°C, but took 12.2 ± 0.2 days at 17°C. The optimum temperature for development was found to be 28°C, as measured by percentage hatch of eggs and minimum developmental period of nymphs (Sayed, Soliman and Salama, 1962b).

1.3.3 Control

P. citri is not easily controlled with chemicals, indeed many authors attribute its recent importance as a pest to the widespread use of inorganic pesticides (Wood, 1963, Guerrero, 1969, Dean et al., 1971; Bedford, 1973; DeBach, 1974, Khoo, 1974, Meyerdirk et al., 1981). Very little of the literature on this pest advises or supports the use of

insecticides as the best control measure. Even in the early part of the century there were reports of marked conflict amongst growers and investigators as to the efficacy of fumigation (using hydrocyanic acid gas) in controlling the mealybug. The application of large quantities of water under high pressure has been suggested as a more effective measure than a number of possible chemical pesticides (Clausen, 1915, Quayle, 1938).

P. citri tends to congregate in protected and inaccessible parts of the plant and is difficult to reach with sprays (Doutt, 1952; French and Reeve, 1979).

Despite these early problems and repeated warnings of the dangers of indiscriminate pesticide use by eminent researchers in the field, chemicals have been used continually ever since (Pickett, 1949; DeBach and Bartlett, 1951; Bartlett, 1957; DeBach, 1974). Recent reports on the efficacy of commercially applied pesticides and insect growth regulators do not indicate much improvement in the degree of control possible. Up to 97% mortality of 1st instar crawlers can be achieved, but 2nd instars are more difficult to kill and 3rd instars and adults cannot be effectively suppressed (French and Reeve, 1979; Meyerdirk et al., 1981). In orchards where all stages are found on the trees at the same time (Clausen, 1915) satisfactory control is unlikely.

Berlinger, Tzahor and Gol'berg, (1979) sum up the use of chemicals against P. citri as follows. "Chemical control is possible, is expensive, might be inefficient and has the well-known unwanted side-effects of insecticides."

Attempts at controlling P. citri biologically started in Citrus orchards in California. An account of the early history of the biological control of mealybugs is given by Smith and Armitage, (1931).

The first predator to be introduced was the Australian Coccinellid Cryptolaemus montrouzieri Mulsant (see plates 3 and 4). At first, reports of its efficiency were variable, and in the early 1900's a large variety of predators and parasitoids was imported, due to the rapidly increasing importance of the pest (Clausen, 1915; Smith and Armitage, 1931). The first indications of success came in 1919, when a rapid decline in the pest population in Ventura County was attributed to the combination of C. montrouzieri and the Encyrtid parasitoid Leptomastidea abnormis (Girault), which had been introduced from Sicily in 1914 (Smith and Armitage, 1931). By 1958 good control was being achieved, and an extensive survey by Bartlett and Lloyd, (1958) found C. montrouzieri to be the best at reducing heavy infestations of P. citri, followed by Sympherobius californicus Banks and another lacewing Chrysopa sp. These authors also give an extensive list of the important parasitoids found during their survey and contributing to control.

There are now numerous reports of at least partially successful biological control of P. citri around the world (Dean et al., 1971; Greathead, 1976; Panis, 1979; Berlinger et al., 1979, Krambias and Kontzonis, 1980). The comments of Rust (1914), Guerrero (1969) and Khoo (1974) make it apparent that in parts of the world where it is native and where broad-spectrum insecticides have not been used, P. citri is held at low levels by natural enemies and does not reach pest status.

In glasshouses, chemical control of mealybug is also undesirable though for slightly different reasons. One major disadvantage is that of phytotoxicity. Many ornamental plants are damaged by insecticidal sprays, suffering such symptoms as leaf scorch, leaf hardening, leaf fall and retardation of growth and budding (Becker 1974; Panis unpublished data).



PLATE 3. The predator Cryptolaemus montrouzieri: adult.
(Magnification x 20 approx., plates 3 and 4).



PLATE 4. C. montrouzieri: final instar larva, devouring mealybug egg mass.

On top of this there is also the problem of public access to displays of ornamentals in botanical gardens, amenity areas and offices. Here the use of chemicals is obviously ill-advised (Hussey and Scopes, 1977; Copland, 1982).

The same factors which make glasshouses almost ideal environments for the mealybug, along with the fact that large numbers of hosts occur in a relatively limited area, offer excellent conditions for the efficient use of biological control (Doutt, 1951).

Practical control of P. citri was achieved in glasshouses in Massachusetts using C. montrouzieri, provided that the infestation of mealybugs was large enough to ensure adequate food for the predator to establish itself, and provided that chemicals needed to control other pests were used with care. Temperature was found to be critical in determining the success of this predator, but if temperatures were maintained at around 21°C satisfactory control could be achieved (Whitcomb, 1940). Similar results have been reported using this predator in glasshouses in France (Panis and Brun, 1971). Pritchard (1949) stated that both C. montrouzieri and L. abnormis had been used in gardenia ranges by a number of growers, with variable results. In general mealybug populations were reduced but not eliminated. However, the use of these two insects and several other parasitoids and predators produced successful control of heavy infestations of P. citri on gardenias in a trial by Doutt, (1951). The most effective species were Exochomus flavipes (Thunberg), Chrysopa californica Coquillett, and Anagyrus kivuensis Compere. In another trial on glasshouse grown Stephanotis, virtual eradication of the mealybug was reported, using C. montrouzieri and Leptomastix dactylopii and the author was very optimistic about the potential for the use of biological control against P. citri in glasshouses (Doutt 1952). The same combination of natural

1.4 THE PARASITIDS

Anagyrus pseudococci (Girault)

1.4.1 Life history and biology

A. pseudococci (Hymenoptera: Chalcidoidea) is a solitary internal parasitoid of the family Encyrtidae. The adults show marked sexual dimorphism (see plates 5 and 6). The female is about 1.5-2 mm in length and brown, with distinctive black and white banded antennae, whilst the male is smaller (approximately 0.8-0.9 mm in length) and black in colour. A full description of the morphology of the adults and developmental stages is given by Rosen and Rossler (1966), and Niyazov (1968).

A. pseudococci is arrhenotokous, virgin females producing male offspring only. The preferred hosts are 3rd instar mealybug nymphs, but females will also attack 2nd and final instars. The female lays a single egg in each host. The size of the host affects the sex of the offspring, eggs laid in smaller hosts generally producing males (Chandler, Meyerdirk, Hart and Garcia, 1980). Oviposition proceeds as follows: the female parasitoid examines the host with its antennae for several seconds, before turning its caudal end towards the mealybug and inserting the ovipositor between the wax filaments on the lateral margins of the host. The wings and hind legs are held aloft during this process, which generally lasts about 15-40 seconds (Avidov, Rossler and Rosen, 1967). The larva completely consumes the contents of the mealybug, going through 5 instars before pupating within the mummified skin of its host (Zinna, 1960, Rosen and Rossler, 1966). The adult emerges through an irregular exit hole gnawed at the posterior end of the mummy.



PLATE 5. Female Anagyrus pseudococci (Magnification x 50 approx.).



PLATE 6. Male Anagyrus pseudococci (Magnification x 50 approx.).



PLATE 7. Female Leptomastix dactylopii (Magnification x 50 approx.).



PLATE 8. Male Leptomastix dactylopii (Magnification x 50 approx.).

L. dactylopii has been little studied in the laboratory, but it appears that its biology is strongly influenced by temperature.

1.4.4 Status as a natural enemy

In the field the indications are that this parasitoid is monophagous, with P. citri as its only host. However, females are known to oviposit in Pseudococcus adonidum L., P. maritimus (Ehrh) and Phenacoccus solani Ferris, although progeny production in all but the latter is negligible. Occasional reports have been received of it also attacking other mealybug species (Lloyd, 1958). L. dactylopii was originally introduced into the U.S.A. from Brazil as a biocontrol agent in 1934 (Flanders, 1951) and has been reported attacking P. citri in Trinidad (Entwistle, 1972), Texas (Meyerdirk, Hart and Dean, 1978), California (Bartlett and Lloyd, 1958), Bermuda (Bennett and Hughes, 1959) Cyprus (Krambias and Kontzonis, 1980) and France (Panis, 1979). Attempts have also been made at introducing the parasitoid into Italy and Spain (Greathead, 1976). L. dactylopii was released in California at the rate of 10 million per year for the 15 years leading up to 1953, without establishment (Flanders, 1953). Despite this, it is still mass produced at the Associate Insectary, Santa Paula and commercial distribution has also been taken up by others (Steiner and Elliott, 1983). This parasitoid has frequently been used in glasshouses against P. citri and some success has been reported (Doutt, 1952, Copland et al., 1985).

Leptomastidea abnormis (Girault)

1.4.5 Life history and biology

L. abnormis is also a solitary, internal parasitoid of the family Encyrtidae. It is smaller than the other two parasitoids (0.75-1.5 mm in length) and is distinctive in that it holds its wings up at 90 degrees to the body, and with one at a strange angle as though broken at the base (see plate 9). In both sexes the body is yellowish, marked with dusky black and the wings have three, blackish bands across them. The sexes may be distinguished by the smaller size of the male and the conspicuous hairs on the male's antennae (Viereck, 1915). A very full description of its biology, life-history and introduction into the United States is given by Smith (1917).

Its preferred hosts are second instar mealybugs, but it will also readily attack the 1st and 3rd instars. Like the other two species it is arrhenotokous and has similar developmental biology. As with the other two species, the biology of this parasitoid is influenced by temperature. Little work has been done on this insect using constant temperatures, but the indications from laboratory work done under fluctuating conditions are that the developmental period and adult longevity are temperature dependent (Viereck, 1915, Rivnay and Perzelan, 1943).

1.4.6 Status as a natural enemy

L. abnormis has been reported parasitising Pseudococcus sacchari (Smith, 1917), Pseudococcus citriculus (then wrongly identified as P. comstocki (Rosen and Rossler, 1966)) and P. citri, the latter being its preferred host (Rivnay and Perzelan, 1943).



PLATE 9. Female Leptomastidea abnormis (Magnification x 50 approx.).

L. abnormis was originally introduced into the U.S.A. in 1914 from Sicily by H.L. Viereck, and named Paraleptomastix abnormis. It was found to be important in maintaining P. citri at low density in Californian Citrus orchards, although it seemed less effective at high pest density (DeBach and Bartlett, 1951). It has also been recorded in Cyprus (Wood, 1963), Israel, then Palestine (Rivnay and Perzelan, 1943, Avidov and Harpaz, 1969; Berlinger, 1977) and Bermuda (Bennett and Hughes, 1959). In the U.S.A. it has been released in glasshouses in attempts to control P. citri, with varying degrees of success (Pritchard, 1949, Douth, 1951).

SECTION 2: LABORATORY PRODUCTION OF INSECTS

SECTION 2: LABORATORY PRODUCTION OF INSECTS

Introduction

It is of fundamental importance to the development of a biological control programme, that the natural enemy concerned can be artificially cultured on a large scale. A reliable source of control agents is required, particularly if releases are to be made in more than one area or in succession. It also allows production of adequate numbers for experimental investigation of the insect's biology. The goal of any culture programme is to produce the maximum number of fertile females of the entomophagous species with the minimum man hours and space, in as short a time and as inexpensively as possible (Debach, 1964).

Generally, there are three stages to the production of natural enemies:- propagation of the host plant or a substitute, rearing of the host insect (generally the pest itself) and rearing of the natural enemy. P. citri has an enormously wide host range, giving a large choice of culture plant. However, it was the finding that P. citri thrived on sprouted potato tubers Solanum tuberosum, which opened the door to practical mass production of the mealybug. Following this discovery in the early part of the century, a large number of insectaries were set up in California for the production of the mealybug predator Cryptolaemus montrouzieri and later of the parasitoid Leptomastix dactylopii. Mass production techniques for these natural enemies have thus been tried and tested and are now well established. Production of L. dactylopii by the Associates Insectary in Santa Paula was estimated at around 42,575,000 per annum in 1963 (Fisher, 1963).

At Wye College the aim was to culture the three mealybug parasitoids A. pseudococci, L. dactylopii and L. abnormis in numbers large enough to

provide the insects needed for experimental work. The intention was to adapt the methods described by Fisher, (1963) to laboratory scale production of the three species mentioned above. Expansion was then envisaged for small scale commercial production for use in the glasshouse. Under glasshouse conditions, fewer natural enemies are required than for release in the field as dispersal is restricted and the environment is generally more stable.

Materials and methods

Propagation of the host plant

Potatoes were chosen as the host plant as they have been found most suitable in other culture programmes, and due to their ready availability, low cost and ease of handling. Where possible small, ware potatoes (those which would pass through a 50 mm griddle) were purchased, as these were of the most convenient size for experimental purposes, and were cheaper. Several changes were made in propagation method from those described by Fisher, (1963), either for convenience or due to differences in facilities.

Variety Several varieties were tried including Pentland Crown, Javelin, King Edward, Maris Piper and Desiree. The latter were most readily available and were found suitable.

Storage The potatoes were bought in 25 kg sacks, and placed in storage at $2 \pm 0.5^{\circ}\text{C}$ until required. Potatoes bought in November could generally be stored in this way through to March, although some deterioration did occur over this period. Further supplies purchased in January or February would last through to the following November.

Sprouting: Sacks were removed from storage 2 weeks before required during the summer, and three weeks before during the winter. Several techniques for producing sprouts were tried:

- 1) The tubers were spread out in "Sharpak" transparent, polythene punnets 167 mm x 110 mm x 50 mm (approx. 6 per punnet). Punnets were placed on metal trays in a controlled temperature room 2.25 m x 1.85 m x 1.2 m held at $26 \pm 2^{\circ}\text{C}$, with ventilation supplied by an "Airflow Developments" blower, type 40BTFL. The room was lit with one fluorescent strip light (80 W "daylight").
- 2) The tubers were spread in punnets placed on aluminium staging in a glasshouse cubicle. Punnets were then covered with black polythene. The temperature varied with season. No ventilation was supplied.
- 3) The tubers were left in sacks in the glasshouse cubicle. Sprouting took place within the sacks, and tubers were spread out in the punnets when required for mealybug cultures.

The sprouts were used when 20-200 mm long, 40-50 mm was found to be ideal. It was found that both green and etiolated sprouts were acceptable to mealybugs.

Culture of mealybugs

Initial cultures: Set up by Dr. M.J.W. Copland from an infestation in the Wye College conservatory, during 1977. Identified by Dr. C.J. Hodgson as Planococcus citri (Risso).

Cages: The cultures were confined in large (820 mm x 610 mm x 535 mm), perspex cages with metal trays as bases. Access was through a 380 mm x 310 mm opening at the front. This had a nylon gauze cover (30 mesh/cm), closeable with "Velcro" 30 mm, white, polyester fastening tape.

Ventilation was provided through 2 gauze covered apertures (100 mm diameter) at the back. Cages could hold 42 punnets in two layers, with the upper layer on staging.

Culture rooms: 2 constant temperature rooms were used

Room 1: 2.25 m x 1.85 m x 1.2 m with space for 10 cages, on a wooden wall rack 700 mm deep. Shelves on the rack were fitted with "Dexion" wheels and runners for easy handling of the cages. The temperature was maintained at $26 \pm 2^{\circ}\text{C}$, and ventilation was supplied. Air from a fan passed down 80 mm Osma plastic tubing to six outlets, spaced so as to coincide with the apertures at the back of the cages. Lighting was provided by one 80 W "daylight" fluorescent tube.

Room 2: 2.99 m x 2.82 m x 1.95 m with space for 8 cages. Cages were kept on the floor or on wooden staging. The room was constantly ventilated, and maintained at $26 \pm 0.5^{\circ}\text{C}$. Light was supplied by 50 x 80 W "daylight", 20 x 30 W "daylight" and 20 x 30 W "white" fluorescent tubes.

Maintenance of cultures: At 26°C development of P. citri from egg in the first generation to egg in the second generation takes approximately 4 weeks. Two new cultures were set up weekly, i.e. 84 punnets containing about 500 sprouted tubers. Punnets containing the clean, sprouted tubers were placed in previously washed, perspex, culture cages in one of the culture rooms (see plate 10).

In order to infest the clean potatoes with mealybugs, "transfer plants" were used. A variety of hedgerow plants were tried, but during the summer freshly picked shoots (150-200 mm long) of nettle plants (Urtica dioica L.) were found to be most successful, and during the winter twigs of the ornamental shrub, Cotoneaster sp.

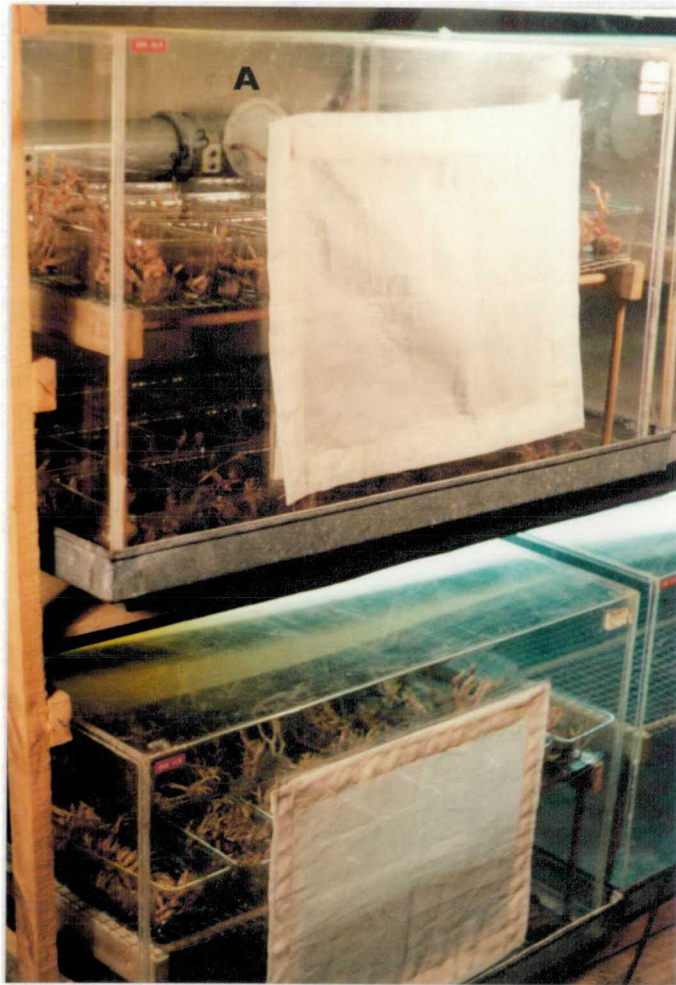


PLATE 10. Mealybug culture cages in culture room 1.
(A - indicates ventilation through gauze covered apertures.)

"Transfers" were laid across the punnets in a cage containing hatching egg masses of the mealybug i.e. a culture about 4-5 weeks old. The "transfer plants" were left overnight (about 15 hours), during which time large numbers of "crawlers" (1st instar mealybug nymphs) moved onto them. They were then moved to newly cleaned cages and laid over punnets containing the sprouted potatoes. Crawlers left the transfers as they dried out, settling on the sprouts. This method produced new mealybug cultures of relatively uniform age.

The mealybugs from these cultures were used as host material for culturing the parasitoids A. pseudococci, L. dactylopii and L. abnormis and the predator Cryptolaemus montrouzieri.

Culture of parasitoids

The same procedure was adopted for all parasitoid species. Any differences are mentioned below.

Initial cultures Set up by Dr. M.J.W. Copland with stocks received from the following, whose help is gratefully acknowledged:

A. pseudococci from Dr. M.J. Berlinger - Israel.

L. dactylopii from Mr. C. Farrarr - Filmore Insectary, California, U.S.A.,
Prof. G. Viggiani - Italy, Dr. M.J. Berlinger - Israel.

L. abnormis from Prof. G. Viggiani - Italy.

Along with the L. dactylopii received from the Filmore insectary came another small, black parasitoid Coccidoxenoides peregrinus (Timberlake) (previously Pauridia peregrina). This insect was cultured separately for some time, but became a serious problem, first as a contaminant of the other parasitoid cultures, and then in the mealybug culture room. Attempts

to remove it from these places are described below.

All the parasitoids were imported under MAFF licence no. PHF64/9 issued under the Import and Export (Plant Health) (Great Britain) Order 1980 and the Plant Pests (Great Britain) Order 1980.

Cages. Cultures were kept in two types of container.

1) Stock cultures were maintained in "Stewart" plastic boxes (173 mm x 115 mm x 63 mm), with ventilation supplied through rectangular openings 62 mm x 32 mm cut in each side and covered with nylon gauze (40 mesh/cm)(See plate 11).

Mass cultures were maintained in perspex cages (400 mm x 610 mm x 535 mm) with metal bases. Access was gained through a rectangular opening (380 mm x 300 mm) with a nylon cover (30 mesh/cm), closeable with "Velcro". Ventilation was supplied through a single, gauze (30 mesh/cm) covered aperture 100 mm in diameter. The cages could hold 18 punnets in two layers, with the upper layer on staging (see plate 12).

Culture room. All three parasitoids were cultured in the same constant temperature room held at $26 \pm 0.5^{\circ}\text{C}$ and 45-60% r.h. Light was supplied by a combination of fluorescent tubes.

Hosts Different stages of the host P. citri are preferred by the different parasitoids.

A. pseudococci - The preferred stage is 3rd instar nymphs (Chandler et al., 1980). Using the methods described above this corresponds to mealybugs from cultures 3 weeks old.



PLATE 11. Parasitoid stock culture, in ventilated, plastic box.



PLATE 12. Parasitoid mass culture cage.

L. dactylopii - The preferred stages are 3rd instar nymphs and adults (Bartlett and Lloyd, 1958, Fisher, 1963). Again, mealybugs from 3 week old cultures were used.

L. abnormis - The preferred stages are 1st and 2nd instar nymphs (Smith, 1917). This corresponds to mealybugs from cultures 2 weeks old.

Plate 13 shows a sprouted tuber infested with mealybug and ready for use in the culture of either A. pseudococci or L. dactylopii.

Maintenance of cultures: At 26°C development from egg to adult takes approximately 2 weeks for L. dactylopii and A. pseudococci, and approximately 3 weeks for L. abnormis.

Stock cultures - Sprouted potato tubers infested with mealybug were removed from the appropriately aged culture and placed in plastic boxes (3 per box). Transfer of parasitoids to these was simply achieved by changing the lid for one from a culture containing large numbers of newly emerged adult parasitoids. The lid was then tapped hard to dislodge the parasitoids and the original lid quickly replaced. Such new cultures were set up twice weekly.

Mass cultures - 9 punnets of sprouted tubers infested with the appropriate stage of mealybug were placed on the top layer of one of the small (400 mm x 610 mm x 535 mm), perspex culture cages. The bottom layer contained punnets full of sprouted potatoes covered with previously parasitized mealybugs, from which adult parasitoids were hatching. These were then left for one week. At the end of this period the bottom layer of punnets was removed and discarded, and the top layer moved to the bottom ready to infest the next new culture a week later.

Collection of parasitoids from mass cultures: The parasitoids show positive phototaxis (Fisher, 1963), and thus tend to congregate on the top surface of the cage. Collection of large numbers of adults can simply be achieved by "scraping" round the top, using a plastic box angled at about 30° to the surface. As the edge of the box approaches, the parasitoids jump up and land in the box. Once the whole of the top surface of the cage has been scraped in this way, the lid of the box is quickly replaced. 300-400 parasitoids may be collected at one time using this method.

Dispatch of control agents: Two boxes of parasitoids, collected using the technique described above were treated with CO₂ to anaesthetize the parasitoids. The contents of the two boxes were then poured through a funnel into a plastic (30 mm x 10 mm), Gallenkamp BTS 430 000S screw-top, specimen tube. A piece of filter paper liberally streaked with honey solution (about 50.50 honey· water) was also placed in the tube, to provide a source of food for the parasitoids. As soon as the parasitoids started to recover, the lid of the tube was screwed on. Tubes were then thoroughly wrapped to protect them from breakage, and sent by post to the release sites.

Results and discussion

Several changes were made from the methods described by Fisher (1963). Potatoes were not planted in soil or potting compost. There were two reasons for this. Firstly, it was found that clean soilless tubers were easier to deal with for culturing, and far more useful for experimental purposes. Secondly, it alleviated problems with tubers rotting, and rots spreading through the soil.



PLATE 13. Sprouted potato tuber infested with Planococcus citri and ready for use in parasitoid cultures.

(A - young adult female; B - 3rd instar nymph; C - 2nd instar nymph; D - crawlers).

Fisher, (1963) used long sprouts (300-450 mm), but it was found that short sprouts (40-50 mm) were easier to handle, more convenient for experimental work and just as acceptable to the mealybugs.

Sprouting method

Three methods were used to sprout the potatoes, as described above. Each had advantages and drawbacks.

- 1) Sprouting in the constant temperature room: Excellent sprouts were produced - etiolated, but thick and healthy. However, there was a problem with mealybug infesting the sprouting tubers, due to the proximity of P. citri cultures. This led to an uneven age distribution, when the potatoes were used for the mealybug cultures, and also gave a reservoir for any contaminants.
- 2) Sprouting in the glasshouse: Good sprouts were produced for most of year. Sprouting was slower in winter, and in the summer low humidity led to the sprouts drying out, and thus being of poorer quality. Etiolated or green sprouts could be produced by this method depending on whether or not the tubers were protected from the light. In the summer a black polythene cover kept the humidity higher, whilst encouraging etiolation.
- 3) Sprouting in sacks: This produced sprouts of adequate quality throughout most of the year. The main problem came in late summer and autumn, with the potatoes getting older, a larger number began to rot. Within sacks, rotting tubers go unnoticed and the rot can spread rapidly. Also, care must be taken not to damage the sprouts as they are tipped from the bag.

None of the above methods were ideal. The best environment would be a C.T. room with adjustable lighting, used exclusively for potato sprouting. This

can be done when mass production is undertaken in an insectary. For small scale production this is probably impractical, due to lack of space and resources. It is, however, possible to produce sprouted tubers of adequate quality without a specifically designed sprouting room, as has been shown above.

Yield

Two large perspex cages were set up each week using about 500 tubers, with an estimated mealybug production of about 100,000 per week. Of these, 18 infested tubers were used per week for parasitoid stock cultures in plastic boxes, producing up to 300 parasitoids of each species, and about 160 infested tubers were used for parasitoid mass production in cages with a yield of up to 2000 of each parasitoid species per week. The remaining mealybugs were used for the maintenance of cultures of the predator Cryptolaemus montrouzieri.

Hygiene

Contaminants can get in at all stages of the natural enemy production process - rots in the potato tubers, insect contaminants on sprouts (e.g. aphids), moulds on mealybug honeydews, predators, parasitoids and other insects on transfers, mites and/or parasitoids in host cultures, and mixing of the parasitoid species in their respective cultures. Hygiene is thus of great importance and any possible sources of contamination should be identified and eliminated as soon as possible at all stages in the process. This is a case where prevention is undoubtedly better than cure, and the following precautions were taken in order to avoid contamination:

Sprouting: Potatoes were examined regularly throughout the sprouting process and before being infested with mealybug. Any tubers showing signs of rots, mould or contaminants of any kind were removed. Potatoes were selected and spaced so that there was no contact between tubers during sprouting, in order to minimise the spread of any rots which did occur.

Where potatoes were sprouted in the room containing the mealybug cultures some sprouts became contaminated with mealybug. The crawler stage was the major culprit and covering or caging the tubers did not prevent their entry, due to their small size. Sprouting in this room was thus discontinued. Where potatoes were sprouted within a glasshouse cubicle some contamination with aphids occurred, but this was minimised by covering with black plastic.

Culture maintenance: A number of measures were taken in order to prevent contamination of the cultures. A regular cycle was maintained with each culture being kept a maximum of six weeks, before being removed. As the tubers age, they can provide a medium for the growth of moulds and mites (Tyrophagus sp.) and thus must not be allowed to accumulate in old cultures. After six weeks any remaining potatoes were thrown out, the cages washed and brushed out with hot water and then left empty for a minimum of two weeks.

Any insects present on the transfer plants were removed as they were picked, in order to avoid the introduction of anthocorid bugs and other predators.

Mites can easily be mistaken for mealybug crawlers and care was taken to avoid transferring mites from a contaminated source, when setting up new cultures. Thus cultures to be used as a source of crawlers were carefully

examined, before transfers were introduced. If any mites or parasitoids were present, these were not used unless absolutely necessary, transfers being placed in a source culture without contaminants.

Further precautions were taken by planning movements so that host plant cultures were visited first, followed by host cultures, followed by parasitoid and predator cultures. When possible different cultures were visited on different days. In this way the risk of cross contamination was minimised.

Despite this care, some problems were still encountered with both moulds and mites in culture room 1. This was due to high humidity, which despite forced air circulation to the cages, could not be avoided due to the age and design of the building. No such problems were encountered in culture room 2, where the humidity could be kept low. During the autumn mealybug cultures had to be confined to culture room 2 and room 1 left empty for several months before it could be used again.

Other contaminants of the mealybug cultures were the parasitoids C. peregrinus and L. abnormis. These were probably originally introduced on the clothing of the culture technician and once present were extremely difficult to eradicate. The females of C. peregrinus are thelytokous and can lay almost 100 eggs in their lifetime. Just one individual can lead to large scale contamination within a few months. Although this parasitoid proved useless as a control agent in the glasshouse, it was capable of an extremely high rate of increase when given optimum conditions of the culture room, and an environment in which hosts were abundant and virtually no searching was required in order to locate them. L. abnormis generally built up more slowly, but was also difficult to remove. When these parasitoids were present as contaminants, clean mealybug cultures had to be

set up by removing adult female mealybugs, using a fine paint brush and transferring them onto clean sprouted potatoes. Approximately 10 females were placed on each sprout, and the tubers placed in separate boxes. These were then placed in incubators or a C.T. room away from the contaminated cultures and observed regularly to check that they remained uncontaminated. These clean cultures were allowed to pass through one generation and the infested tubers were then used to restart cage cultures.

Whilst the clean cultures were being built up, the least contaminated cultures were used to maintain a cycle so that adequate numbers of mealybug were available for the parasitoids and C. montrouzieri. As soon as the new clean cultures were sufficiently productive, the contaminated cultures were run down, the cages carefully washed and the cages and culture room left empty for at least 5 weeks before being reused. This period was long enough to allow any parasitoids to complete development within their hosts, emerge and die, without finding further hosts.

Some mixing of the parasitoid species in the parasitoid cultures also occurred. This was difficult to avoid as the cultures were kept in the same room, where ideally separate rooms should be used. However, stock cultures in plastic boxes could be kept pure relatively simply, by setting up fresh cultures using individually selected parasitoids. This was done by anaesthetising a culture containing newly emerged adults and removing individuals of one species only, using an aspirator. These could then be introduced into a box containing tubers infested with mealybugs of the appropriate stage.

Following these problems, small stock cultures of both mealybugs and parasitoids were kept in a location other than the normal culture rooms. This provided a source of uncontaminated culture in case anything was to go wrong with the main cultures.

Costing

Inputs - Potatoes.

Punnets.

Labour.

Energy - heating, lighting, etc.

Equipment - cages, etc.

The cost of parasitoid production during this study cannot be stated exactly, as cultures of the mealybug predator C. montrouzieri and scale insects and their parasitoids were maintained using the same facilities, host plant and employees. However, an estimate is made below:

Potatoes - 1 x 25 kg sacks/month @ $\pm$ £2.50/sack = $\pm$ £ 30/year

Punnets - 42/week @ $\pm$ £0.02 each = $\pm$ £ 45/year

Labour - $\pm$ 10 man hours/week @ $\pm$ £3.00/hour = $\pm$ £1560/year

Total - = $\pm$ £1635/year

It is difficult to estimate the cost of other inputs, for example electricity to heat and light culture rooms, and the equipment necessary to culture insects, but these are low in comparison to the major inputs listed above. The use of perspex culture cages is relatively expensive, but is unnecessary. Cages could be much simpler and less costly.

Estimated production of the parasitoids was $\pm$ 450,000/year i.e. less than 0.5 p each. The market value of other parasitoids used in biological control e.g. Dacnusa sibirica and Diglyphus isaea is £12.00 - £15.00/100 (1983 prices) (Bassett, 1983), and thus it would appear that costs could easily be covered.

Possibility for mass production

As mentioned in the introduction this has been proven in the U.S.A. (Fisher, 1963). Providing facilities are available it is relatively easy to scale up production to produce larger numbers.

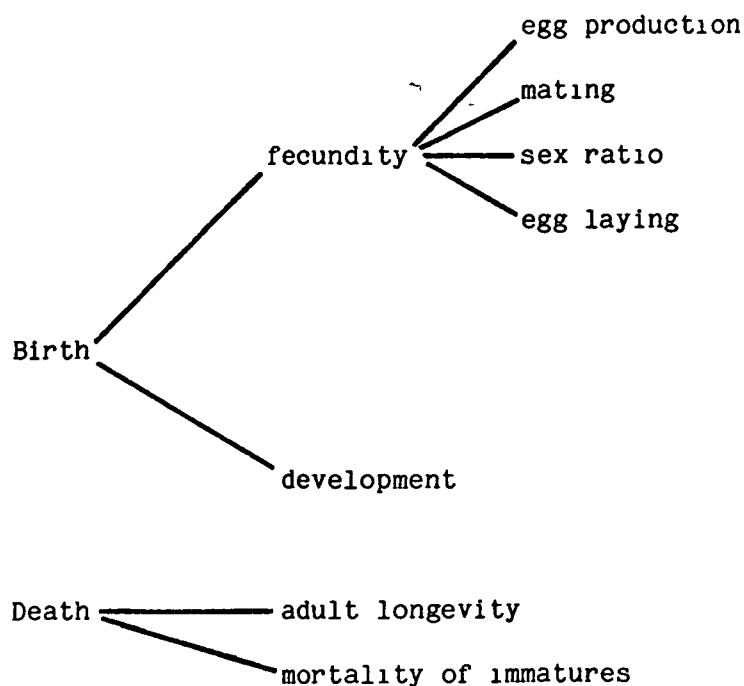
SECTION 3: LABORATORY STUDIES

SECTION 3: LABORATORY STUDIES

INTRODUCTION

Biological control is based on ecological principles and requires a thorough understanding of the population dynamics of the pest and its natural enemies within the context of the ecosystem concerned. One approach to developing this kind of understanding is to study the various component factors which cause changes in insect abundance, and how they are influenced by environmental conditions. With data of this kind and knowledge of how the factors interact, a conceptual or computer model can be developed to predict changes in the insect population under differing conditions.

The numbers of an insect species are related to the ratio of recruitment (= births) to losses (= deaths and dispersal) within a given population over a given time period. The birth rate into the population and the population death rate are governed by a number of factors:



These are all affected by a number of external factors, of which environmental conditions are amongst the most important.

In attempting to understand the population dynamics of P. citri and its parasitoids within the glasshouse, a knowledge of the effects of temperature on these aspects of the biology is particularly relevant. Observations made under natural fluctuating conditions are difficult to interpret, as the relative contribution of various parts of the fluctuating cycle cannot be separated. Laboratory studies at constant temperatures have thus been widely used to determine the thermal influences on the biology of insects, as the data from this type of work can be easily interpreted and compared. The results can then be used to construct a model, and predictions made as to what might happen under field conditions. However, it is doubtful whether the results of constant temperature studies can be applied directly in a field situation. Work under fluctuating conditions is useful in providing an indication of the way in which shorter periods at different temperatures affect insect biology and in testing the predictions made from constant temperature studies.

This section describes experiments carried out at constant and cycling temperatures to determine the thermal influences on development, mating, egg laying, searching behaviour and longevity of the mealybug parasitoids Anagyrus pseudococci, Leptomastix dactylopii and Leptomastidea abnormis and on the development and fecundity of their host.

3.1 THE EFFECTS OF TEMPERATURE ON DEVELOPMENT

3.1.1 Development of *P. citri* at various constant temperatures

Introduction

The developmental period of poikilothermic animals is known to vary with temperature (Andrewartha and Birch, 1954, Campbell and Mackauer, 1975, Kitching, 1977). The relationship between temperature and development has been widely studied over the last two centuries, but none of the numerous attempts at a mathematical description of the relationship have met with universal acceptance. However, the most commonly used and the most simple is probably the day-degree concept. This is based on a linear relationship between temperature and the rate of development, from which may be derived 2 constants to characterise the developmental process, the lower threshold for development, T_l and the thermal constant, K . These may be calculated from the formulae $T_l = -a/b$ and $K = 1/b$, where the constants a and b are determined by linear regression, using the least-sum-of squares method.

There are several reports of the life history and development of *P. citri* under glasshouse or field conditions (Myers, 1932, Sutherland, 1932, Sayed et al., 1962b), but the results of these are of limited value in quantifying the effects of temperature on mealybug development. Studies made under controlled, constant temperatures have shown that percentage mortality of both eggs and nymphal stages is greatly affected by temperature, as is the developmental period. Eggs developed most quickly at 33°C and nymphs at 28°C, with the developmental period being greatly lengthened at 17°C. The thermal constant and lower threshold for development were calculated as 258.7 D° and 13.3°C respectively for female nymphs and 264.5 D° and 12.8°C for males (Sayed et al., 1962a).

The aim of this investigation was to determine the effects of temperature on the duration of development using controlled constant temperatures. The difficulty in following the development of individuals through to completion was avoided by measuring the period from the time when the first crawler hatched in the first generation, to first crawler hatch in the second.

In this study standard techniques were then used to determine the thermal constant and lower thresholds for development for the entire life cycle, to allow comparison with the results of other workers. In section 3.1.5, more sophisticated and potentially more accurate methods will be used to describe the temperature/development relationship and predict developmental periods under glasshouse conditions.

Materials and methods

The experiments were carried out in 4 different constant temperature rooms, held to within $\pm 0.5^{\circ}\text{C}$ of the required temperature. The developmental periods were recorded at 18°C , 22°C , 26°C and 30°C . Light was supplied on an 18/6 h light/dark regime, by 5 fluorescent tubes of 30 W, 40 W and 65-80 W. Relative humidity ranged from 45-60%.

Three replicates were done at each temperature and each replicate was set up as follows. Two sprouted potatoes were placed in a clean, ventilated, plastic sandwich box (173 mm x 115 mm x 65 mm). Twenty fully completed egg masses were removed from the mealybug culture held at 26°C and dissected under a binocular microscope (45 x magnification) and the number of eggs in each one recorded. The mean number of eggs per egg mass at this temperature was then calculated. Two egg masses were then removed from the same culture and transferred onto one of the potatoes using a fine paint

brush. The second potato was provided as an alternative food source for crawlers dispersing from the other potato. The boxes were then held at 26°C and observed twice daily until the first crawler was seen to have hatched. They were then transferred to the experimental temperatures and the period from hatch of the first crawler to hatch of the first crawler in the subsequent generation was recorded.

Results

The mean developmental periods from first crawler in the parent generation to first crawler in the F1 generation are presented in table 1.

TABLE 1. Effect of various constant temperatures on the minimum life cycle length of Planococcus citri.

TEMP (°C)	LIFE CYCLE LENGTH (DAYS)		
	n	$\bar{x}$	s.d.
18	3	80.7	3.21
22	3	46.0	2.00
26	3	31.7	1.15
30	3	29.3	0.58

n = number of replicates. The mean number of eggs/egg mass was found to be 248 ± 73.71 and each replicate contained 2 egg masses. The data are therefore based on approximately 1500 individuals at each temperature.

Analysis of variance shows that temperature has a very highly significant effect on the developmental period ($P < 0.001$). The duration of development is significantly greater at 18°C than at any other temperature, and significantly greater at 22°C than at 26°C or 30°C (l.s.d. at the 0.1% level). There is no significant difference between the developmental periods at 26°C and 30°C.

The reciprocal of the developmental period (i.e. development rate) is used to determine the lower threshold for development and the thermal constant. Figure 1 shows the effect of temperature on the developmental rate of P. citri, with the regression line fitted. The data for development at 30°C was discarded as it deviated from the linear, indicating that the rate was declining due to the deleterious effects of high temperature (see Campbell et al., 1974). The lower threshold of development (T_l) was found to be 12.86°C and the thermal constant (K) 416.667 D°.

Discussion

The results confirm that temperature greatly affects the developmental rate of P. citri, the developmental period increasing at lower temperatures.

The calculated value of T_l was 12.86°C which compares well with that determined by Sayed et al. (1962) as 13.3°C, although this latter estimate was made using development from egg to adult only.

The indications from this study are that the developmental period of the mealybug is quite extended at low temperatures. However, once temperatures rise above about 22°C, the developmental period decreases considerably, and the rate of turn over of generations will therefore increase markedly. At 26°C the developmental rate is still increasing, but at 30°C the developmental rate does not show a great increase and the developmental period at this temperature is not significantly different from that at 26°C. Moderate temperatures appear to be most favourable to the mealybug.

Figure 1

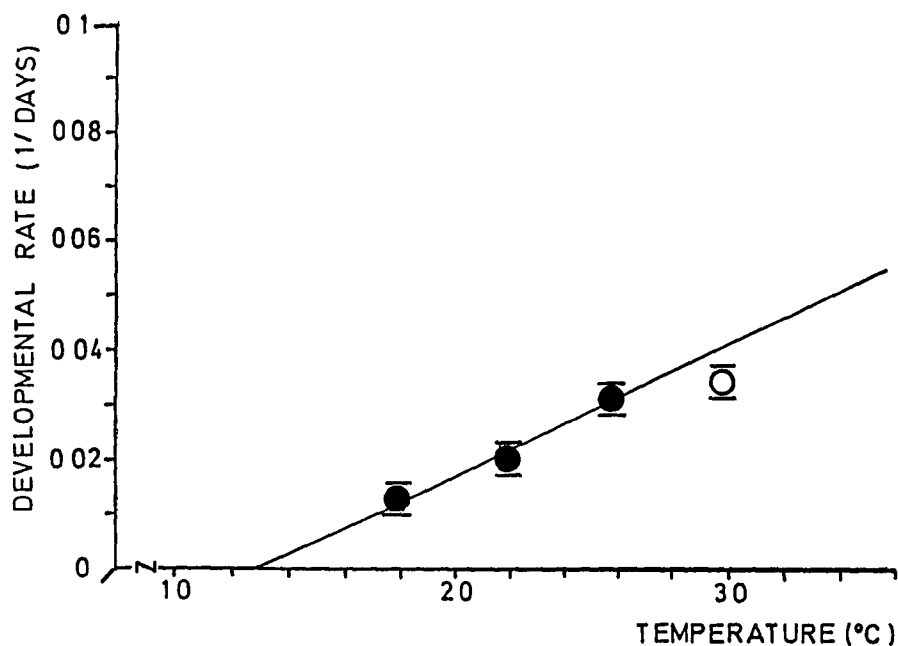


FIGURE 1 The effect of temperature on the developmental rate of Planococcus citri

The developmental period from first crawler in parent generation to first crawler hatch in F1, was measured at various constant temperatures. The rate of development was calculated by taking the reciprocal.

Points represent mean of 3 replicates (involving approx 1500 individuals) with standard deviation.

Open circles - points not used in fitting regression line (see text)

Linear regression $Y = -0.0308666667 + 0.0024 X$
 $r^2 = 99.97$

Lower threshold for development $(-a/b) = 12.86^\circ\text{C}$
 Thermal constant $(1/b) = 416.667 \text{ D}^\circ$

3.1.2 Development of the parasitoids at various constant temperatures

Introduction

Very little information is available on the influence of temperature on the biology of the mealybug parasitoids Anagyrus pseudococci (Girault), Leptomastix dactylopii Howard and Leptomastidea abnormis (Girault).

Rather more data has been published on A. pseudococci, than on the other two species. Avidov et al., (1967) measured the duration of development at 4 constant temperatures and under variable temperature regimes. They calculated the lower threshold as being 11.4°C for females and 12°C for males, with 297 and 285 day-degrees respectively, being required for complete development. The results of Chandler et al., (1980), who found mean developmental periods of 15.4 days and 15.6 days at 25.6°C, for males and females respectively, are in good agreement with those of Avidov et al.

L. dactylopii was found to require 18-25 days for complete development at 28°C (Zinna, 1959), and 28 days at 24°C (Fisher, 1963).

No precise data is available for the developmental period of L. abnormis.

The aim of this study was to elucidate the influence of temperature on the developmental rates of the three parasitoids and to use the data to determine their lower thresholds for development and thermal constants.

Materials and methods

The same experimental procedure was adopted for each of the three species: Fifteen females were taken at random from a culture containing parasitoids less than one week old. The parasitoids were anaesthetised using carbon dioxide and removed by means of an aspirator. 5 females were placed in each of three ventilated, plastic boxes (173 mm x 115 mm x 65 mm) streaked at either end with honey solution (approximately 50:50 honey to water). These were then left approximately 15 h to allow full recovery from anaesthetic and starve the females of hosts. The following day, 3 sprouted potatoes heavily infested with mealybugs were introduced into each box. The females were then allowed to oviposit for a period of 2 hours in the first series of experiments and subsequently for 5 hours, before being anaesthetised and removed. The boxes containing the parasitised mealybugs were then immediately transferred to a constant temperature room or incubator held at the experimental temperature. The offspring were allowed to develop under constant conditions and emergence was monitored twice daily. Parasitoids were removed after emergence.

Development was observed at seven different constant temperatures. Experiments at 18°C, 24°C, 26°C, and 30°C were carried out in constant temperature rooms controlled to within $\pm 0.5^\circ\text{C}$. The light was provided by 5 fluorescent tubes of 30 W, 40 W and 65-80 W, on an 18/6 h light/dark cycle. Relative humidity varied between 45% and 60%. Constant ventilation was provided. Experiments at 20°C, 35°C, and 40°C were carried out in Gallenkamp compenstat R, illuminated, cooled incubators (1.30 m x 0.50 m x 0.50 m) controlled to within $\pm 0.5^\circ\text{C}$. Constant light was provided by 4 x 8 W fluorescent lamps.

Data was recorded as the total developmental period for each individual i.e. the number of days from oviposition to emergence of the adult. The reciprocal of this value represents the rate of development, which was used to determine the lower threshold and the thermal constant for development.

Significant differences in the developmental period at different temperatures were analysed using an F-test. The significance of differences in the developmental period between species was analysed using an F-test and l.s.d. at the 5%, 1% and 0.1% levels. Differences between the sexes were analysed using a t-test (Bailey, 1959).

Results

Anagyrus pseudococci

Data for males and females are presented in table 2.

TABLE 2. Effect of various constant temperatures on the developmental period of Anagyrus pseudococci.

MEAN DEVELOPMENTAL PERIOD (DAYS)									
TEMP (°C)	MALES			FEMALES			SEXES POOLED		
	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.
17.5	17	40.5	1.87	12	40.5	2.15	29	40.5	1.96
20	32	28.9	1.77	2	31.5	0.71	34	29.0	1.84
24	15	16.7	1.44	8	17.0	2.0	23	16.8	1.61
26	42	13.5	0.55	45	14.1	0.60	87	14.0	0.82
30	8	11.1	0.35	22	11.7	0.55	30	11.6	0.57
35	26	10.1	0.67	13	11.1	0.55	39	10.5	0.79
40	-	-	-	-	-	-	-	-	-

n = number of individuals

At each temperature the developmental period was shorter than that of L. dactylopii ($P < 0.05$) or L. abnormis ($P < 0.001$). The mean developmental period was found to decrease with increasing constant temperature up to 40°C , at which temperature no development occurred. A constant 40°C was lethal to A. pseudococci. The effect of temperature on development was very highly significant, analysis of variance giving F values with $P < 0.001$ for both sexes. Mean developmental time was less for males than for females at 20°C ($P < 0.05$), 26°C ($P < 0.001$), 30°C ($P < 0.05$), and 35°C ($P < 0.001$).

Linear regression analysis of the data for the mean rate of development against temperature is shown in figure 2. The lower threshold for development, T_l was found to be 13.28°C for males and 12.99°C for females. The thermal constant, K was 182.812 D° for males and 192.730 D° for females.

Leptomastix dactylopii

Data for males and females are presented in table 3.

At each temperature the mean developmental period was shorter than that of L. abnormis, but longer than that of A. pseudococci. The mean developmental period was found to decrease with increasing temperature as it was for A. pseudococci. A constant 40°C was lethal. Differences between temperatures were very highly significant, analysis of variance giving F values with $P < 0.001$ for both sexes. Males developed faster than females at 17.5°C ($P < 0.01$), 24°C ($P < 0.001$) and 30°C ($P < 0.01$).

Figure 2

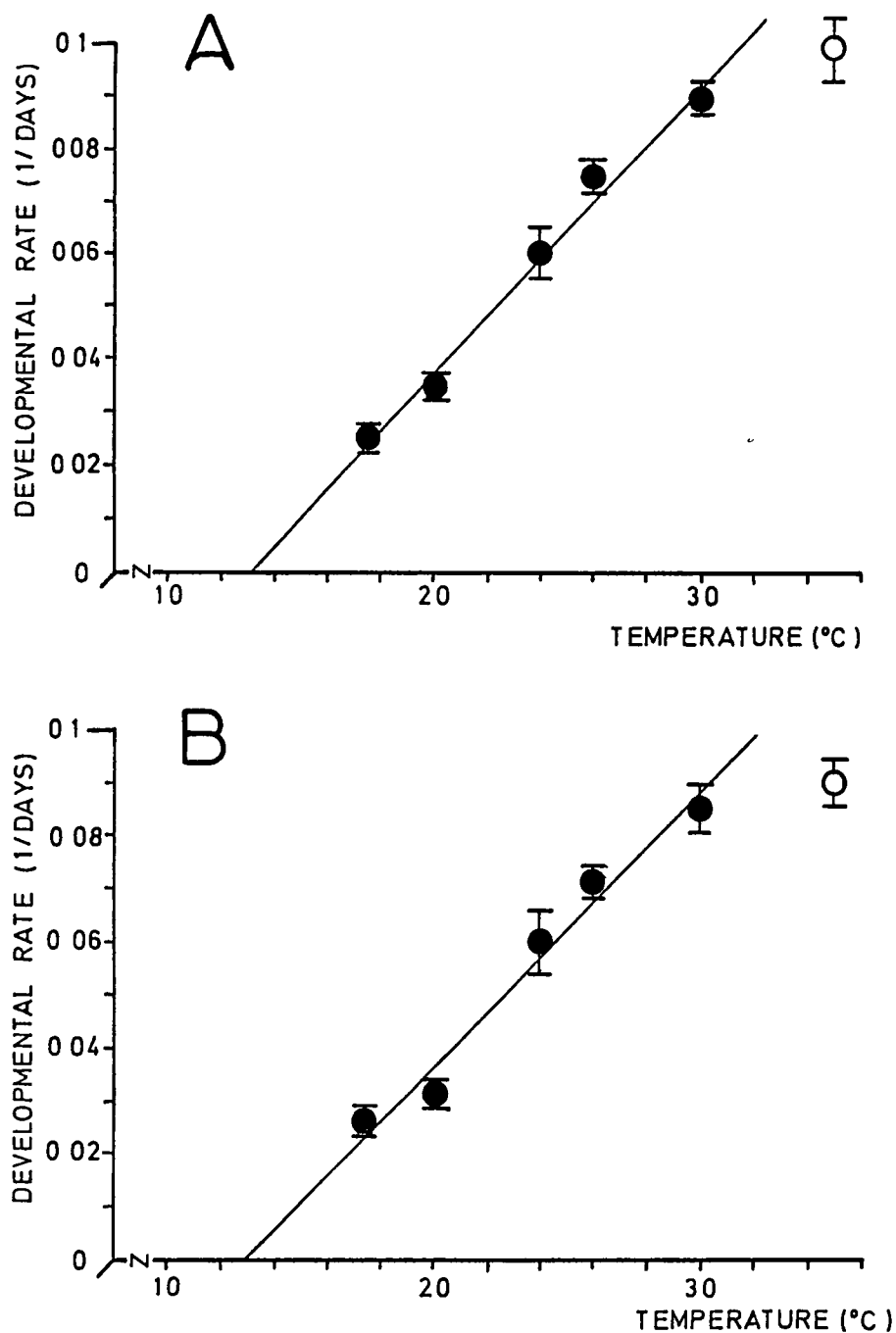


Figure 2 The effect of temperature on the developmental rate of *Anagyrus pseudococci*

Points represent mean developmental rate with standard deviation
Open circles - points not used in fitting regression line (see text)

A Males Linear regression $Y = -0.0717746907 + 0.00547041237 X$
 $r^2 = 98.65$

Lower threshold for development ($-a/b$) = 13.28°C
Thermal constant ($1/b$) = 182.812 D°

B Females Linear regression $Y = -0.0674235051 + 0.00518865979 X$
 $r^2 = 97.51$

Lower threshold for development ($-a/b$) = 12.99°C
Thermal constant ($1/b$) = 192.73 D°

TABLE 3. Effect of various constant temperatures on the developmental period of Leptomastix dactylopii.

MEAN DEVELOPMENTAL PERIOD (DAYS)									
TEMP (°C)	MALES			FEMALES			SEXES POOLED		
	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.
17.5	6	44.2	0.81	12	45.9	2.15	18	45.3	1.94
20	24	33.1	0.61	3	34.3	2.31	27	33.3	0.94
24	12	18.1	0.29	7	19.1	0.38	19	18.5	0.61
26	14	14.6	0.51	27	15.6	1.33	41	15.3	1.22
30	13	12.3	0.63	8	12.9	0.83	21	12.5	0.75
35	9	11.5	0.53	19	12.6	0.69	29	12.3	0.85
40	-	-	-	-	-	-	-	-	-

n = number of individuals

Linear regression analysis of the data for the mean rate of development at various constant temperatures is shown in figure 3. The lower threshold T_l is 13.22°C and 13.16°C for males and females respectively. The thermal constant K is 199.013 D° for males and 208.559 D° for females.

Leptomastidea abnormis

Data for males and females are presented in table 4.

Again, the mean developmental period decreased with increasing temperature, but the lethal constant temperature was found to be lower, occurring at 35°C. Analysis of variance showed the relationship to be very highly significant ($P < 0.001$) for both sexes. Males developed faster at 20°C and 30°C ($P < 0.05$).

Figure 3

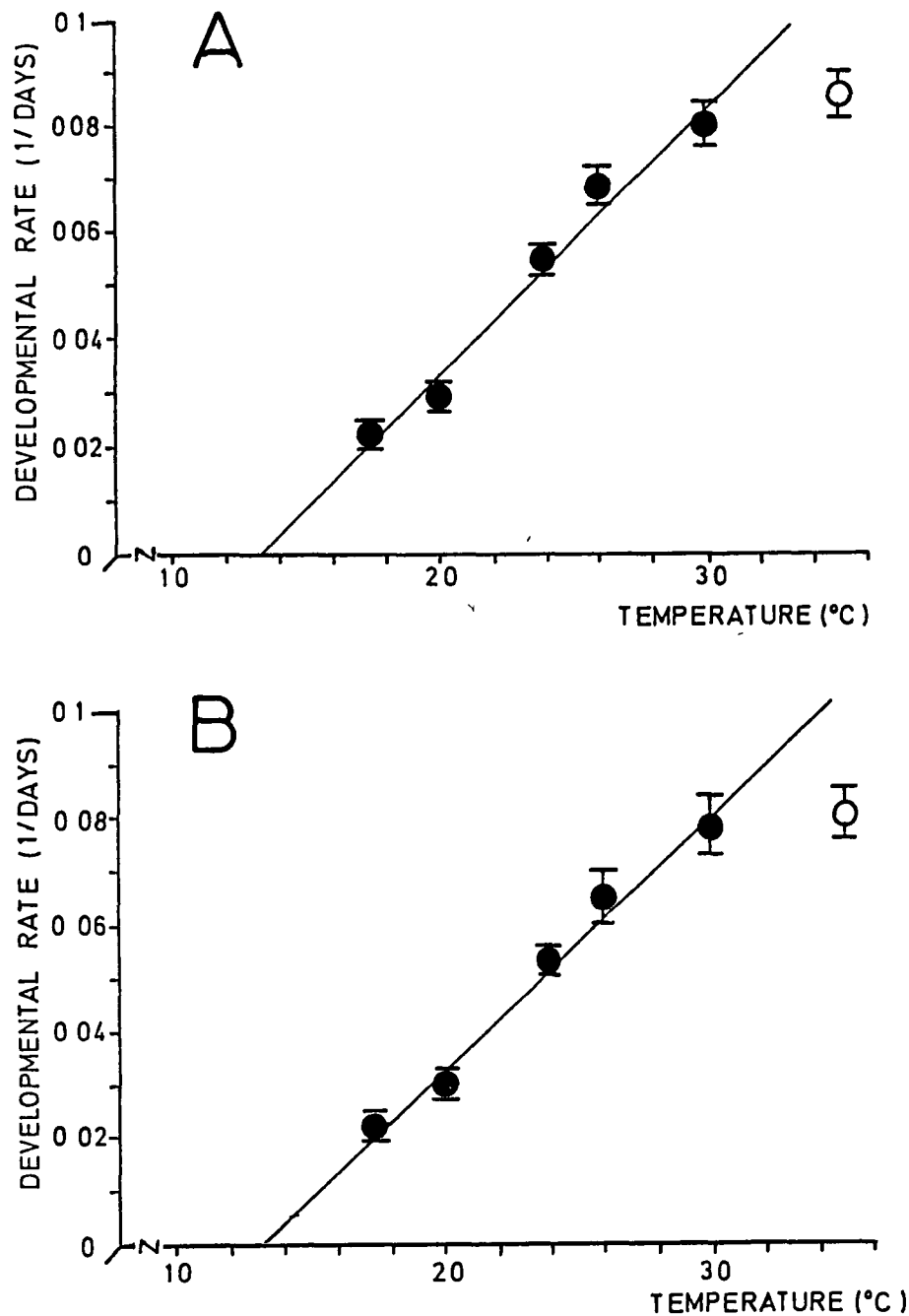


Figure 3 The effect of temperature on the developmental rate of *Leptomastix dactylopii*

Points represent mean developmental rate with standard deviation
Open circles - points not used in fitting regression line (see text)

A Males Linear regression $Y = -0.0664363277 + 0.00502476288 X$
 $r^2 = 97.55$

Lower threshold for development ($-a/b$) = 13.22°C
Thermal constant ($1/b$) = 199.013 D°

B Females Linear regression $Y = -0.0625008865 + 0.00474948453 X$
 $r^2 = 98.29$

Lower threshold for development ($-a/b$) = 13.16°C
Thermal constant ($1/b$) = 208.559 D°

TABLE 4. Effect of various constant temperatures on the developmental period of Leptomastidea abnormis.

MEAN DEVELOPMENTAL PERIOD (DAYS)									
TEMP (°C)	MALES			FEMALES			SEXES POOLED		
	n	x	s.d.	n	x	s.d.	n	x	s.d.
18	19	45.6	1.86	4	47.5	3.00	23	45.9	2.14
20	7	40.3	2.50	21	44.0	3.98	28	43.1	3.98
24	21	26.0	2.05	10	26.9	3.00	31	26.3	2.38
26	6	22.5	2.66	10	22.8	1.93	16	22.7	2.15
30	7	17.9	1.34	12	20.5	2.28	19	19.5	2.34
35	-	-	-	-	-	-	-	-	-
40	-	-	-	-	-	-	-	-	-

n = number of individuals.

Linear regression analysis of the data for the mean rate of development at various constant temperatures is shown in figure 4. The lower threshold T_1 is 11.03°C for males and 10.07°C for females, with the values of the thermal constant being 336.791 D° and 386.698 D° for males and females respectively.

Discussion

As expected, the developmental period of all species decreased as the temperature rose. The maximum developmental rate occurred at around 35°C for both A. pseudococci and L. dactylopii and at around 30°C for L. abnormis. A constant 40°C was lethal to all species, and L. abnormis also failed to develop at a constant 35°C. However, this does not necessarily mean that short periods at these temperatures would also prove lethal (see section 3.1.3 and 3.1.4). In general high temperatures will cause rapid development, hence increasing the birth rate into the population and are thus likely to be favourable to parasitoid populations.

Figure 4

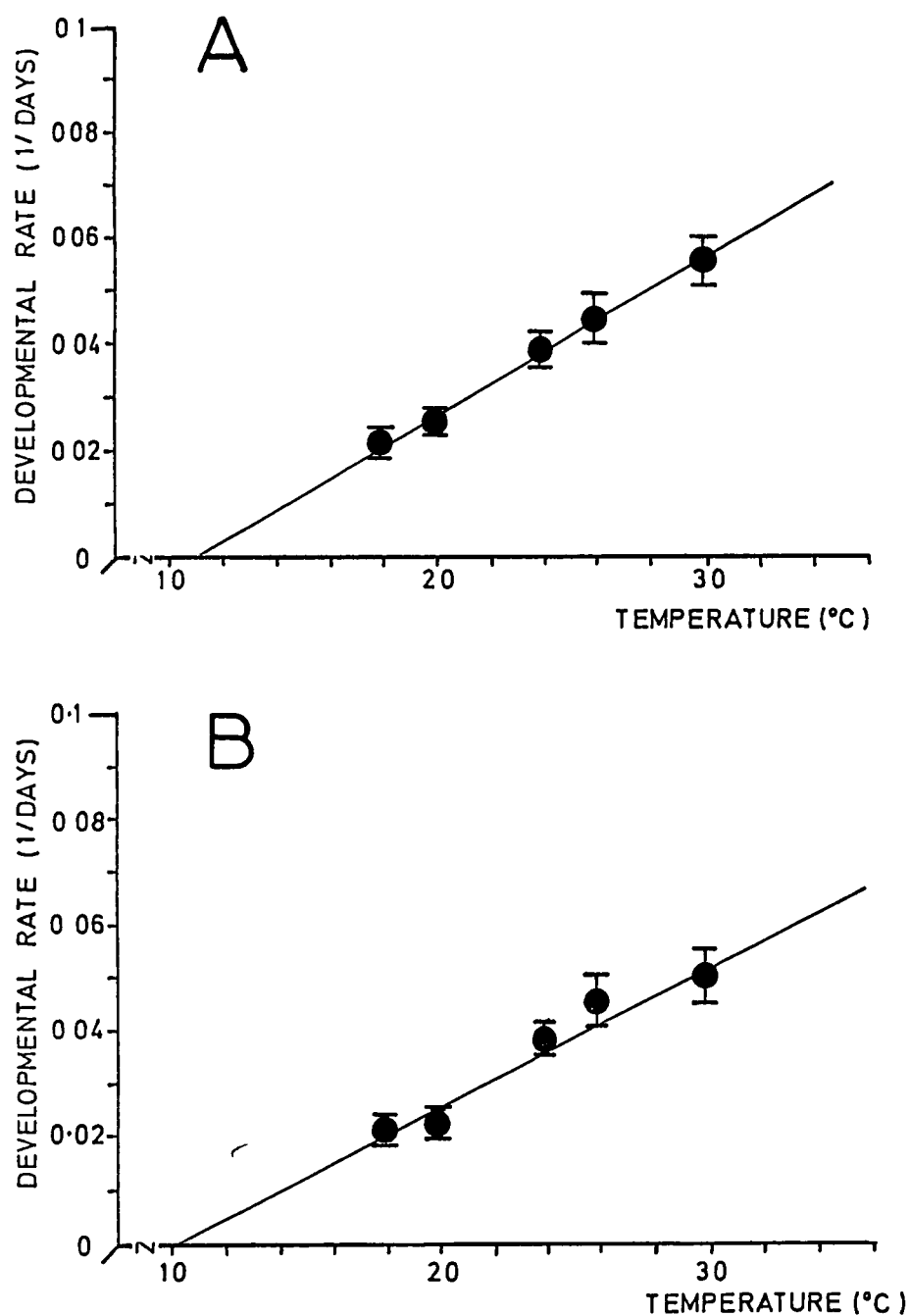


Figure 4 The effect of temperature on the developmental rate of *Leptomastidea abnormis*

Points represent mean developmental rate with standard deviation

A Males Linear regression $Y = -0.0327373683 + 0.00296921052 X$
 $r^2 = 99.21$

Lower threshold for development ($-a/b$) = 11.025°C
 Thermal constant (1/b) = 336.791 D°

B Females Linear regression $Y = -0.026046842 + 0.00258605263 X$
 $r^2 = 94.96$

Lower threshold for development ($-a/b$) = 10.07°C
 Thermal constant (1/b) = 386.698 D°

A. pseudococci developed more quickly than the other two species, and L. dactylopii developed more quickly than L. abnormis at all temperatures. However, the differences between L. dactylopii and L. abnormis were least pronounced at lower temperatures.

The results are in good agreement with those of Avidov et al., (1967), who determined the developmental period of A. pseudococci at four constant temperatures. They also found that males generally developed slightly faster, although they did not test the significance of the differences. These authors also calculated the threshold of development ($T_l = 11.4^{\circ}\text{C}$ and 12.0°C for males and females respectively) and the thermal constant ($K = 285 \text{ D}^{\circ}$ for males and 297 D° for females), but this was done from data on the developmental period under variable temperatures. The values they give are at variance with those presented herein. There are two possible reasons for this. Firstly, the highest constant temperature used in their experiments was 32°C . At this temperature the data was probably deviating from the assumed linear relationship, and if included in the regression analysis would have affected the calculated value of T_l and K . Secondly, their values were determined using the average temperature of a variable regime. It has frequently been noted that this practice produces different results from those obtained using constant temperatures (Huffaker, 1944; Pradhan, 1945, Lin, Hodson and Richards^h, 1954; Messenger, 1964a, Eubank et al., 1973; Siddiqui et al., 1973; Yeargan, 1983).

Data on the rate of development of L. dactylopii and L. abnormis are sparse. A developmental period of 18-25 days at 28°C for L. dactylopii (Zinna, 1959) seems somewhat slower than those found in this study. No comparable data are available for L. abnormis.

Figure 5

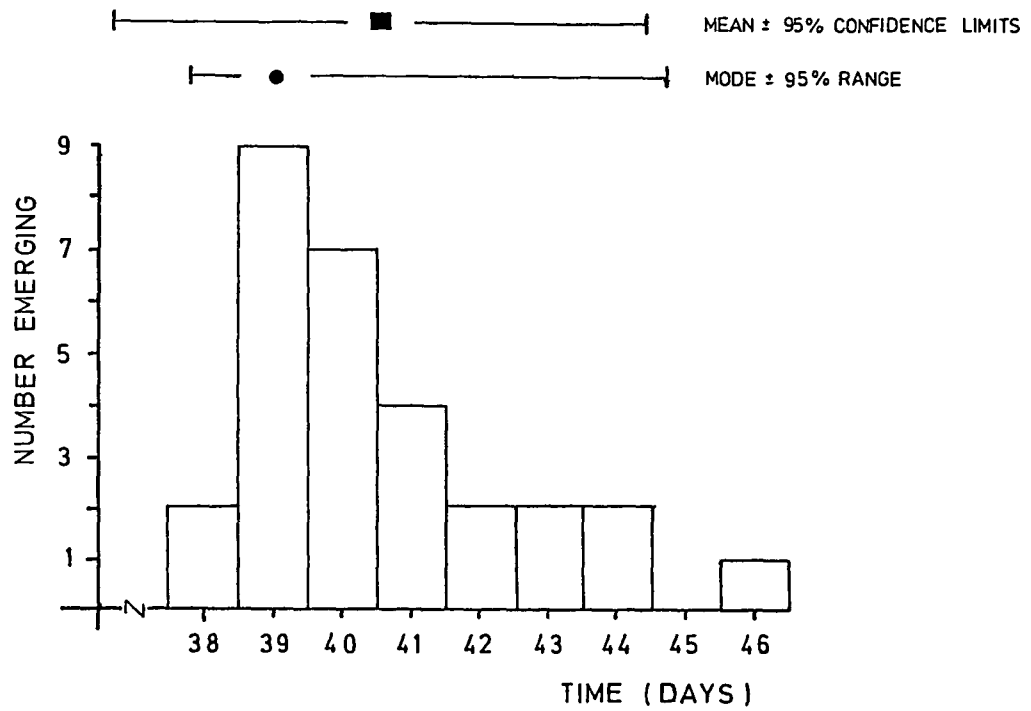


Figure 5 Distribution of emergence times for Anagyrus pseudococci at 17.5°C

Note positive skew

Mean $\pm$ 95% confidence interval

Mode $\pm$ 95% range

The results have been presented above using standard techniques, data for the developmental period being presented as a mean and standard deviation, allowing standard statistical techniques to be used for analysis. In doing this the assumption is made that the data is normally distributed. In practice the distribution of development times shows a distinct positive skew and thus the standard deviation does not give an accurate measure of the variation around the mean. This is illustrated in figure 5 using the data for the developmental period of A. pseudococci at 17.5°C.

Several attempts have been made to overcome this problem by using another measure of central tendency (Howe, 1967; Eubank et al., 1973) or by developing a stochastic model of development times (Stinner et al., 1975; Sharpe et al., 1977). The use of the mode or median as an alternative to the mean presents problems if statistical analysis of the resulting data is required and has not been generally accepted. The stochastic approach adds another dimension to the handling of data, but has been used in more sophisticated models of insect development. It has been demonstrated that the use of development rate diminishes the observed skew (Sharpe et al., 1977). In general the errors incurred in representing developmental periods by a mean value are probably small, but the distribution of emergence can be more accurately represented using the 95% range (see section 3.1.5).

3.1.3 The effect of cycling temperatures on development of the parasitoids

Introduction

It is universally accepted that the simplest way to determine the relationship between temperature and development is to observe development at various constant temperatures (Sharpe et al., 1977). However, there is some dispute as to whether the results of such constant temperature studies may be directly related to the fluctuating temperatures of the field. Several authors have reported acceleration or retardation of developmental processes, when comparisons are made between the developmental periods at cycling temperatures, and a constant temperature equivalent to the mean of the cycling regime (Huffaker, 1944; Pradhan, 1945; Lin et al., 1954; Messenger, 1964a; Kindler and Staples, 1970; Eubank et al., 1973, Siddiqui et al., 1973; Yeargan, 1983).

A second problem with the use of constant temperatures is that developmental periods can only be determined at temperatures within the favourable range. The effects of temperatures outside this range must then be estimated by extrapolation from the relationship within the favourable range, on a purely theoretical basis (Andrewartha and Birch, 1954).

The use of cycling temperature regimes provides a method of gaining experimental data on the effects of short periods at temperatures which produce high mortality or other detrimental effects, when held constantly. Assuming that the rate of development at any given temperature is a constant, then the proportion of an insect's development which is completed in an hour at any temperature can be calculated as $1/24 \times 1/\text{developmental period at that temperature}$. The developmental period under a cycling

regime can then be determined by summing the increments for each hour at each temperature. If a 12/12 h cycle is used, with half of the time at a favourable temperature (e.g. 26°C) and half at a low temperature, then the lower threshold could be determined if the time at the lower temperature contributes nothing to the developmental period. Progressively lower temperatures could be used until the developmental period under the 12/12 h cycling regime is not significantly different from twice that at a constant 26°C. The lower temperature would then be making no significant contribution to the development, and could be taken as the lower threshold.

Similarly the effects of high temperatures, which are lethal when held constantly, could be determined in this way. Shorter periods of time at these temperatures may be detrimental, without actually killing the insect. Using the logic described above, the upper threshold could be found using a 12/12 h cycling regime with half the time at 26°C and half at progressively higher temperatures until there is no significant difference between the developmental period at the cycling regime and at a constant 26°C.

If predictions are to be made of insect development under variable temperatures i.e. in the field or glasshouse, then it is essential that the effects of fluctuating conditions be understood. The aim of this study was to determine whether development of A. pseudococci, L. dactylopii, and L. abnormis speeds up under rhythmically fluctuating temperature regimes and secondly to use cycling regimes to determine the upper and lower thresholds for development.

Materials and methods

The experimental procedure was similar to that used in the constant temperature studies, females being allowed to oviposit for 5 hours at 26°C. The experimental temperatures were achieved using the Gallenkamp compenstat R incubators mentioned above. The incubators were set on a 12/12 h cycle with the lower temperature starting at 2200, and the higher temperature starting at 1000. A constant light regime was employed.

The temperatures used were. 26°C/12°C; 26°C/15°C; 26°C/18°C; 26°C/38°C; 26°C/40°C, with each temperature controlled to within $\pm 0.5^\circ\text{C}$.

Significance of differences were tested using Students t-test if variances could be assumed equal after an F-test, and d-test where variances could not be assumed equal (Bailey, 1959).

Results

A. pseudococci

The effects of several cycling temperature regimes on the developmental period of A. pseudococci are shown in table 5. Developmental periods at a constant 20°C, 26°C and 30°C are also included for comparison.

The mean developmental period for both males and females, under the 26/18°C regime was significantly shorter than that under the 26/15°C regime ($P < 0.001$), which in turn was significantly shorter than under the 26/12°C regime ($P < 0.001$). In all three cases development proceeded faster than 2 x that at a constant 26°C ($P < 0.001$).

TABLE 5. Effect of various cycling temperature regimes on the developmental period of Anagyrus pseudococci.

MEAN DEVELOPMENTAL PERIOD (DAYS)									
TEMP (°C)	MALES			FEMALES			SEXES POOLED		
	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.
26/12	13	26.0	1.87	7	26.3	0.49	20	26.1	1.52
26/15	33	23.8	1.48	59	25.4	1.55	92	24.8	1.71
26/18	44	20.9	1.3	36	21.6	0.84	80	21.2	1.17
26/38	11	13.7	1.19	5	14.0	0.71	16	13.8	1.05
26/40	5	14.2	0.45	5	14.6	0.55	10	14.4	0.52
Const 20	32	28.9	1.77	2	31.5	0.71	34	29.0	1.84
Const 26	42	13.5	0.55	45	14.1	0.60	87	14.0	0.82
Const 30	8	11.1	0.35	22	11.7	0.55	30	11.6	0.57

All regimes alternating 12 hours at each temperature.
n = number of individuals.

Under the 26/38°C regime the developmental period was not significantly different from than at a constant 26°C, but under the 26/40°C regime, the developmental period was 14.4 days (sexes pooled), significantly longer than at a constant 26°C ($P < 0.05$).

L. dactylopii

The effect of cycling temperatures on the developmental period are presented in table 6. The data for a constant 26°C, 20°C and 30°C are included for comparison. The trends are similar to those reported for A. pseudococci.

There was no significant difference between the mean developmental periods under the 26/12°C regime and at 2 x constant 26°C.

TABLE 6. Effect of various cycling temperature regimes on the developmental period of Leptomastix dactylopii.

MEAN DEVELOPMENTAL PERIOD (DAYS)									
TEMP (°C)	MALES			FEMALES			SEXES POOLED		
	n	x	s.d.	n	x	s.d.	n	x	s.d.
26/12	22	29.0	1.67	53	30.7	1.68	75	30.2	1.83
26/15	29	28.1	1.3	17	28.8	0.81	46	28.4	1.18
26/18	25	21.4	0.82	51	22.3	0.84	76	22.0	0.94
26/40	1	17.0	0.0	-	-	-	1	17.0	0.0
Const 20	24	33.1	0.61	3	34.3	2.31	6	33.3	0.94
Const 26	14	14.6	0.51	27	15.6	1.33	41	15.3	1.22
Const 30	13	12.3	0.63	8	12.9	0.83	21	12.5	0.75

All regimes alternating 12 hours at each temperature.
n = number of individuals.

Under the 26/18°C regime the mean developmental period was significantly less than under the 26/15°C regime ($P < 0.05$ for males and $P < 0.001$ for females), and the mean developmental period was significantly less under the 26/15°C regime than under the 26/12°C for both males and females ($P > 0.05$). Pooled data for males and females, showed that the developmental period under both these regimes was significantly shorter than at 2 x 26°C ($P < 0.001$).

Under the 26°C/40°C regime mortality was very high, only one male emerging. This male took significantly longer to develop than would have been expected at a constant 26°C ($P < 0.01$).

L. abnormis

The results are presented in table 7, along with data for a constant 26°C and 30°C. Again the trends are similar to those above.

TABLE 7. Effect of various cycling temperature regimes on the developmental period of Leptomastidea abnormis.

MEAN DEVELOPMENTAL PERIOD (DAYS)									
TEMP (°C)	MALES			FEMALES			SEXES POOLED		
	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.
26/12	20	38.4	1.43	20	39.8	1.20	40	39.1	1.48
26/18	46	30.4	2.48	27	33.1	2.20	73	31.4	2.70
26/40	8	20.5	2.27	11	22.0	2.14	19	21.5	2.35
Const 26	6	22.5	2.66	10	22.8	1.93	16	22.7	2.15
Const 30	7	17.9	1.34	12	20.5	2.28	19	19.5	2.34

All regimes alternating 12 hours at each temperature.
n = number of individuals.

The mean developmental period under the 26/18°C regime was significantly shorter than that under the 26/12°C regime ($P < 0.001$). In both cases the developmental period was significantly less than at 2 x that at a constant 26°C ($P < 0.001$: sexes pooled).

There was no significant difference between the mean developmental period under the cycling 26/40°C regime and that at a constant 26°C.

Discussion

Evidence of an accelerating effect of fluctuating temperatures: The standard procedure for identifying an effect of this type has been to compare the developmental period under alternating regimes with that at a constant temperature equivalent to the mean of the alternating regime. When treated in this way the data presented for the developmental periods of the parasitoids, can be shown to exhibit an accelerating effect of fluctuating temperatures. For example, comparing the developmental periods

of A. pseudococci and L. dactylopii under the 26/15°C regime (equivalent mean 20.5°C) with those at a constant 20°C, suggests that the fluctuating temperature may indeed be speeding up development. Development under the fluctuating regime is significantly faster than that at the equivalent constant temperature ($P < 0.01$) for both species. However, the developmental period under the 26/40°C regime (equivalent mean 33°C) is significantly slower than that at a constant 30°C and a constant 35°C (no data for constant 33°C), suggesting that in this case the fluctuating regime has a considerable retardative effect. Similarly conflicting results have been found by previous workers with other insects (Siddiqui et al., 1973). Pradhan, (1945) showed how this type of effect could be produced as an artifact, due to interpreting the temperature development relationship as linear. It can be seen from figures 2, 3, and 4 that this is obviously not an accurate description of the relationship towards the temperature extremes. The overall shape of the curve tends towards the sigmoidal. Direct comparison should not therefore be made between the developmental period at a constant temperature and that at an alternating regime with the equivalent mean temperature. If this comparison is made, any accelerating effect of fluctuating temperature is due to mathematical misinterpretation and cannot be attributed to any real effect on developmental processes.

Evidence for an accelerating effect of fluctuating conditions could be shown if the developmental period observed under a given fluctuating regime was shorter than that calculated from the hourly increments at each of its constituent temperatures (Messenger, 1964a). For example, if the developmental period observed under a 12 h 18°C/12 h 26°C regime is significantly shorter than the developmental period calculated by summing the contributions made to the development of each hour at a constant 18°C and a constant 26°C, there would be support for the hypothesis that

fluctuating conditions do accelerate the developmental rate.

Table 8 shows the developmental periods of the three parasitoids under a 12 h 26/12 h 18°C alternating regime, calculated as described above, by determining the amount that development is incremented by each hour at the constituent temperatures. Unfortunately, data at a constant 18°C were not available for either A. pseudococci or L. dactylopii, and the data for 17.5°C were used instead. The calculated developmental periods of these two parasitoids could therefore be expected to be rather longer than would be true if data at 18°C were available. However, the calculated developmental period under the fluctuating regime for A. pseudococci was in fact shorter than the actual observed developmental period under this regime. The calculated results for all three species are in close agreement with the observed developmental periods under the 26/18°C regime. There is thus no evidence of an accelerating effect of fluctuating temperatures, and the indications are that thermal summation on an hourly basis is a valid way of treating development data. These results are at variance with those of Messenger (1964a). However, these experiments were carried out using insects from the same cultures, over the same time period, using the same facilities as those at constant temperatures. Messenger compared his results under fluctuating conditions with those of another worker, who had carried out experiments at constant temperatures some 4 years earlier. Despite the author's justification for this, some doubt must remain as to the differences in the conditions and the clones of aphid used over such an extended time period.

TABLE 8. Calculation of developmental periods at cycling temperatures from constant temperature data.

	<u>A. pseudococci</u>	<u>L. dactylopii</u>	<u>L. abnormis</u>
Dev. period at 18°C	40.52 days	45.33 days	45.95 days
Dev. completed/day	0.0246791	0.0220604	0.0217627
Dev. completed/12 hours	0.0123395	0.0110302	0.0108813
Dev. period at 26°C	14.05	15.27	22.69
Dev. completed/day	0.0711743	0.0654878	0.0440722
Dev. completed/12 hours	0.0355871	0.0327439	0.0220361
Dev. completed at 18/26°C (24 h)	0.0479266	0.0437741	0.0329174
Calc. Dev. period 18/26°C	20.86 days	22.84 days	30.38 days
obs. dev. period at 18/26°C	21.19 days	22.01 days	31.39 days

Threshold determination: Following from this, it is possible to determine lower and upper thresholds from experiments under alternating temperature regimes. The lower threshold is defined as the temperature at which no development occurs (Wilson and Barnett, 1983). Temperatures at the lower threshold will not, therefore, contribute significantly to the development, when cycled 12 h/12 h with a favourable temperature e.g. 26°C. The upper threshold is less well defined, but may be taken as that at which the developmental rate begins to decrease. Temperatures at the upper threshold will not change the developmental period, compared with that at 26°C, when cycled in the same manner.

The lower threshold for development determined in this way, is below 12°C for A. pseudococci, as the developmental period under the 26/12°C regime was shorter than that at 2 x a constant 26°C. For L. dactylopii the lower threshold lies between 12°C and 15°C, as the developmental period under the 26/15°C was shorter than that at 2 x a constant 26°C, whereas the developmental period under the 26/12°C regime was not. The lower threshold lies well below 12°C for L. abnormis, as the developmental period under the 26/12°C for this species, is considerably shorter than that at 2 x a

constant 26°C. These experimentally determined thresholds can be compared with estimates of 12.71°C; 13.145°C and 10.28°C respectively, determined by linear regression (see section 3.1.1). With the exception of the estimate for A. pseudococci, these appear to be relatively consistent. However, these estimates have no biological basis, and the experimentally determined thresholds are likely to be more reliable.

The effects of high temperatures, when cycled with a temperature of 26°C, can also be used to locate upper thresholds for the three species. The 26/38°C regime resulted in a developmental period very similar to that at a constant 26°C for A. pseudococci, whilst the 26/40°C retarded its development. The upper threshold for A. pseudococci thus lies around 38°C. Unfortunately no data is available for the development of L. dactylopi at 26/38°C, but it is apparent that at the 26/40°C this insect is nearing the limits of its temperature tolerance. Its upper threshold is probably considerably below 40°C. L. abnormis, on the contrary appears very tolerant of high temperature and its upper threshold is around 40°C. Under the 26/40°C regime its developmental period is not significantly different from that at a constant 26°C. This is in marked contrast to the results of constant temperature studies, where L. abnormis failed to develop at a constant 35°C (section 3.1.2). This may have been a result of the effect of temperature on the host. L. abnormis parasitizes relatively young mealybug instars, which are likely to be more susceptible to high temperatures than are older stages. Dessication and death of the host is likely to result in death of the parasitoid as well, and it may be that the hosts are better able to tolerate 12 hours at high temperature, interspersed with periods at a favourable temperature than slightly lower temperatures, constantly. Further work would be necessary to confirm this, as the parasitoid itself may also be less tolerant to extended periods at high temperature.

Another feature worthy of note is that there is evidence of increasing mortality towards the temperature extremes. This is apparent in all three species at high temperatures, with fewer individuals emerging in the experiments using the 26/38°C and 26/40°C regimes than in those at medial temperatures. A. pseudococci and L. abnormis also seem to show susceptibility in this way at low temperatures, as fewer individuals emerged in the 26/12°C regime. A. pseudococci shows this trend most strongly, but it is not apparent in the data for L. dactylopii. Oviposition took place at 26°C in all cases, and thus the number in each sample could be expected to be similar. However, egg laying is extremely variable in these parasitoids (see section 3.4.2) and the differences observed can only be taken as an indication that low and high temperatures may be producing higher levels of mortality.

3.1.4 The effect of high temperature on the survival of immature stages of the parasitoids

Introduction

The experiments carried out at constant temperatures showed that above a certain temperature, development could not proceed to completion. For the two parasitoid species A. pseudococci, L. dactylopi development was not completed above 40°C, whilst for L. abnormis the limiting temperature was 35°C. In contrast, the experiments carried out under alternating temperature regimes, suggested that L. abnormis could tolerate temperatures of 40°C for 12 hours a day, provided the rest of the time was at a favourable temperature. L. dactylopi seemed less tolerant of the cycling 26/40°C regime than L. abnormis or A. pseudococci, with only one male surviving through to adulthood. The data from alternating temperature studies also indicated that mortality was occurring at high temperatures, even when cycled with 12 h at 26°C.

In nature, constant high temperatures of this kind would never be encountered, but temperatures even higher than 40°C may, at times, occur in the field or glasshouse for short periods. In the Wye College conservatory, hourly maximum temperatures of 39.5°C have been recorded.

The California red scale parasitoids Aphytis melinus and A. chrysomphali were found to succumb within a short period at temperatures between 40-48°C, depending on the developmental stage, A. melinus being the more tolerant of the two species. For both species, eggs were found to be the most susceptible to high temperature and pupae the most tolerant (Abdelrahman, 1974).

The aim of this study was to determine the effects of short periods of high temperature on the mortality of immature stages of the three parasitoid species. The developmental stages may have different temperature tolerances and thus the influence of high temperatures was studied on eggs, larvae and pupae separately.

Materials and methods

Parasitized mealybugs containing 1 day old eggs, 6 day old larvae and 13 day old pupae (for A. pseudococci and L. dactylopii) or 19 day old pupae (for L. abnormis) were obtained using a similar procedure to that in the constant temperature studies. All were raised to the appropriate stage at a constant 26°C. Potatoes infested with these parasitized mealybugs were transferred into glass jars with screw caps, incubating in water baths held at the experimental temperatures 40°C, 42°C, 45°C and 50°C. Fluctuations in these temperatures were so small as to be immeasurable with a normal thermometer. The mealybugs were left for periods of 1 h, 4 h and 16 h before being transferred back into ventilated, plastic boxes and incubated at 26°C until the parasitoids emerged. At 50°C the 4 h and 16 h incubations were omitted, but the 1 h incubation was replicated three times. Emergence was monitored daily and adult parasitoids removed.

Controls were treated in exactly the same way, but were placed in a water bath at 26°C for the 16 h duration of the experiment.

Results and discussion

The results are presented for the three species separately in tables 9, 10 and 11.

The experiments were not repeated and thus it is not possible to make statistical comparisons between the number of offspring emerging from the different treatments. However, it appears that relatively long exposures at high temperatures are necessary to produce 100% mortality of immature stages of the parasitoids. Eggs, larvae and pupae of all three species were killed by exposure to a temperature of 45°C for 16 hours. In contrast to the results of Abdelrahmen, (1974), there was no evidence that any of the developmental stages were more susceptible to high temperatures than any other, and no evidence of differences between the species.

In the glasshouse, temperatures would only reach 40°C for a few hours a day, and thus it is unlikely that high levels of temperature induced mortality will occur in practice.

TABLE 9. Effect of high temperature on survival of immature stages of Anagyrus pseudococci

		NUMBER OF ADULTS EMERGING							
Temperature (°C)	26	40		42		45		50	
exposure time (h)	16	1	4	16	1	4	16	1	1
eggs	9	13	3	17	15	3	5	4	22
larvae	16	5	4	24	41	18	14	10	5
pupae	27	43	23	32	33	20	13	53	60
								0	0
								6	3
								8	14
								20	24
									54

Number of adults emerging after exposure as eggs, larvae, or pupae to various periods at a number of high temperatures.

TABLE 10. Effect of high temperature on survival of immature stages of Leptomastix dactylopii

		NUMBER OF ADULTS EMERGING									
Temperature (°C)		40			42			45			50
exposure time (h)											
	16	1	4	16	1	4	16	1	4	16	1 1 1
eggs	16	34	4	0	17	13	1	8	1	0	5 8 3
larvae	22	13	18	7	15	29	17	27	10	0	12 4 5
pupae	45	126	73	70	6	5	38	12	35	0	5 23 37

TABLE 11. Effect of high temperature on survival of immature stages of Leptomastidea abnormis

		NUMBER OF ADULTS EMERGING									
Temperature (°C)		40			42			45			50
exposure time (h)											
	16	1	4	16	1	4	16	1	4	16	1 1 1
eggs	13	8	4	7	4	4	1	1	6	0	11 5 7
larvae	17	18	3	11	5	3	0	6	17	0	9 4 5
pupae	12	8	5	1	26	20	1	25	6	0	21 19 91

Number of adults emerging after exposure as eggs, larvae, or pupae to various periods at a number of high temperatures.

3.1.5 Prediction of developmental periods under fluctuating conditions from constant temperature data

Introduction

The aim of carrying out constant temperature studies on the development of P. citri and its parasitoids was to determine the relationship between temperature and development. Once this relationship has been defined it can be used to predict how development will proceed under the fluctuating temperature of the glasshouse. Interest in this type of prediction dates back to the 1800's and numerous models have been used in an attempt to get accurate results. The simplest use a rate summation approach based on mean developmental rate at each temperature, whilst the most complex involve stochastic elements to take account of the distribution of emergence times and non-linear functions to predict from these across temperature (Wagner et al., 1984). Polynomial regression analysis was found to give an excellent, empirical description of developmental rates at various constant temperatures for the McDaniel spider mite (Tanigoshi et al., 1975), but has not been used to predict development under fluctuating conditions.

Whatever the method used the predictions must be tested against actual developmental periods under natural conditions. The measurement of natural temperatures can be made in a number of ways, again involving different degrees of complexity. The simplest is a maximum/minimum thermometer, read once a day and the most sophisticated might involve hourly records of the microclimate of a particular environment, using extremely accurate electronic sensors.

In general the method used to predict development under fluctuating conditions should be selected to match the accuracy of actual temperature

readings (Howe, 1967). Several authors have found that predictions from more complex descriptions of the temperature/development relationship do not give significant improvement over day degree estimations, when field temperatures are calculated from the minimum and maximum, using Allen's sine wave method (Whalon and Smilowitz, 1979; Allsopp, 1981).

The aim of this study was to compare the accuracy of predictions of the day degree method and a polynomial regression technique with actual development times under glasshouse conditions using hourly records of ambient temperature obtained from electronic sensors in aspirated screens.

Materials and methods

Developmental periods under glasshouse conditions. The same procedure was adopted for each parasitoid species. Five female parasitoids, 1-7 days old were transferred from a culture to a plastic box (173 mm x 155 mm x 65 mm), of which the top and bottom had been removed and replaced with 40 mesh/cm nylon gauze. The ends of the box had previously been streaked with honey solution. The females were left about 15 h at 26°C. Three mealybug infested, sprouted potatoes were then introduced and left for 5 h, after which time the female parasitoids were removed under carbon dioxide anaesthesia. The boxes were then taken to the Wye College conservatory and suspended from the branches of a Guava bush or a large Ficus benjamina, by means of a short length of string (see plate 14). This ensured that air movement around the box was unrestricted and that the boxes were partially shaded from direct sunlight. The boxes were located near to the temperature sensors, so that ambient temperature was similar to that being recorded.



PLATE 14. Ventilated, plastic box used for determination of developmental periods under glasshouse conditions, in the Wye College conservatory.

The procedure with P. citri was different. Two mature egg masses were removed from culture and placed on a clean, sprouted potato tuber. The tuber was placed in a similar box to those used for the parasitoids, and left at 26°C. The egg masses were examined twice daily until the first crawlers were seen, and the box was then taken and suspended in the conservatory, as described above.

Emergence of parasitoids and second generation mealybug crawlers was monitored twice daily.

Temperature records: The temperature in the conservatory was monitored constantly by electronic sensors, and hourly mean temperatures were recorded and stored on floppy disc (filename "Glassdata"; see appendix 1).

Day degree predictions: These were carried out by a simple program written in "BASIC" for a micro-computer (see appendix 1a). The inputs were the constants T1 and K, calculated from constant temperature data (see sections 3.1.1 and 3.1.2), with data for males and females pooled (in the case of the parasitoids). The hourly temperatures were read off disc and any temperature over the lower threshold summed until the total reached the value of K. The time at which this occurred corresponded to the developmental period.

Polynomial predictions: The mean developmental rate was calculated from the raw data on developmental periods (sexes combined) at each constant temperature. In addition the mean rate of development at 12°C (all parasitoid species), 15°C (A. pseudococc1 and L. dactylopii only), 38°C (A. pseudococc1 only) and 40°C (all parasitoid species), was calculated from cycling temperature data. In section 3.1.3 it was shown that development could be incremented on an hourly basis, assuming that the

contribution made by an hour at any given temperature may be derived from constant temperature data. It thus follows that the reverse process is valid, and that developmental rates (or periods) under constant temperatures can be deduced from fluctuating temperature data. This allows estimates to be made of developmental rates at temperatures which are outside the favourable range, and which are lethal or immeasurable using experiments at constant temperature. The calculations are shown in table 12.

The data on developmental rates were fed into a polynomial regression program for a micro-computer, and the best fit regression line was selected. The coefficients of this line were then fed into a second program, which would compute the proportion of the total life cycle completed in an hour at any given temperature, and sum these increments until they reached unity, at which point development was complete (see appendix 1b). Development was not incremented at any temperature outside the 12°C to 40°C range. A print out then gave the number of days to complete development and the day and time at which mean emergence of adults would be expected. Hourly temperatures were read off disc, as for the day-degree predictions.

The variation in development time under fluctuating temperatures was calculated using the same shape theory of Wagner et al., (1984). Raw data on daily emergence at each constant temperature was fed into a computer program written in SAS, which normalised the cumulative frequency curves for each temperature around their median values, calculated the weighted mean of the tau distributions for these, estimated the Weibull parameters eta, beta and gamma and fitted a Weibull curve.

TABLE 12. Calculation of the developmental rate and developmental period at constant temperature from alternating temperature data.

Regime	dx	$\frac{1}{dx}$	d26	$\frac{1}{d26}$	$\frac{1}{d26} \times \frac{1}{2}$	$\frac{1}{dx} - \frac{1}{d26/2}$	$\frac{1}{dc}$	dc
<u>A. pseudococci</u>								
26/12	26.1	0.0383141	14.05	0.0711743	0.0355871	0.0027270	0.0054540	183.3
26/15	24.7	0.0404448	14.05	0.0711743	0.0355871	0.0048577	0.0097154	102.9
26/38	13.8	0.0723981	14.05	0.0711743	0.0355871	0.0368110	0.0736221	13.6
26/40	14.4	0.0694444	14.05	0.0711743	0.0355871	0.0338573	0.0677146	14.8
<u>L. dactylopii</u>								
26/15	28.4	0.0351679	15.27	0.0654964	0.0327482	0.00241966	0.00483932	206.6
26/40	17.0	0.0588235	15.27	0.0654964	0.0327482	0.02607530	0.05215060	19.2
<u>L. abnormis</u>								
26/12	40.1	0.0249376	22.69	0.0440771	0.0220385	0.00289910	0.00579820	172.5
26/40	21.5	0.0465116	22.69	0.0440771	0.0220385	0.02447310	0.04894620	20.4

dx = developmental period under cycling regime

d26 = developmental period at a constant 26°C

dc = calculated developmental period at a constant 12°C, 15°C, 38°C or 40°C depending on cycling regime used.

From this the values on the normalised curve at which 2.5% and 97.5% of individuals would emerge could be calculated. When the mean development time at any temperature was multiplied by these values, it gave the range in which 95% of individuals could be expected to emerge.

Each prediction was thus composed of a mean developmental period and an estimate of the range within which 95% of the individuals would have completed development.

Significant differences in the methods of prediction were tested using data pooled across species, by means of an F-test. Evidence of any bias in the predictions was tested using a t-test (Clewer, pers. comm.), where:

$$t = \bar{x} / s/\sqrt{n}$$

Results

The best fit polynomials to temperature/development data for each species are shown in figures 6, 7, 8, and 9. In all cases excellent fits were achieved to the observed data, as has been found by previous workers using this technique (Tanigoshi et al., 1975).

The actual mean developmental periods of the parasitoids observed in the Wye College conservatory at 4 different times of year, are shown along with predicted values in tables 13, 14 and 15.

No significant difference could be found in the accuracy of the two methods of prediction ($F_{(1,1)} = 2.31$ n.s., residuals pooled across species).

Figure 6

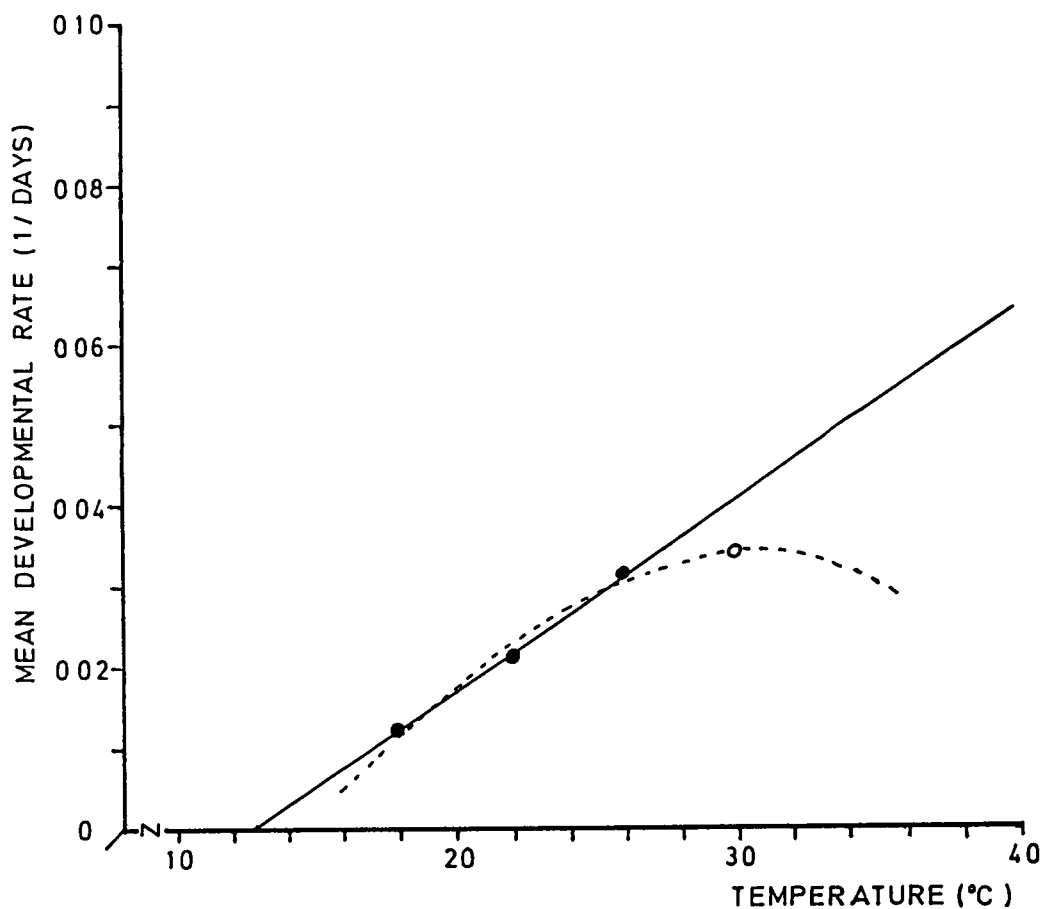


FIGURE 6 Regression lines for predicting development of Planococcus citri from constant temperature data

Points represent mean of 3 replicates (incorporating approx 1500 individuals), with standard deviation

Open circles - points not used in fitting linear regression line

Solid line - best fit linear regression line

Dashed line - best fit polynomial regression line

Linear regression $Y = -0.03087 + 0.0024 X$
 $r^2 = 99.97$

Polynomial regression Best fit polynomial order 2

$Y = -0.079909 + 0.0070475 X - 0.000178 X^2$
 $r^2 = 96.99$

TABLE 13. Prediction of developmental period of Anagyrus pseudococci under fluctuating conditions

DEVELOPMENTAL PERIOD IN DAYS										
REGIME	Actual emergence			polynomial prediction			day-degree			
	first	$\bar{x}$	last	-95%	$\bar{x}$	+95%	residual	$\bar{x}$	residual	residual
18°C/26°C	19	21.19	26	18.5	20.4	23.6	-0.79	20.97	-0.22	
15°C/26°C	22	24.72	31	21.7	23.9	27.7	-0.82	25.0	0.28	
12°C/26°C	23	26.1	29	23.5	25.7	29.8	-0.4	30.97	4.87	
26°C/38°C	12	13.58	16	12	13.3	15.4	-0.28	10.1	-3.48	
26°C/40°C	14	14.77	15	12.7	14.0	16.2	-0.76	9.6	-5.17	
1st July 1983	15	15.67	17	14.0	15.4	17.8	-0.27	16.37	0.7	
13th May 1983	20	20.69	22	17.5	19.3	22.4	-1.39	19.23	-1.46	
28th Sep 1983	27	29.36	32	25.3	27.9	32.3	-1.46	28.78	-0.58	
19th Dec 1983	32	34.08	37	30.9	34.1	39.5	0.1	33.8	-0.28	

All regimes fluctuating between two temperatures remained 12 hours at each. Dated regimes were at conservatory temperatures, starting from date shown. For actual emergence, the number of days to first and last emergence are given along with the calculated mean developmental period. For predictions the mean developmental period is given with calculated 95% range. Residuals are the differences between actual and predicted mean developmental periods for a given regime.

Figure 7

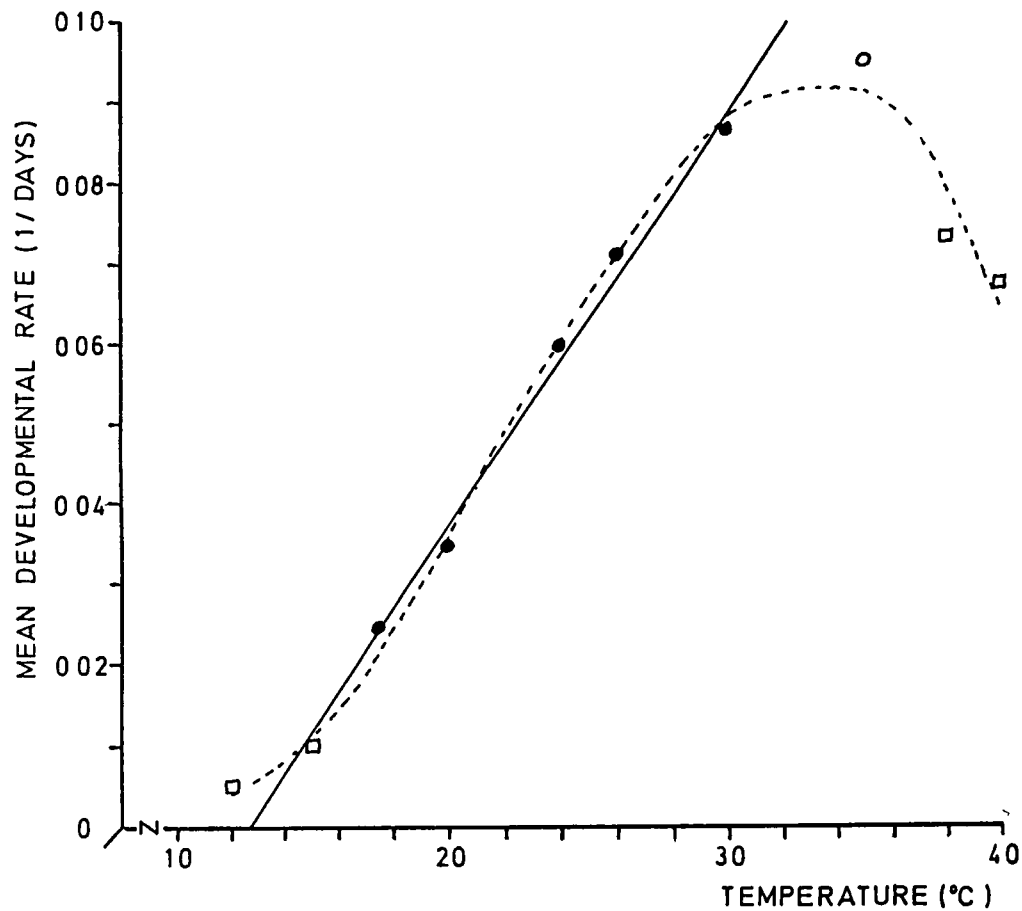


FIGURE 7 Regression lines for predicting development of Anagyrus pseudococci from constant temperature data

Points represent mean developmental rate of males and females combined
 Open circles - points not used in fitting linear regression line
 Open squares - points estimated from experiments using cycling temperatures, not used in fitting linear regression line
 Solid line - best fit linear regression line
 Dashed line - best fit polynomial regression line (all points used)

Linear regression $Y = -0.06524 + 0.0075135 X$
 $r^2 = 98.89$

Polynomial regression Best fit polynomial order 3

$Y = 0.089595 - 0.017448 X + 0.001055 X^2 - 0.00001586 X^3$
 $r^2 = 98.75$

TABLE 14. Prediction of developmental period of Leptomastix dactylopi under fluctuating conditions

DEVELOPMENTAL PERIOD IN DAYS										
REGIME	Actual emergence		polynomial prediction				day-degree			
	first	$\bar{x}$	last	-95%	$\bar{x}$	+95%	residual	$\bar{x}$	residual	
18°C/26°C	20	22.01	24	20.8	22.3	24.9	0.29	23.23	1.22	
15°C/26°C	27	28.43	32	24.8	26.5	29.6	-1.93	27.97	0.47	
12°C/26°C	27	30.22	36	27.6	29.5	32.9	-0.72	35.13	4.91	
26°C/40°C	17	17.00	17	15.3	16.4	18.3	-0.6	10.36	-6.64	
1st July 1983	15	16.33	17	15.5	16.5	18.5	0.17	18.07	1.73	
13th May 1983	20	21.35	22	19.9	21.3	23.8	-0.05	21.02	-0.33	
28th Sep 1983	29	32.71	36	31.0	33.1	37.0	0.39	33.25	0.54	
19th Dec 1983	38	42.17	45	37.1	39.7	44.3	-2.47	39.04	-3.13	

All regimes fluctuating between two temperatures remained 12 hours at each. Dated regimes were at conservatory temperatures, starting from date shown. For actual emergence, the number of days to first and last emergence are given along with the calculated mean developmental period. For predictions the mean developmental period is given with calculated 95% range. Residuals are the differences between actual and predicted mean developmental periods for a given regime.

Figure 8

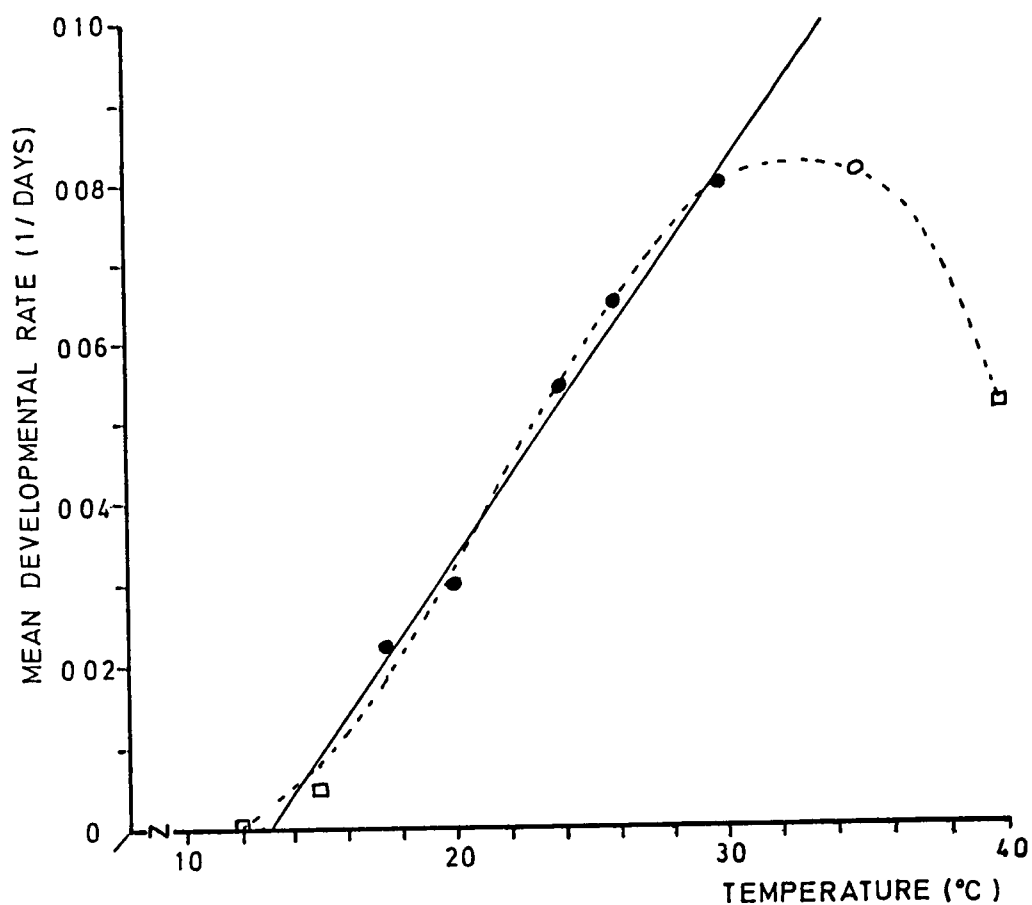


FIGURE 8 Regression lines for predicting development of Leptomastix dactylopii from constant temperature data

Points represent mean developmental rate of males and females combined
 Open circles - points not used in fitting linear regression line
 Open squares - points estimated from experiments using cycling temperatures, not used in fitting linear regression line
 Solid line - best fit linear regression line
 Dashed line - best fit polynomial regression line (all points used)

Linear regression $Y = -0.06392 + 0.0048613 X$
 $r^2 = 98.47$

Polynomial regression Best fit polynomial order 4

$Y = 0.088097 - 0.018544 X + 0.001167 X^2 - 0.00001992 X^3 + 0.0000000445 X^4$
 $r^2 = 98.75$

TABLE 15. Prediction of developmental period of Leptomastidea abnormis under fluctuating conditions

DEVELOPMENTAL PERIOD IN DAYS										
REGIME	Actual emergence			polynomial prediction			day-degree			
	first	$\bar{x}$	last	-95%	$\bar{x}$	+95%	residual	$\bar{x}$	residual	
18°C/26°C	27	31.39	38	27.4	30.4	35.9	-0.99	31.91	0.52	
12°C/26°C	37	40.10	42	35.3	39.2	46.3	-0.90	42.89	2.79	
26°C/40°C	17	21.50	26	18.8	20.9	24.7	-0.6	16.46	-5.04	
1st July 1983	23	24.50	26	22.1	24.5	29.0	0.00	27.04	2.54	
13th May 1983	26	27.35	28	25.6	28.4	33.6	1.05	29.05	1.70	
28th Sep 1983	35	38.00	40	36.1	40.1	47.3	2.10	41.77	3.77	
19th Dec 1983	44	46.00	49	40.5	45.0	53.2	-1.00	45.98	-0.02	

All regimes fluctuating between two temperatures remained 12 hours at each. Dated regimes were at conservatory temperatures, starting from date shown. For actual emergence, the number of days to first and last emergence are given along with the calculated mean developmental period. For predictions the mean developmental period is given with calculated 95% range. Residuals are the differences between actual and predicted mean developmental periods for a given regime.

Figure 9

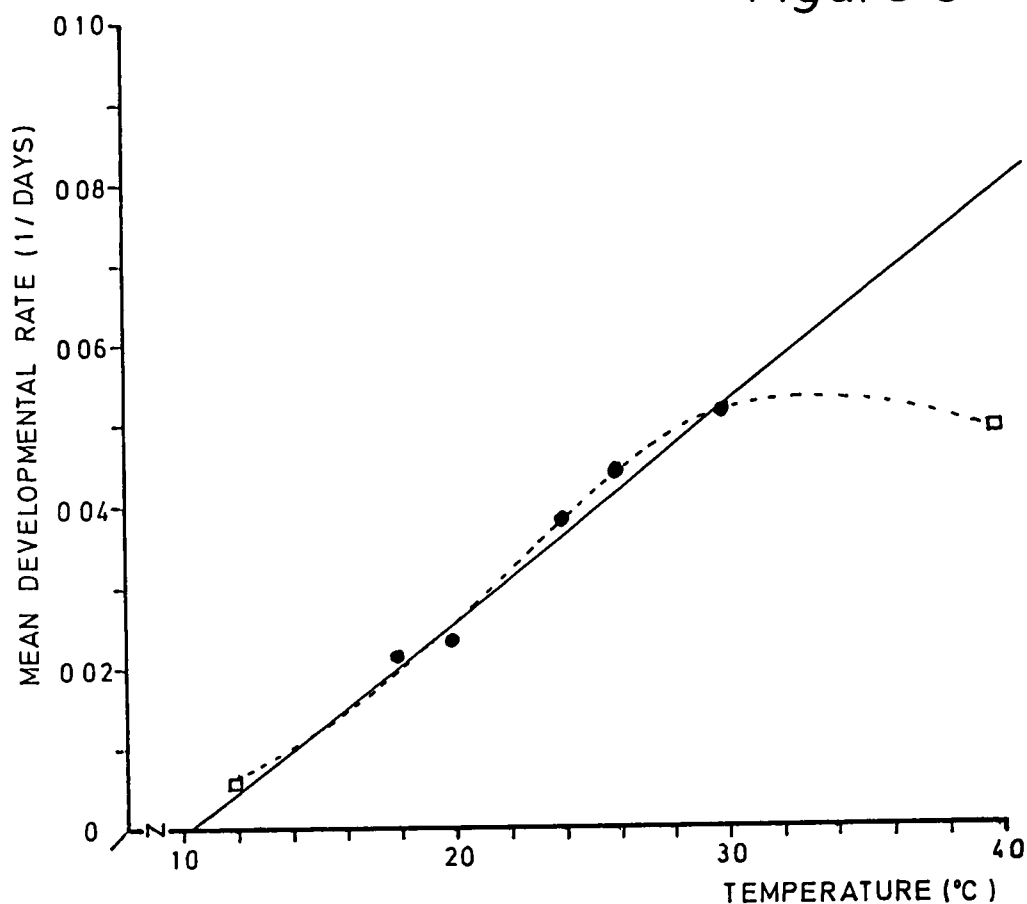


FIGURE 9 Regression lines for predicting development of *Leptomastidea abnormis* from constant temperature data

Points represent mean developmental rate of males and females combined
Open squares - points estimated from experiments using cycling temperatures, not used in fitting linear regression line

Solid line - best fit linear regression line

Dashed line - best fit polynomial regression line (all points used)

Linear regression $Y = -0.02748 + 0.0026737 X$
 $r^2 = 96.30$

Polynomial regression Best fit polynomial order 3

$Y = 0.016386 - 0.004181 X + 0.000340 X^2 - 0.00000538 X^3$
 $r^2 = 98.64$

This data also gave no evidence of bias in the predictions towards under or over predicting ($t = -0.672$ n.s. for polynomial, $t = 0.811$ n.s. for D°), but did reveal that the mean square error (MSE) for the day-degree predictions (MSE = 3.318) was substantially larger than that for the polynomial method (MSE = 1.412).

Predictions were also made of development under cycling temperature regimes using both methods and tested against observed data. These results are also shown in tables 13, 14 and 15.

There was a very highly significant difference between the residuals from the polynomial predictions and those from day-degrees ($F_{(11,11)} = 55.364^{***}$). The polynomial method being the more accurate, although there was a tendency for this method to be biased towards under-predicting ($t = -4.7587^{***}$), whilst the day-degree method showed no bias ($t = -0.4819$ n.s.). The mean square error for the day-degree predictions (MSE = 13.835) was much greater than that for polynomials (MSE = 0.7486).

Discussion

Both the day degree and polynomial methods gave good predictions of the development of the parasitoids under fluctuating conditions in the conservatory. There was no significant difference in the accuracy of the two methods, but the indications are that the polynomials give more consistent predictions. Either method can be used to give predictions of development time under natural conditions accurate to within 10%. Thus, despite extremely accurate hourly temperature readings, the findings are similar to those reported by other workers that the more sophisticated mathematical descriptions of the temperature development relationship, give little advantage over the simple day-degree method of predicting insect

development under natural, fluctuating temperatures (Whalon and Smilowitz,¹⁹⁷⁹ Allsopp, 1981; Goodenough et al., 1983). However, the use of the two methods to predict development under rhythmically cycling regimes between 2 temperatures, shows up weaknesses in the day-degree method. Here, the polynomials continue to predict with a similar degree of accuracy, but the day-degree predictions become extremely inconsistent. Where extended periods at extreme temperatures are involved the D° method vastly under-predicts at high temperature, and over-predicts at low temperature. This occurs due to the deviation of the temperature/development relationship from the linear at extreme temperatures. Thus even if the lower threshold has been determined with a reasonable degree of accuracy (see section 3.1.3), predictions will tend to be inaccurate at high temperature. This demonstrates that the D° method is limited in its application, and can produce poor results. The polynomial method however, remains reasonably consistent under all conditions tested and should thus prove useful for predicting development in temperate glasshouses, which operate for periods near to the temperature extremes.

The range values determined using the same shape theory of Wagner et al., (1984) also gave consistently good estimates of the first and last emergence, observed under fluctuating conditions. This information may also prove useful for modelling host/parasitoid relationships in the glasshouse, allowing the spread of developmental periods to be quantified.

Unfortunately only 2 replicates were done for the development of P. citri under glasshouse conditions. Both day degree and polynomial predictions were reasonably accurate. Running predictions for P. citri development at the times corresponding to those observed for the parasitoids, shows how season affects the rate of development. During the height of summer predicted development time for the mealybug is 40.3 days. In this time,

under the same conditions A. pseudococci could complete 2.46 generations, assuming that hosts were found and eggs layed the day after adult eclosion; L. dactylopi could complete 2.3 generations, and L. abnormis 1.58 generations. During the autumn predicted development time for P. citri is 65.6 days, during which time the parasitoids could complete 2.27, 1.92 and 1.59 generations respectively, whilst during the winter P. citri completes its development in 67.5 days in which time the parasitoids complete 1.92; 1.66 and 1.47 generations respectively.

It seems unlikely that these differences in themselves are likely to produce any great differences in the performance of the parasitoid populations relative to that of the host. The indication is that L. abnormis is the best adapted to its host, as season has least effect on its synchrony with the host's development.



PLATE 15. Male and female Leptomastix dactylopii in courtship.

3.2 THE EFFECT OF TEMPERATURE ON MATING OF THE PARASITIDS

Introduction

The three mealybug parasitoids A. pseudococci, L. dactylopii and L. abnormis are all arrhenotokous species, producing females from fertilised eggs and males from unfertilised ones. Fertilisation is thus important if females are to be produced. Fertilisation of the females depends on successful mate location, courtship and copulation. All these aspects of the parasitoid's behaviour could be affected by temperature. Mate location is almost certainly temperature dependent as it involves active searching.

The aim of this study was to determine whether temperature has an effect on the rate of success in mating, when males and females are confined together and the need for mate location is removed.

Materials and methods

Mummified mealybugs were removed from the parasitoid cultures and isolated individually in gelatin capsules (size 00) at $26 \pm 0.5^{\circ}\text{C}$, 45-60% r.h. with an 18/6 h light/dark photoperiod (light being provided by 5 fluorescent tubes). Emergence of adult parasitoids from the mummies was monitored twice daily. After emergence the gelatin capsules were placed at one of five experimental temperatures, namely $18 \pm 0.5^{\circ}\text{C}$, $20 \pm 1^{\circ}\text{C}$, $22 \pm 0.5^{\circ}\text{C}$, $26 \pm 0.5^{\circ}\text{C}$ and $30 \pm 0.5^{\circ}\text{C}$, and allowed to acclimatize.

In order to determine whether age affected mating, males and females were divided into three batches. Each batch comprised 10 male and 10 female insects. Those in the first batch were kept in isolation for 1-24 h, the

second for 24-72 h, and the third for more than 72 h, before being placed together in one gelatin capsule for one hour. After one hour the sexes were again separated and the females dissected to determine whether mating had taken place. This was done by examination of the spermatheca for active sperm; if none were seen the female was presumed to be virgin. A fourth batch of females (of mixed ages) were confined for one hour with males which had already mated. Controls were dissected after a period of 24 h confined alone.

Results were tested for significance using a d-test for comparing two percentages based on large samples ($n > 30$) (Bailey, 1959).

Results

The data for the different age intervals were pooled and tested for any overall effect of temperature, and the results are shown in table 16.

TABLE 16. The effect of temperature on mating of the parasitoids Anagyrus pseudococci, Leptomastix dactylopii and Leptomastidea abnormis.

PROPORTION MATING (p)						
TEMP (°C)	<u>A. pseudococci</u>		<u>L. dactylopii</u>		<u>L. abnormis</u>	
	p	s.e.	p	s.e.	p	s.e.
18	0.50	0.09	-	-	-	-
20	0.63	0.09	0.7	0.08	0.37	0.08
22	0.60	0.09	0.9	0.05	0.37	0.08
26	0.40	0.09	0.73	0.08	0.3	0.08
30	0.53	0.09	0.73	0.08	0.67	0.09

Figures represent proportion of sample of 40 individuals which had mated, after sexes were confined together in a gelatin capsule for 1 hour. Standard error calculated as $\sqrt{p(1-p)/n}$ (Bailey, 1959).

From these results it would appear that there is no consistent effect of temperature on the proportion of the population mating. A. pseudococci and L. dactylopii show no significant differences in the proportion mating at the different temperatures. L. abnormis showed a significantly greater proportion mating at 30°C than at 20°C, 22°C ($P < 0.05$) or 26°C ($P < 0.01$), but there were no significant differences between the other temperatures.

Table 17 shows the effect of age on the proportion mating, for data pooled across temperature. The results are different for each species.

TABLE 17. The effect of age on mating of the parasitoids Anagyrus pseudococci, Leptomastix dactylopii and Leptomastidea abnormis

PROPORTION MATING (p)						
AGE (h)	<u>A. pseudococci</u>		<u>L. dactylopii</u>		<u>L. abnormis</u>	
	p	s.e.	p	s.e.	p	s.e.
< 24	0.74	0.06	0.62	0.08	0.3	0.07
24-72	0.48	0.07	0.92	0.04	0.42	0.07
> 72	0.38	0.07	0.75	0.07	0.55	0.08
exp	0.26	0.06	0.67	0.07	0.4	0.08

exp = experienced males, which had already mated.

p = proportion of sample which had mated after males and females were confined together in a gelatin capsule for 1 hour. $n = 50$ for A. pseudococci and $n = 40$ for the other two species. Standard error calculated as $\sqrt{p(1 - p)/n}$.

A. pseudococci

The proportion of the sample mating appears to decrease with increasing age, the proportion mating when <24 h old being significantly greater than the proportion mating in the 24-72 h age interval ($P < 0.01$) or the >72 h age interval ($P < 0.001$).

There may be some evidence that previous mating experience in the males has an effect on the success of subsequent mating. A significantly smaller proportion of females mated with experienced males, than with virgin males (data for all age intervals pooled, $P < 0.001$).

L. dactylopis

There is no consistent trend in the proportion mating with age, the proportion mating in the 24-72 h age interval being significantly greater than either younger or older individuals ($P < 0.01$). There is no evidence of an effect of previous mating in males.

L. abnormis

The trend here is in the opposite direction to that shown by

A. pseudococci, the proportion mating increasing with age, although the only significant difference is between the proportion mating in the >72 h age interval and in the <24 h age interval ($P < 0.05$). There is no evidence of an effect of previous mating in males.

Discussion

From these results, there is no evidence of an effect of temperature on the proportion of females successfully fertilised, when confined together with a single male. This suggests that once a male finds a female, courtship and copulation behaviour are not affected by temperatures within the range tested. At more extreme temperatures these behavioural processes may be affected, but within the glasshouse, temperatures outside the 18-30°C range occur only for relatively short periods and are unlikely to significantly effect the overall proportion of successful matings.

The age of the parasitoids does appear to affect the success of fertilisation. A. pseudococci mates more readily soon after eclosion, whilst L. abnormis shows a higher success rate when several days old. Further data would be necessary to confirm this trend, as it could be significant in determining their efficiency as biological control agents. If, as these data suggest, A. pseudococci mates less readily as time goes on, it will be less effective under conditions which reduce the chance of rapid mate finding, i.e. low parasitoid density (which will tend to coincide with low host density unless inoculative releases have been made), or low temperature. If this were to occur, the presence of a majority of unmated females in the population would lead to a shift in sex ratio towards the production of males, so reducing the potential to control host numbers in the succeeding generation (Waage and Hassell, 1982). It is likely that this effect would be less pronounced with L. abnormis, indicating that it may be the more successful of the two species at low density. L. dactylopii seems to be intermediate between the other two species in this respect.

3.3 THE EFFECT OF TEMPERATURE ON SEARCHING BY THE PARASITIDS

Introduction

A parasitoid must have a high intrinsic searching capacity, in order to be effective as a biological control agent. The searching behaviour and strategies of parasitoids in relation to host density and distribution have been the subject of a good deal of investigation (Holling, 1959a; 1959b, van Lenteren and Bakker, 1976; Luck, van Lenteren, Twine, Kuenen and Unruh;¹⁹⁷⁹ Waage, 1979, Waage and Hassell, 1982) and a number theoretical models have been proposed to explain the effects on population dynamics (Hassell and May 1973; Cook and Hubbard, 1977; Comins and Hassell, 1979, Hassell, 1980, Hassell, 1982).

Comparatively little attention has been given to the environmental influences on searching behaviour, despite their potentially important effects on population systems (Kitching, 1977). Temperature is generally the most important environmental factor influencing insect biology and it has been shown to have a significant effect on the searching capacity of several Trichogramma species (Biever, 1972).

There are a number of ways in which temperature may affect the ability of a parasitoid to search for hosts. As poikilotherms all biochemical and physiological processes are dependant on temperature for their rates of operation and thus locomotory activity in general is affected by thermal changes (Barbosa and Frongillo, 1977; Barrass, 1970; Kitching, 1977). Temperature may also affect behavioural patterns and the speed at which various activities are carried out. Both these aspects are considered in this study.

3.3.1 The effect of temperature on parasitoid activity

Introduction

Temperatures which impair the activity of a parasitoid will make it less able to search for and locate hosts. In attempting to understand parasitoid population dynamics it is therefore important to determine the effects of temperature on activity. It has been shown that the locomotory and flight activity of the chalcid Brachymeria intermedia increase with rising temperature and that activity levels follow a diurnal pattern (Barbosa and Frongillo, 1977). Diurnal patterns in the activity of Glossina morsitans were found to be dependant on photoperiod and levels of activity increased with rising temperature up to an optimum and then declined (Barrass, 1970). A similar effect was found with sheep blowflies by Kitching (1977). The aim of this study was to evaluate the influence of temperature on the activity levels of the three mealybug parasitoids Anagyrus pseudococci, Leptomastix dactylopii and Leptomastidea abnormis.

Materials and methods

Male and female parasitoids of each species, aged between 1 and 7 days were removed from the cultures and isolated in clean, glass, 100 mm diameter petri dishes. These were then placed in one of four constant temperature rooms held at $18 \pm 0.5^{\circ}\text{C}$, $22 \pm 0.5^{\circ}\text{C}$, $26 \pm 0.5^{\circ}\text{C}$ and $30 \pm 0.5^{\circ}\text{C}$ respectively, and left for 1 h to acclimatise. Light was provided by fluorescent tubes at $8.55 \pm 0.5 \text{ W/m}^2$. Relative humidity was between 45% and 65%. Each individual was then observed for 10 seconds every 5 minutes during a 15 minute period and scored as either active or resting. The parasitoid was considered to be resting if it was motionless except for occasional movement of the antennae, otherwise it was scored as active.

Three 15 minute observation periods were spread over the day in order to take account of any diurnal variation in activity levels. The first began at 1130, the second at 1400 and the third at 1600. A total of at least 90 ten second observations were made for each sex of each species at each temperature.

Results

Results from the three observation periods were pooled as sampling periods were the same at each temperature.

For all three species the proportion of the population active generally increased with rising temperature up to an optimum (Figure 10). The significance of differences across temperature were tested using a d-test, based on a normal approximation to the binomial distribution (Bailey, 1959).

For both sexes of all species the proportion active was significantly greater at 22°C, 26°C and 30°C than at 18°C ($P < 0.001$). Above 22°C the proportion active tends towards an optimum. The trends within the individual species are explained below.

A. pseudococci

The proportion active continued to rise up to 30°C for both sexes. Significantly fewer females were active at 26°C than at 22°C ($P < 0.05$), although the proportion active then rose again at 30°C, and was significantly greater than at 22°C ($P < 0.05$) or 26°C ($P < 0.001$). A significantly greater proportion of females than males were active at 22°C ($P < 0.01$) and 30°C ($P < 0.001$).

Figure 10

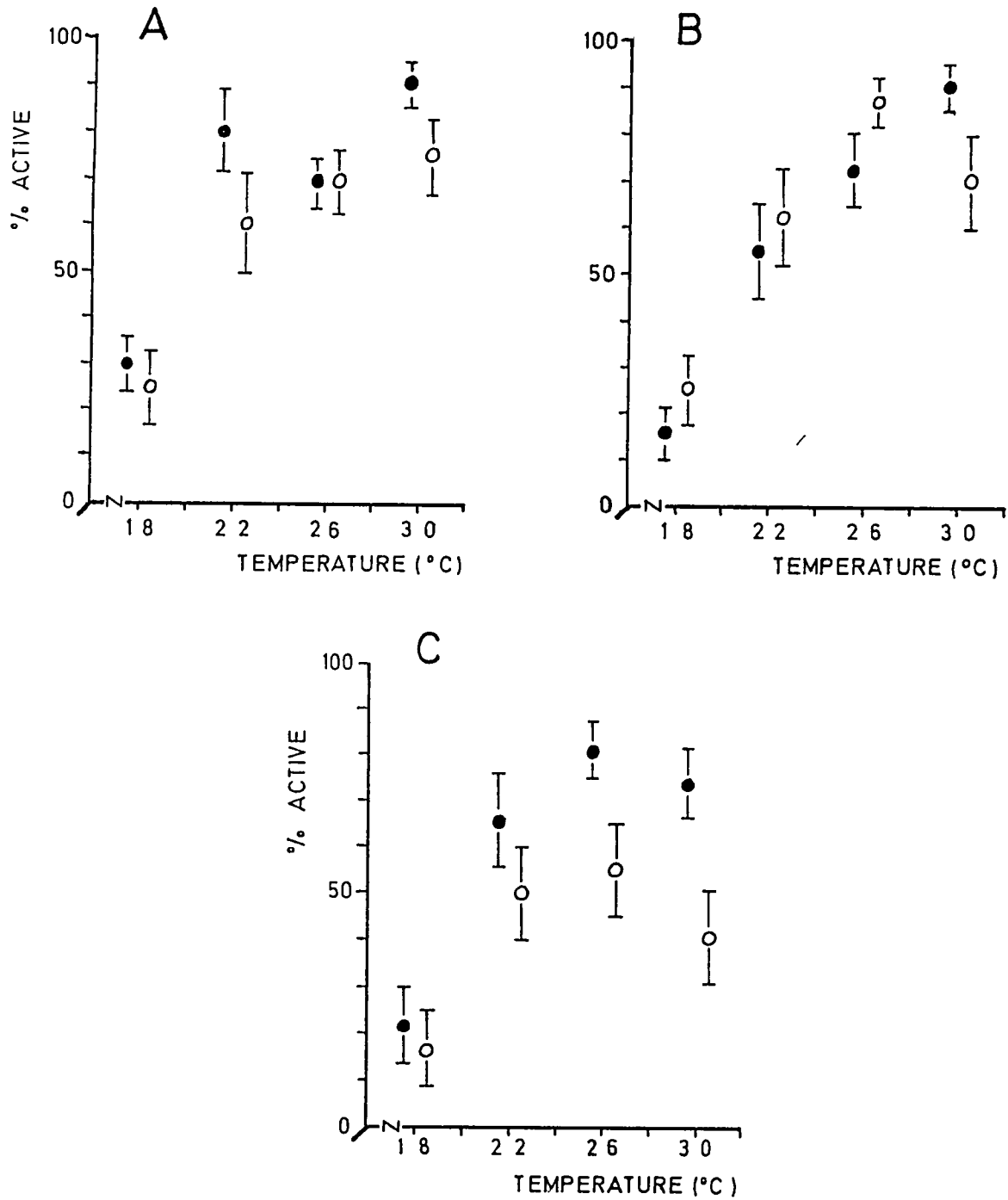


Figure 10 Percentage of population of parasitoids active during spot checks at different constant temperatures

Points represent mean percent of sample active with 95% confidence interval

○ Males ● Females

A *Anagyrus pseudococci*
 B *Leptomastix dactylopii*
 C *Leptomastidea abnormis*

L. dactylopii

A significantly higher proportion of females were active at 26°C than at 22°C ($P < 0.01$) and at 30°C than at 26°C or 22°C ($P < 0.001$). The proportion of males active increased up to 26°C, but then fell at 30°C ($P < 0.001$). A significantly greater proportion of females were active than males at 30°C ($P < 0.001$), but at 26°C the proportion of males active was greater than the proportion of females ($P < 0.01$).

L. abnormis

Both sexes showed less activity at 30°C than at 26°C, but the difference was only significant for males. A significantly higher proportion of females were active at 26°C than at 22°C ($P < 0.01$). The proportion of females active was significantly greater than the proportion of males at 22°C ($P < 0.05$), 26°C and 30°C ($P < 0.001$).

Discussion

The results show that for all three species, activity is impaired at low temperature. Below 22°C only a small proportion of a given population will be active, and thus at these low temperatures both host location by females and mate location by males will be greatly reduced.

These results are consistent with those found for the chalcid Brachymeria intermedia, which showed far higher levels of activity at 21°C than at 15.5°C, but less difference in activity levels between 21°C and 26.5°C (Barbosa and Frongillo, 1977).

3.3.2 The effect of temperature on searching behaviour of parasitoids on a leaf surface

Introduction

The walking pattern of a parasitoid is a useful indication of its searching behaviour. If searching is random, the rate at which randomly distributed hosts are located is entirely dependant on the walking speed of the parasitoid and the host density and size (van Lenteren, Nell, Sevenster-van der Lelie and Woets, 1976). It is likely that the amount of time spent walking would also have an effect. The walking speed of the parasitoid Trichogramma is highly dependant on temperature, as is the proportion of time spent walking (Biever, 1972). Despite the fact that very few hosts are randomly distributed, and most parasitoids show non-random searching (Murdie and Hassell, 1973; Yano, 1978), temperature is likely to have an important effect on the percentage parasitism achieved at a given host density and therefore on the effectiveness of the parasitoid.

The walking speed of many parasitoid species is affected by the presence of certain host cues, for example Microterys flavus shows reduced locomotory activity in response to honeydew secreted by its host Coccus hesperidum (Vinson, Harlan and Hart, 1978). Nemeritis canescens shows similar behavioural changes in the presence of chemicals produced by its host, and in addition the wasp shows klinotaxis in response to removal of those stimuli. These orthokinetic and klinotactic responses are major factors leading to arrestment in patches of greater host density, which has important implications for the population dynamics of the host/parasitoid system (Waage, 1978; 1979).

In the experiments described below observations were made of the searching patterns of the parasitoids Anagyrus pseudococchi, Leptomastix dactylopii and Leptomastidea abnormis on "clean" and host infested leaf surfaces at different temperatures, in order to determine how these factors influence their behaviour.

Materials and Methods

Unless otherwise stated the same experimental procedures were carried out on the 3 species. Parasitoids aged between one and seven days were removed from cultures kept at $26 \pm 0.5^{\circ}\text{C}$ and isolated in 100 mm diameter, glass petri dishes. 5 males and 5 females were then placed in one of 4 constant temperature rooms set at $18 \pm 0.5^{\circ}\text{C}$; $22 \pm 0.5^{\circ}\text{C}$, $26 \pm 0.5^{\circ}\text{C}$, and $30 \pm 0.5^{\circ}\text{C}$, respectively, and allowed to acclimatise to the experimental temperature for one hour. Light was provided by fluorescent tubes at approximately $8.55 \pm 0.5 \text{ W/m}^2$. Relative humidity ranged from 30-50% at 30°C to 65-75% at 18°C .

Two sets of experiments were carried out, observing the parasitoid's searching behaviour on "clean" and "infested" leaves taken from the ornamental plant Catalpa terminalia. "Clean" leaves were rinsed with water to remove any surface contaminants. "Infested" leaves had 30-50 P. citri of mixed ages feeding on them and were also covered in patches of honeydew and sooty moulds.

The parasitoids were kept in petri dishes until required for observation. The lid of the petri dish with the parasitoid on it was then positioned over the leaf and the parasitoid tapped down onto the leaf surface. Recording began once the parasitoid was on the leaf.

Observations of an individual parasitoid's activity were made over a 5 minute period, during which time the walking path was traced onto paper, and a stop-watch used to record the time spent in the following activities: walking, antennating, palpating, preening, resting and host handling.

These categories were defined as follows.

walking - where the parasitoid is moving over the leaf surface, generally tapping the antennae infrequently.

antennating - where the parasitoid is stationary and drumming on the leaf surface with its antennae.

palpating - where the parasitoid is stationary and sensing the leaf surface with its mouthparts, or actually feeding.

preening - where the parasitoid is stationary and preening itself. This process may involve rubbing the back legs together, rearranging the wings with its legs, "combing" its antennae with its front legs, etc.

resting - where the parasitoid is motionless.

host handling - where the parasitoid is in contact with a host. This may involve antennating the host, palpating the host or ovipositing into the host.

The number of jumps made was also noted. A separate record was kept of activities and walking path on the leaf surface itself and on the glass lid. The distance walked was measured using a map measurer and an estimate made of the number of major changes in direction (i.e. a turn through an angle of 90° or more).

Two females and one male were observed in this way during a 20 minute period. Observation periods were spread throughout the day to avoid the effects of any diurnal patterns of activity.

The walking speed was calculated from the distance walked in the time spent walking. The rate of turning was calculated as the number of turns made per second, by dividing the total number of turns made by the time spent walking. The number of turns made per metre was calculated by dividing the total number of turns made by the distance walked.

The data for walking speed and rate of turning were subjected to 2-factorial analysis of variance, and compared using the l.s.d. at the 5%, 1%, and 0.1% levels. This analysis first pools the data for different temperatures to identify any overall effect of infestation, and pools data for clean and infested leaves to show the influence of temperature. These are termed the "main effects". The presence of an "interaction" indicates that the effect of temperature is dependant on whether the leaf is infested or not. If there is evidence of an interaction then the main effects can be ignored. Further partitioning of the sums of squares allowed linear, quadratic or cubic trends within the data to be identified (Kempson, pers. comm.).

Results

Observations on the behavioural patterns of female parasitoids

Two examples of the walking pattern of each of the parasitoids are shown, one at 18°C and one at 30°C, for both clean and infested leaves (figures 11, 12 and 13). The difference in leaf area covered is apparent.

The behaviour of the three species studied was generally similar, and the following description holds for all of them (any differences between the species will be given special mention).

Figure 11

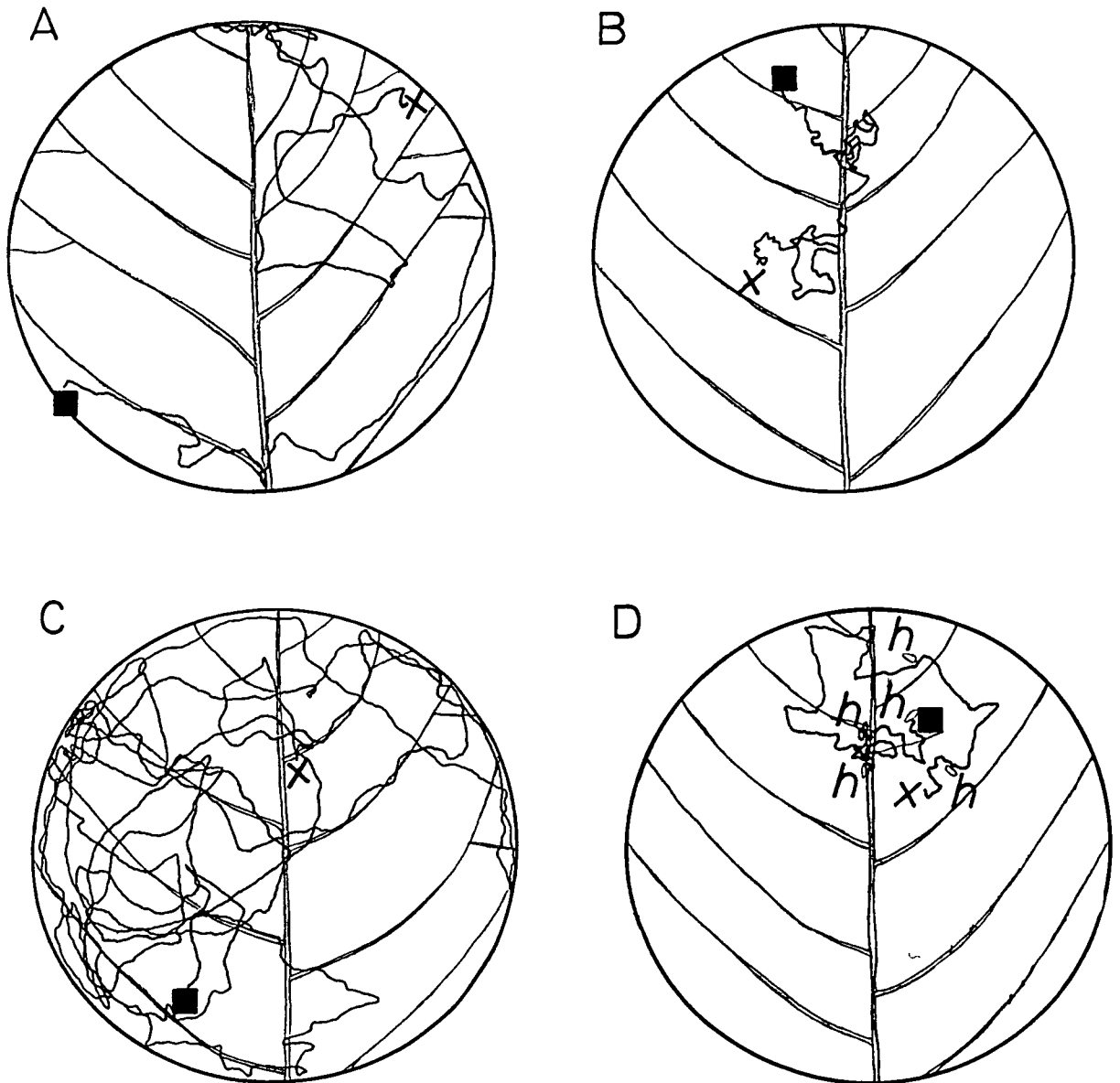


Figure 11 Searching pattern of female *Anagyrus pseudococci* during a five minute period on leaf of *Catalpa terminalia* at different temperatures

- A 18°C on clean leaf
- B 18°C on host infested leaf
- C 30°C on clean leaf
- D 30°C on host infested leaf

X starting point ■ position at end of five minute period

Hosts not shown unless contacted by parasitoid, Position marked h

Figure 12

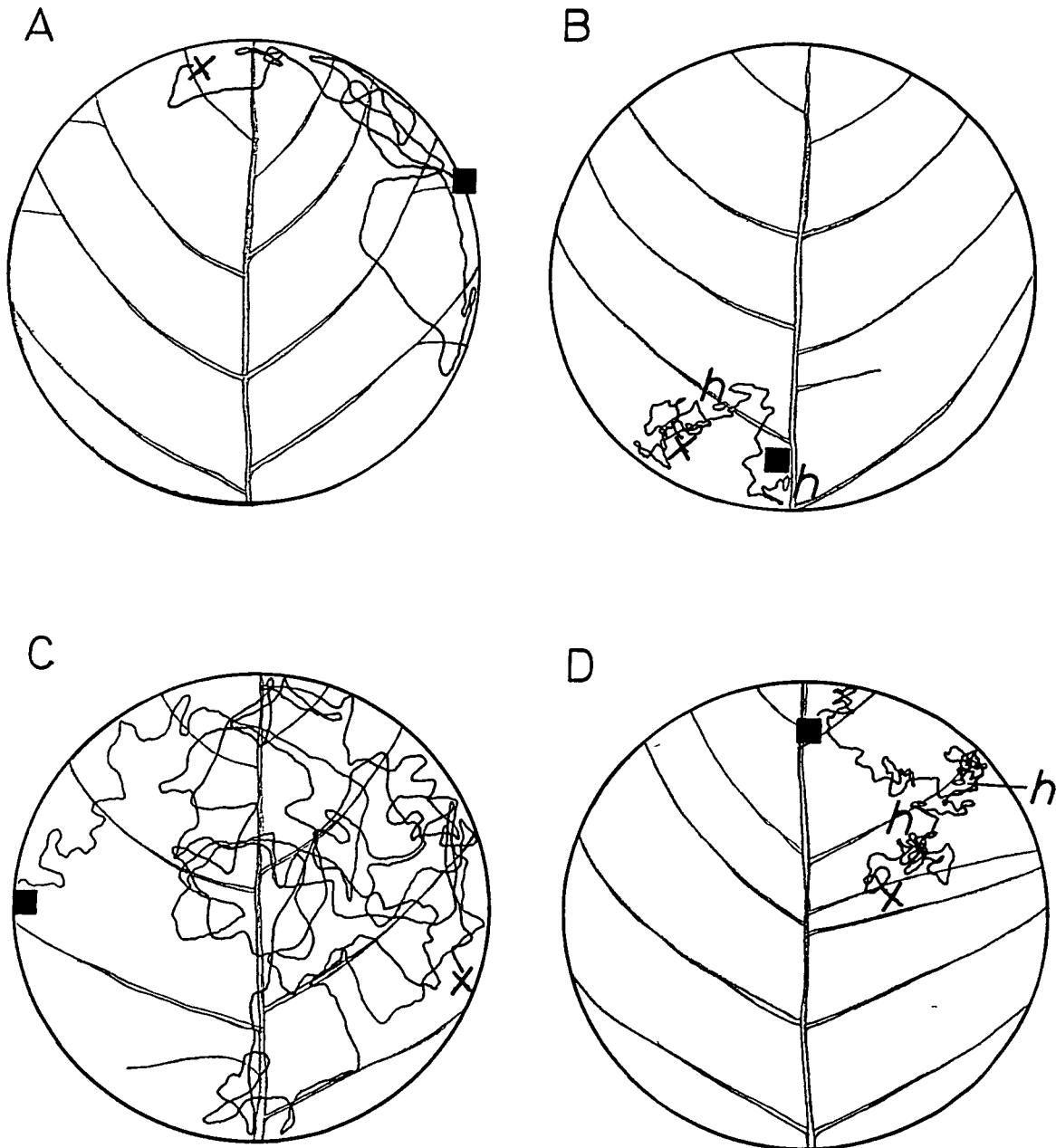


Figure 12 Searching pattern of female *Leptomastix dactylopiid* during five minute period on leaf of *Catalpa terminalia* at different temperatures

- A 18°C on clean leaf
- B 18°C on host infested leaf
- C 30°C on clean leaf
- D 30°C on host infested leaf

X starting point ■ position at end of five minute period

Hosts not shown unless contacted by parasitoid, Position marked h

Figure 13

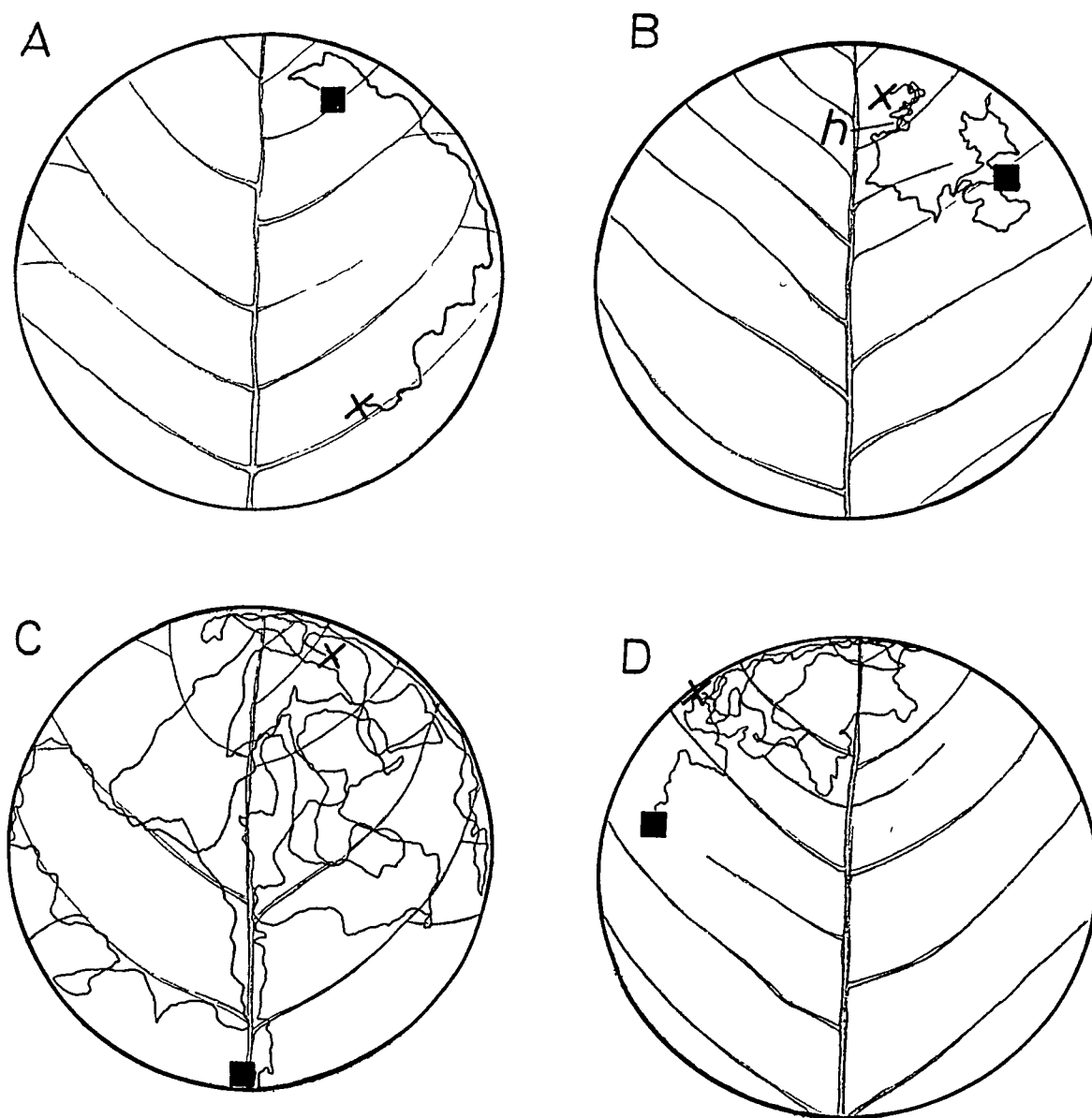


Figure 13 Searching pattern of female *Leptomastidea abnormis* during five minute period on leaf of *Catalpa terminalia* at different temperatures

- A 18°C on clean leaf
- B 18°C on host infested leaf
- C 30°C on clean leaf
- D 30°C on host infested leaf

X starting point ■ position at end of five minute period

Hosts not shown unless contacted by parasitoid, Position marked h

3.3.2.1 Searching a clean leaf

When introduced on to a clean leaf surface the parasitoids generally started to walk immediately. The antennae were held out at an angle of approximately 45° and tapped infrequently on the leaf surface.

The walking path of the parasitoids was generally random over the leaf surface with few major changes of direction, but there was some indication of a tendency to follow leaf veins, particularly the mid-rib. However, not every encounter with a leaf vein resulted in orientation along the vein, and there was great variation in the distance over which the vein was followed. L. abnormis showed a slightly greater tendency to orientate to the topography of the leaf surface than the other two species (see figures 11, 12 and 13).

The parasitoids generally walked at an even speed, occasionally stopping and "antennating" the leaf surface. When this occurred the antennae were brought closer together and "elbowed", so that the angle of contact with the leaf surface was greater. The antennae were then drummed on the leaf surface for a number of seconds. Plates 16 and 17 show the position of the antennae whilst "walking" and "antennating".

The parasitoids were occasionally observed palpating a clean leaf. The reasons for this are unclear, but it may have occurred where the leaf was slightly damaged, and the parasitoids were examining or feeding on plant sap. When palpating, the mouthparts were brought down onto the leaf surface, and the antennae held together and upwards at a steep angle to the leaf surface.



PLATE 16. Female Leptomastidea abnormis, showing position of antennae whilst "walking". (magnification x 50 approx.).



PLATE 17. Female Leptomastidea abnormis, showing position of antennae when "antennating". (Magnification as above).

In general, the searching behaviour was similar at all temperatures, although at lower temperatures the parasitoids moved much more slowly, regularly tapping their antennae on the leaf surface and would occasionally stop and rest with the antennae held still. At higher temperatures walking was much faster and with less frequent antenna tapping.

3.3.2.2 Searching an infested leaf

When introduced onto a host infested leaf surface, the behaviour was quite different. The parasitoids walked much more slowly and changed direction a great deal, giving the impression of scanning the leaf surface with their antennae whilst walking, and frequently tapping the leaf surface. This behaviour seems to be elicited by the presence of host honeydews and wax on the leaf surface. The parasitoids frequently stopped and antennated the leaf surface in the same way as described on a clean leaf, but in this case antennating was often followed by a period of palpating and/or feeding, presumably on honeydews.

As on a clean leaf the pattern of movement over the leaf surface seemed to be random, although again there was a tendency to orientate to leaf veins, but in an inconsistent manner. There was no obvious sign of orientation towards hosts, females frequently passing within a millimetre or two of a mealybug without "noticing" it.

Host encounters occurred when the parasitoid contacted the mealybug with one of its antennae. The female would then turn and antennate the host with its antennae close together and elbowed (as described above), examining its body surface. This process generally lasted at least 5 seconds (although it was very variable) after which the parasitoid either rejected the host and walked away, or began to oviposit. In this case the

female would turn away from the host and unsheath and extend its ovipositor, probing the host surface a little before thrusting backwards to stab the mealybug (see plate 18). Oviposition usually lasted only a few seconds, but was observed to continue for up to several minutes. During longer ovipositions the parasitoid appeared to be probing inside the mealybug with her ovipositor.

On occasion a mealybug would start to move away whilst a female was preparing to oviposit, leaving the parasitoid probing with its extended ovipositor without contacting the host. Following a failure of this type the parasitoid did not always relocate the host, but would merely sheath her ovipositor and walk away.

The pattern of behaviour did not show any marked change after oviposition, except that the parasitoids generally preened for some time. This involved "combing" the antennae with the front legs, rubbing the rear legs together and using them to rearrange the wings.



PLATE 18. Anagyrus pseudococci ovipositing into young adult female
Planococcus citri. (Magnification x 30 approx.).

Analysis of behavioural patterns

From the data collected in this experiment it was possible to evaluate and quantify the effects of temperature on a number of aspects of parasitoid searching behaviour. In general the trends were similar for males and females, but the data for males are not presented, as with only three replicates the data was too variable to be meaningful.

3.3.2.3 Walking speed

The effect of temperature on the walking speed of the three parasitoid species on both clean and mealybug infested leaf surfaces can be seen in figure 14.

The overall trends are similar for all species. The analysis of variance shows that the effect of temperature on the walking speed is dependent on whether the leaf is infested with hosts or not. Walking speed increases with rising temperature on a clean leaf, the data showing a significant linear trend. However, on an infested leaf surface walking speed shows no significant increase with increased temperature.

A. pseudococci

On a clean leaf the walking speed at 30°C is significantly greater than at 18°C, 22°C ($P < 0.001$) or 26°C ($P < 0.01$). At 26°C the walking speed is significantly greater than at 18°C ($P < 0.01$). The parasitoids walk significantly faster on a clean leaf than on a mealybug infested leaf at 22°C ($P < 0.01$), 26°C and 30°C ($P < 0.001$).

L. dactylopii

On a clean leaf the walking speed at both 26°C and 30°C is significantly greater than at 18°C or 22°C ($P < 0.001$). L. dactylopii walks significantly faster on the clean leaf than on the infested one at both 26°C and 30°C ($P < 0.001$).

L. abnormis

On a clean leaf, the walking speed at 30°C is significantly greater than at 18°C ($P < 0.001$), and 22°C ($P < 0.05$), and at both 22°C and 26°C the parasitoids walk faster than at 18°C ($P < 0.05$). At all temperatures except 18°C female L. abnormis walk significantly faster on the clean leaf than on the infested one ($P < 0.001$).

3.3.2.4 Rate of turning

The effects of temperature on the number of major turns made per unit distance, and per unit time can be seen in figures 15 and 16 for each of the three parasitoid species.

As above, the data were analysed using 2-factorial analysis of variance with the l.s.d. calculated at the 5%, 1% and 0.1% levels. Treatment to identify linear, quadratic and cubic trends was also included.

A. pseudococci

Analysis of variance shows that temperature has no significant effect on the number of turns made per metre, but that a significantly greater number of turns are made on an infested leaf than on a clean leaf ($P < 0.001$)(see figure 15).

Figure 14

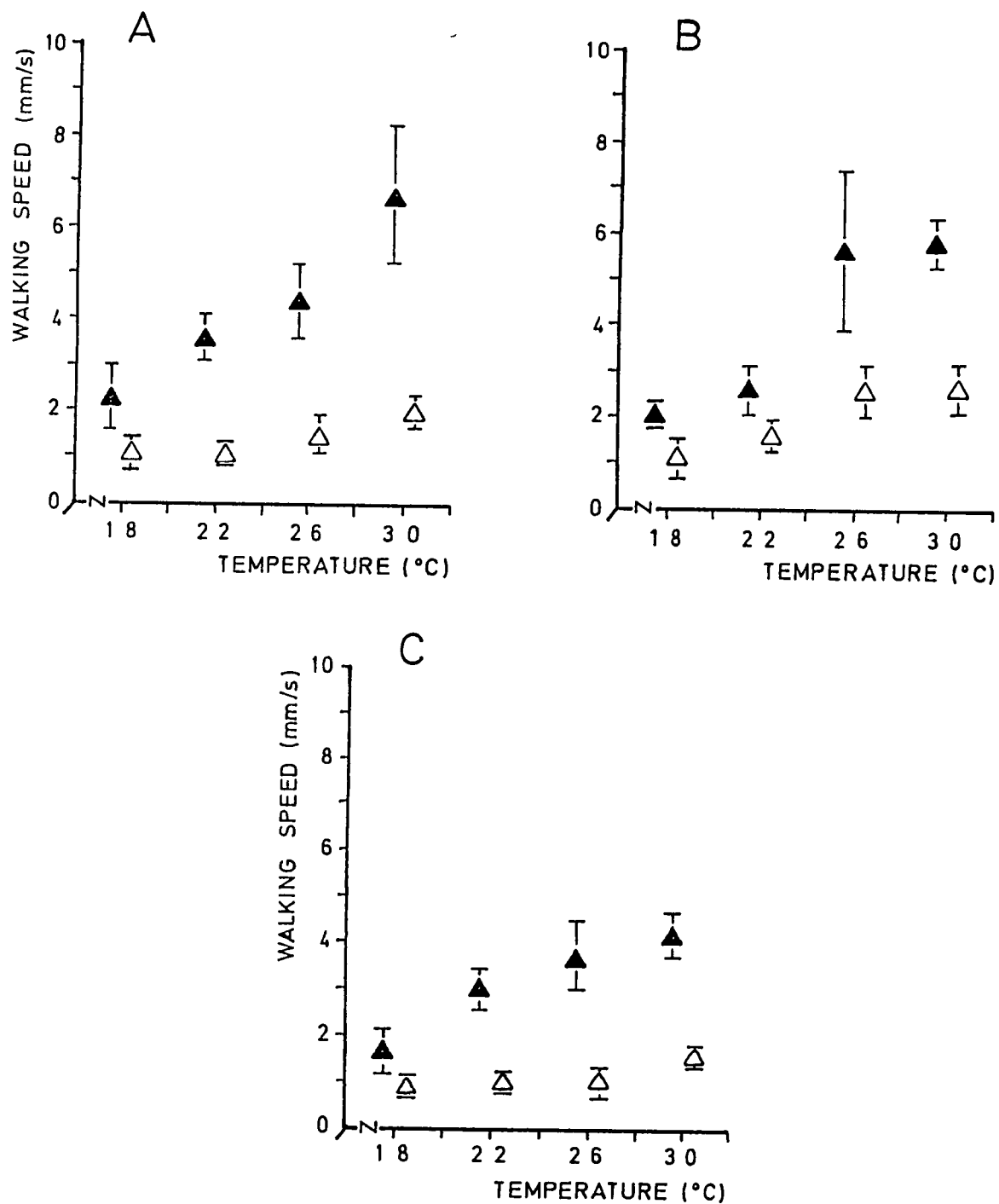


Figure 14 Walking speed of female parasitoids at different constant temperatures whilst searching on clean and host infested leaf surfaces

Points represent mean walking speed of six individuals with standard error

▲ clean leaf △ infested leaf

A *Anagyrus pseudococci*
 B *Leptomastix dactylopii*
 C *Leptomastidea abnormis*

When the data are analysed in terms of the number of turns made per second the results are very different (see figure 16). Analysis of variance indicates that temperature influences the number of turns made per second on a clean leaf, with the data showing a significant linear trend. On a clean leaf the rate of turning increases with increasing temperature, the number of turns made per second being significantly greater at both 26°C and 30°C than at 18°C ($P < 0.001$) or at 22°C ($P < 0.01$).

On an infested leaf the trend is towards increased rate of turning with higher temperature, but there are no significant differences at the 5% level. There are no statistical differences between the number of turns made per second on infested as opposed to clean leaves at any of the four temperatures.

L. dactylopi

Analysis of variance shows that both temperature and infestation affect the rate of turning per unit distance. There are no significant differences in the rate of turning per metre at different temperatures on the clean leaf, but on an infested leaf significantly more turns are made per unit distance at 30°C than at 22°C or 26°C ($P < 0.01$). At all temperatures except 22°C significantly more turns are made per metre on an infested than on a clean leaf ($P < 0.01$ at 18°C; $P < 0.05$ at 26°C, and $P < 0.001$ at 30°C) (see figure 15)

As with the other species, the rate of turning per unit time shows different effects. Analysis of variance indicates that both temperature and infestation have an influence. On a clean leaf significantly fewer turns are made per second at 18°C than at 22°C ($P < 0.05$), 26°C ($P < 0.01$) or 30°C ($P < 0.001$). The data shows a significant linear trend (see figure 16).

Figure 15

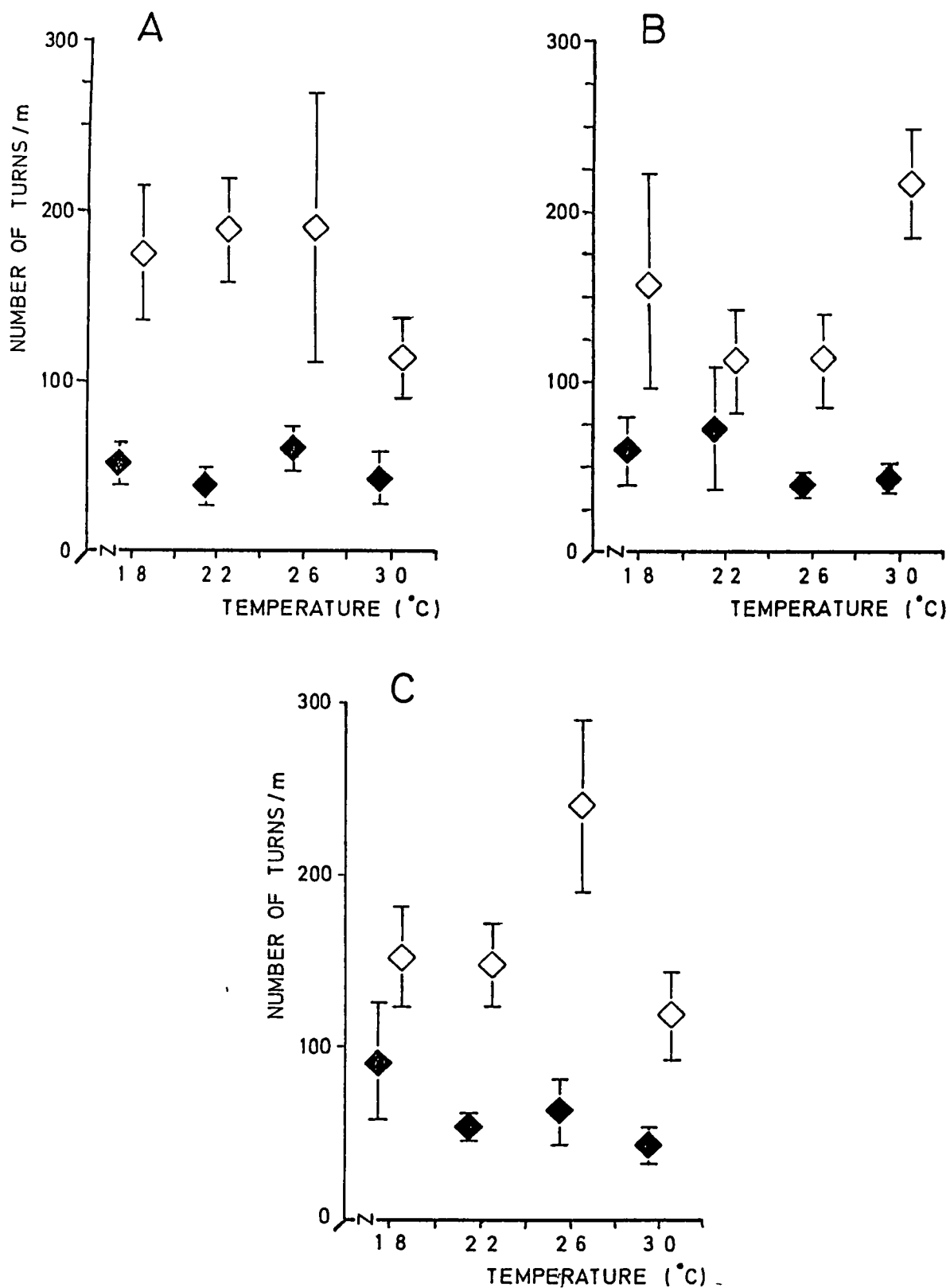


Figure 15 Number of major turns made per meter walked by female parasitoids at different constant temperatures, whilst searching on clean and host infested leaf surfaces

Points represent mean number of turns based on six individuals with standard error

◆ clean leaf ◇ infested leaf

A *Anagyrus pseudococci*
 B *Leptomastix dactylopii*
 C *Leptomastidea abnormis*

On an infested leaf there is also a definite linear effect of temperature, the rate of turning being significantly greater at 30°C than at 18°C, 22°C or 26°C ($P < 0.001$) and greater at 26°C than at 18°C ($P < 0.01$). There are no significant differences in the number of turns made per second on clean versus infested leaves at any temperature except 30°C, where the rate of turning is greater on an infested leaf ($P < 0.001$).

L. abnormis

Analysis of variance shows that both temperature and infestation affect the number of turns made per metre by the parasitoids.

There are no significant differences in the number of turns made per metre at the different temperatures on a clean leaf, but on an infested leaf the rate of turning is greater at 26°C than at both 18°C and 30°C ($P < 0.001$). Significantly more turns are made per metre on an infested leaf than on a clean one at each temperature ($P < 0.05$ at 18°C; $P < 0.01$ at 22°C; $P < 0.001$ at 26°C; $P < 0.05$ at 30°C) (see figure 15).

When the results are analysed in terms of the number of turns made per second, an effect of temperature can be identified, but not one of infestation. When treated for regression the data shows a strong, linear, main effect of temperature. The rate of turning per second is significantly greater at 26°C than at 18°C ($P < 0.001$) or 22°C ($P < 0.05$), and significantly greater at 30°C than at 18°C ($P < 0.01$) (see figure 16)

Figure 16

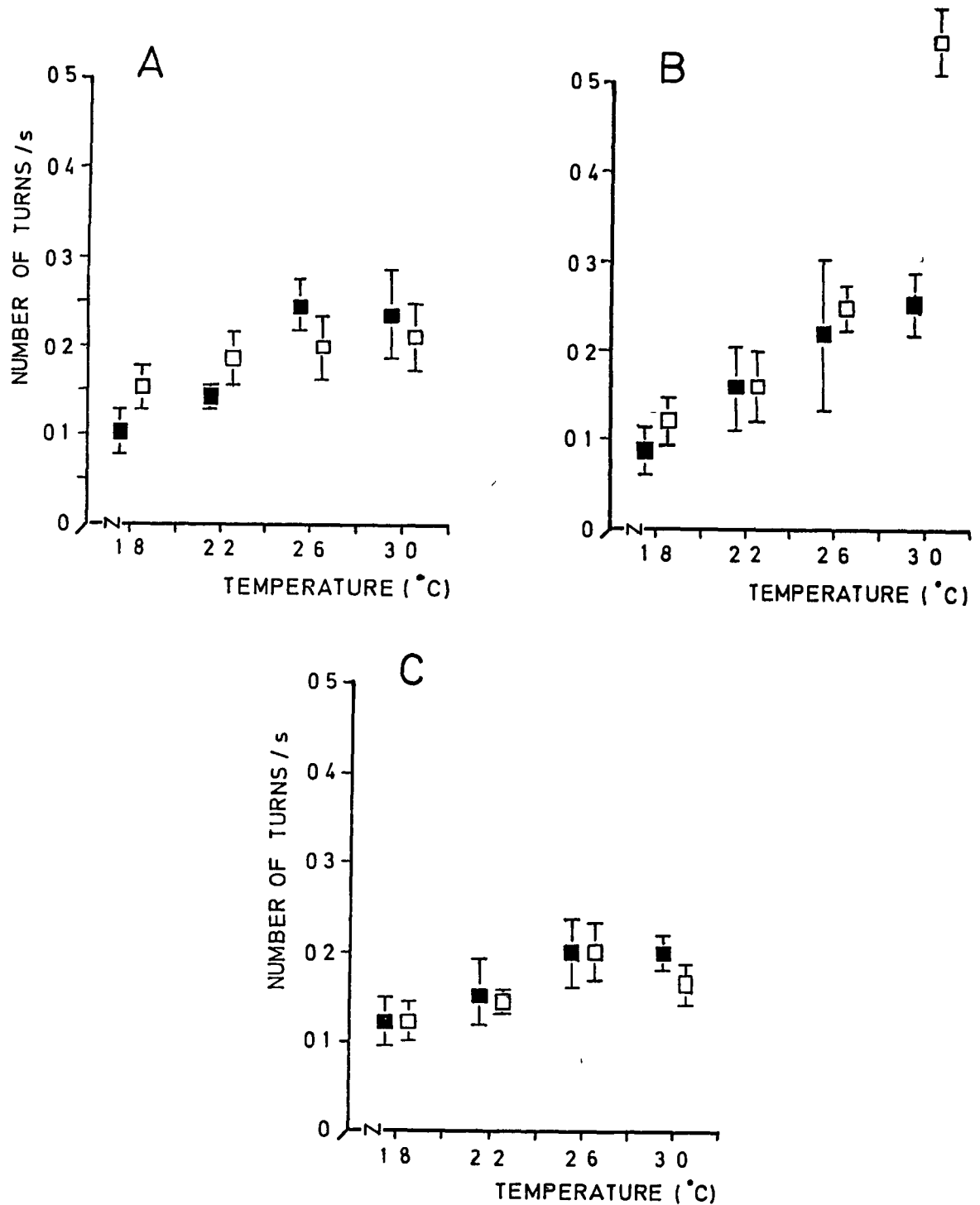


Figure 16 Number of major turns made per second spent walking by female parasitoids at different constant temperatures, whilst searching on clean and host infested leaf surfaces

Points represent mean number of turns, based on six individuals with standard error

■ clean leaf □ infested leaf

A *Anagyrus pseudococci*
 B *Leptomastix dactylopii*
 C *Leptomastidea abnormis*

3.3.2.5 Jumping frequency

The results are shown for each species separately in tables 18, 19 and 20.

A. pseudococci

Analysis of variance fails to show any influence of temperature or infestation on the number of jumps made by the parasitoids during the five minute observation.

TABLE 18. Effect of various constant temperatures on jumping frequency of female Anagyrus pseudococci on clean and host infested leaf surfaces.

		MEAN NUMBER OF JUMPS			
		clean		infested	
TEMP (°C)		$\bar{x}$	s.d.	$\bar{x}$	s.d.,
18	A	0.0	0	0.0	0
22	A	0.0	0	1.0	2.45
26	A	0.17	0.41	0.33	0.52
30	A	0.17	0.41	0.0	0

Means of six individuals observed for 5 min each, with standard deviation. Same letters indicate no significant difference at the 5% level. Capital letters for comparisons within main effects of temperature or infestation

L. dactylopii

Analysis of variance identifies an overall influence of temperature on jumping frequency, with significantly more jumps at 22°C than at 18°C ($P < 0.05$).

L. abnormis

As with A. pseudococci there is no evidence of an effect of either temperature or infestation on jumping frequency.

TABLE 19. Effect of various constant temperatures on jumping frequency of female Leptomastix dactylopi on clean and host infested leaf surfaces.

MEAN NUMBER OF JUMPS					
TEMP (°C)		clean		infested	
		$\bar{x}$	s.d.	$\bar{x}$	s.d.
18	A	0.0	0	0.0	0
22	B	2.33	3.83	0.17	0.41
26	AB	1.00	1.30	0.67	1.21
30	AB	0.33	0.52	0.0	0

TABLE 20. Effect of various constant temperatures on jumping frequency of female Leptomastidea abnormis on clean and host infested leaf surfaces.

MEAN NUMBER OF JUMPS					
TEMP (°C)		clean		infested	
		$\bar{x}$	s.d.	$\bar{x}$	s.d.
18	A	0.17	0.41	0.0	0
22	A	1.67	3.61	0.0	0
26	A	0.33	0.82	0.0	0
30	A	0.33	0.52	0.0	0

Means of six individuals observed for 5 min each, with standard deviation. Same letters indicate no significant difference at the 5% level. Capital letters for comparisons within main effects of temperature or infestation.

3.3.2.6 The frequency of abandoning leaf

The results for the three species are presented separately in tables 21, 22 and 23.

A. pseudococci

Analysis of variance identifies an effect of infestation on the frequency with which the leaf is abandoned, but no effect of temperature. The parasitoids leave an infested leaf less often than a clean one ($P < 0.01$).

TABLE 21. Effect of various constant temperatures on frequency with which female Anagyrus pseudococci abandon clean and infested leaf surfaces.

		MEAN NUMBER OF TIMES LEAF ABANDONED			
		clean		infested	
TEMP (°C)		$\bar{x}$	s.d.	$\bar{x}$	s.d.
18	A	1.0	0.89	0.33	0.52
22	A	1.0	1.09	0.5	0.84
26	A	2.33	2.06	0.33	0.82
30	A	2.00	2.00	0.83	1.6

Means of six individuals observed for 5 min each, with standard deviation. Same letters indicate no significant difference at the 5% level. Capital letters for comparisons within main effects of temperature or infestation.

L. dactylopii

Analysis of variance fails to show any influence of temperature or infestation on the frequency with which a leaf is abandoned.

TABLE 22. Effect of various constant temperatures on frequency with which female Leptomastix dactylopii abandon clean and infested leaf surfaces.

MEAN NUMBER OF TIMES LEAF ABANDONED					
TEMP (°C)		clean		infested	
		$\bar{x}$	s.d.	$\bar{x}$	s.d.
18	A	1.67	1.21	0.67	0.82
22	A	2.00	1.33	2.17	1.60
26	A	2.33	2.06	2.00	2.69
30	A	2.67	1.63	1.03	0.89

TABLE 23. Effect of various constant temperatures on frequency with which female Leptomastidea abnormis abandon clean and infested leaf surfaces.

MEAN NUMBER OF TIMES LEAF ABANDONED					
TEMP (°C)		clean		infested	
		$\bar{x}$	s.d.	$\bar{x}$	s.d.
18	AB	0.83	1.17	0.03	0
22	A	0.50	0.57	0.0	0
26	A	0.17	0.41	0.03	0
30	B	1.50	1.52	0.53	0.84

Means of six individuals observed for 5 min each, with standard deviation. Same letters indicate no significant difference at the 5% level. Capital letters for comparisons within main effects of temperature or infestation.

yes

L. abnormis

Analysis of variance reveals significant effects of both temperature and infestation. A clean leaf is abandoned more frequently than an infested one ($P < 0.01$). The effect of temperature is not a linear one, with the leaf being abandoned more frequently at 30°C than at 22°C ($P < 0.05$) or 26°C ($P < 0.01$), but not at 18°C.

3.3.2.7 Frequency of edge contact

The results are presented for the three species separately in tables 24, 25 and 26.

A. pseudococci

The frequency with which the parasitoids contact the "edge" of the arena is affected by both temperature and infestation. Significantly more edge contacts occur on a clean leaf at 26°C than at 18°C ($P < 0.01$) or 22°C ($P < 0.05$) and at 30°C than at 18°C or 22°C ($P < 0.001$), but there are no significant differences in the number of edge contacts at any of the four temperatures, on an infested leaf. The number of edge contacts is significantly larger on a clean leaf than on an infested one at all temperatures above 18°C ($P < 0.05$ at 22°C; $P < 0.001$ at 26°C and 30°C).

TABLE 24. Effect of various constant temperatures on the number of times that female Anagyrus pseudococci contact the arena edge, on clean and host infested leaf surfaces.

TEMP (°C)	MEAN NUMBER OF EDGE CONTACTS			
	clean		infested	
	$\bar{x}$	s.d.	$\bar{x}$	s.d.
18	5.67 ac	5.61	1.17 c	1.83
22	7.00 a	4.43	1.83 c	2.23
26	13.17 b	5.23	2.83 c	4.40
30	17.67 b	4.93	1.33 c	1.5

Means of six individuals observed for 5 min each, with standard deviation. Same letters indicate no significant difference at the 5% level. Capital letters for comparisons within main effects of temperature or infestation, small letters - comparisons may be made between clean and infested leaves at a given temperature and across temperature for a given leaf type.

L. dactylopii

Analysis of variance shows that there is a linear effect of temperature on the number of edge contacts, with significantly more edge contacts being made at 30°C than at 18°C ($P < 0.001$), 22°C or 26°C ($P < 0.05$). No influence of infestation can be identified.

TABLE 25. Effect of various constant temperatures on the number of times that female Leptomastix dactylopii contact the arena edge, on clean and host infested leaf surfaces.

MEAN NUMBER OF EDGE CONTACTS					
TEMP (°C)		clean		infested	
		$\bar{x}$	s.d.	$\bar{x}$	s.d.
18	A	3.17	3.25	1.33	1.03
22	A	3.00	1.99	4.67	2.42
26	A	4.67	2.27	3.50	3.88
30	B	8.83	4.96	4.33	1.97

TABLE 26. Effect of various constant temperatures on the number of times that female Leptomastidea abnormis contact the arena edge, on clean and host infested leaf surfaces.

MEAN NUMBER OF EDGE CONTACTS					
TEMP (°C)		clean		infested	
		$\bar{x}$	s.d.	$\bar{x}$	s.d.
18		2.17	a 2.40	0.53	a 0.84
22		7.33	b 3.79	0.67	a 1.03
26		8.50	b 6.83	1.50	a 2.81
30		9.50	b 6.74	1.33	a 1.21

Means of six individuals observed for 5 min each, with standard deviation. Same letters indicate no significant difference at the 5% level. Capital letters for comparisons within main effects of temperature or infestation; small letters - comparisons may be made between clean and infested leaves at a given temperature and across temperature for a given leaf type.

L. abnormis

The results are similar to those for A. pseudococci, with analysis of variance showing an effect of both temperature and infestation. On a clean leaf the frequency of edge contacts rises in a linear manner as the temperature increases, with significantly fewer contacts made at 18°C than at 22°C ($P < 0.05$), 26°C or 30°C ($P < 0.01$). There are no significant differences with temperature on an infested leaf. The frequency of edge contacts is significantly greater on a clean leaf than on an infested one at 22°C, 26°C ($P < 0.01$), and 30°C ($P < 0.001$).

3.3.2.8 Number of host encounters and ovipositions

The results are presented in tables 27, 28 and 29.

A. pseudococci

Analysis of variance reveals that temperature effects both the number of host encounters made by this parasitoid, and the number of ovipositions which ensue. Significantly more host encounters are made at 26°C than at 18°C ($P < 0.05$) and at 30°C than at 18°C ($P < 0.01$) or 22°C ($P < 0.05$), whilst significantly more ovipositions are made at 30°C than at 18°C ($P < 0.05$).

L. dactylop11

There is no evidence of an effect of temperature on the number of host encounters or ovipositions made during the observation period by this parasitoid.

L. abnormis

As with L. dactylop11 there is no evidence of an effect of temperature on host encounter or oviposition rate.

TABLE 27. Effect of various constant temperatures on the number of host encounters and ovipositions by female Anagyrus pseudococci whilst searching on a host infested leaf.

TEMP (°C)	host encounters			ovipositions		
	$\bar{x}$		s.d.	$\bar{x}$		s.d.
18	0.17	a	0.41	0.17	d	0.41
22	0.83	ab	1.17	0.83	de	1.17
26	2.83	bc	2.14	0.83	de	1.33
30	3.33	c	2.66	2.67	e	3.27

TABLE 28. Effect of various constant temperatures on the number of host encounters and ovipositions by female Leptomastix dactylopi whilst searching on a host infested leaf.

TEMP (°C)	host encounters			ovipositions		
	$\bar{x}$		s.d.	$\bar{x}$		s.d.
18	0.83	a	1.33	0.33	b	0.82
22	0.50	a	0.55	0.33	b	0.82
26	0.17	a	0.41	0.00	b	0
30	1.00	a	0.89	0.33	b	0.52

TABLE 29. Effect of various constant temperatures on the number of host encounters and ovipositions by female Leptomastidea abnormis whilst searching on a host infested leaf.

TEMP (°C)	host encounters			ovipositions		
	$\bar{x}$		s.d.	$\bar{x}$		s.d.
18	0.33	a	0.52	0.33	b	0.52
22	0.50	a	0.84	0.33	b	0.52
26	0.17	a	0.41	0.17	b	0.41
30	0.67	a	1.63	0.67	b	1.03

Means of six individuals observed for 5 min each, with standard deviation. Same letters indicate no significant difference at the 5% level, comparisons may be made across temperature, but not between encounters and ovipositions.

3.3.2.9 Time apportioned to various activities

The data on the time spent in various activities on the different surfaces, was used to calculate the proportion of time given to each of the categories: walking; antennating, palpating; preening; resting; and host handling whilst on the leaf surface. This data is presented as a series of bar charts (figures 17, 18 and 19), showing the mean values for six individuals with standard errors. Data for each species is presented separately.

Jumping also occupied a finite period and this was taken into account in calculating the proportion of time spent in each of the above activities. However, the proportion of time spent jumping was minimal at all temperatures in both treatments and is thus not discussed along with the other activities. The frequency of jumping (i.e. the number of jumps made in the five minute period) was analysed separately (section 3.3.2.5).

Two separate sets of statistical analysis were performed. In both cases a binomial distribution had to be assumed as the data are in the form of proportions.

Differences in the proportion of time spent in a given activity at different temperatures in the presence or absence of hosts were tested using 2-factorial analysis of variance. The data were first transformed using an arc-sine transformation. Significance of differences was established using the l.s.d. at the 5%, 1%, and 0.1% levels. The data for clean and infested leaves could be treated separately if analysis revealed an interaction between temperature and infestation. If there was no evidence of an interaction then main effects of temperature and infestation could be identified from appropriately pooled data.

A further regression treatment was also included in the analysis in order to expose any linear, quadratic or cubic trends within the data.

Statistical differences in the proportion of time spent in the various activities within treatments were determined using the binomial test of dependant proportions (Kempson, pers. comm.).

A. pseudococci

Analysis of variance shows that the proportion of time spent walking is affected by both temperature and infestation. A significantly greater proportion of time is spent walking at both 26°C and 30°C than at 18°C ($P < 0.01$), and in general a greater proportion of time is spent walking on a clean leaf surface than on an infested one.

The proportion of time spent antennating is not affected by temperature or infestation.

There are also no significant effects of temperature or infestation on the proportion of time spent palpating.

Analysis of variance for the data on preening indicates a significant effect of both temperature and infestation, and identifies a linear trend within the data. The proportion of time spent preening decreases with rising temperature on a clean leaf and increases with rising temperature on an infested leaf. On a clean leaf a significantly greater proportion of time is spent preening at 18°C than at 26°C or 30°C ($P < 0.05$), and at 22°C than at 26°C ($P < 0.05$). On an infested leaf a significantly greater proportion of time is spent preening at 26°C than at 18°C ($P < 0.05$). A significantly greater proportion of time is spent preening on an infested than on a clean leaf at both 26°C and 30°C ($P < 0.01$).

The proportion of time spent resting is significantly greater at 18°C than at 22°C ($P < 0.01$), 26°C or 30°C ($P < 0.001$).

The proportion of time spent host handling increases with rising temperature on an infested leaf, a significantly greater proportion of the time being spent with hosts at 30°C than at 18°C, 22°C ($P < 0.001$) or 26°C ($P < 0.01$).

Temperature affects the proportion of time spent in individual activities differently, resulting in changes in the way in which time is apportioned between the various activities at different temperatures. These changes are also dependent on whether the leaf is infested or not, as can be seen in figure 17.

Clean leaf

At 18°C on a clean leaf no single activity is dominant. The proportion of time spent in any one activity is not significantly different from that spent in any other.

At 22°C a significantly greater proportion of time is spent walking than palpating, preening ($P < 0.05$), or resting ($P < 0.01$).

At 26°C there is no difference in the proportion of time spent walking, antennating or palpating, but a significantly greater proportion of time is spent walking than preening, or resting ($P < 0.001$).

At 30°C the proportion of time spent walking is significantly greater than that spent antennating ($P < 0.05$), preening, or resting ($P < 0.001$).

Figure 17. Histogram showing proportion of time spent in various activities by female Anagyrus pseudococci at different constant temperatures, whilst searching on clean and host infested leaf surfaces.

The behaviour of the parasitoids was observed during a five minute period whilst confined on the leaf in an inverted, 10 cm diameter, glass petri dish.

A Clean leaf B Infested leaf

The columns of the histogram represent the mean proportion of the time spent in the various activities by six individuals, amounting to a total of 30 minutes observation time per treatment.

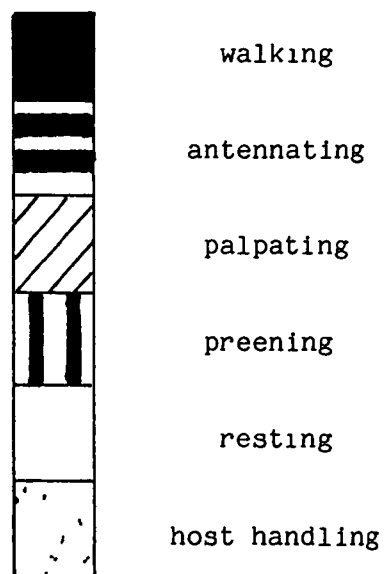
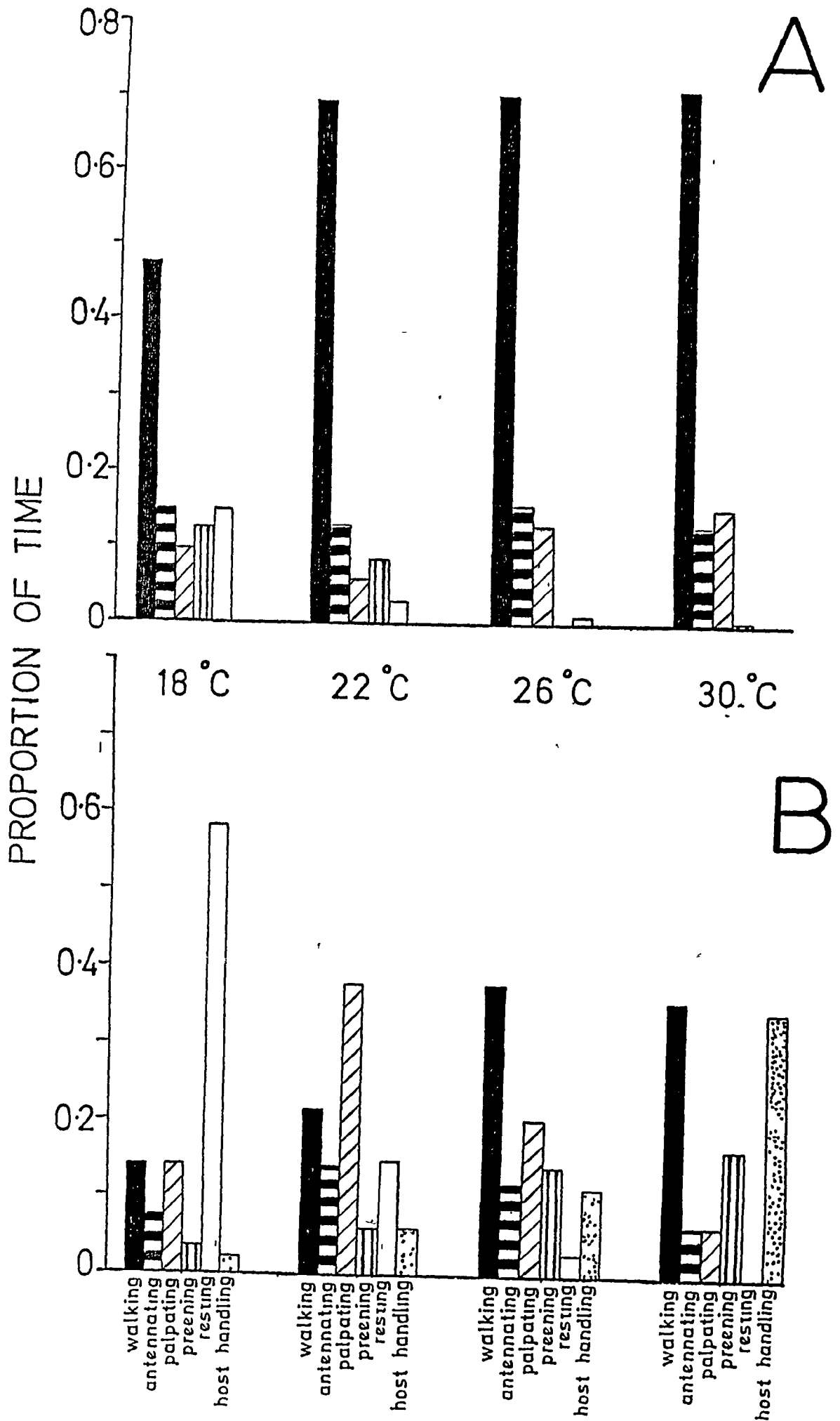


Figure 17



Infested leaf

Time is apportioned very differently at different temperatures on an infested leaf. At 18°C a significantly greater proportion of time is spent resting than antennating ($P < 0.05$), preening, or host handling ($P < 0.001$).

At 22°C, 26°C and 30°C there are no significant differences in the way that time is apportioned between all the activities.

L. dactylopii

Analysis of variance shows that the proportion of time spent walking is affected by both temperature and infestation. On a clean leaf a significantly greater proportion of time is spent walking at 26°C than at 22°C ($P < 0.05$) and there is evidence of a cubic trend within the data. A significantly greater proportion of time is spent walking on an infested leaf than on a clean leaf at 22°C ($P < 0.05$) (see figure 18)

The proportion of time antennating decreases with increasing temperature in a linear manner, the proportion of time spent antennating being significantly less at 30°C than at 22°C ($P < 0.05$). There is no evidence of an effect of infestation.

The proportion of time spent palpating increases with increasing temperature with evidence of a linear trend. On a clean leaf significantly more time is spent palpating at 30°C than at 22°C, whilst on an infested leaf, the proportion of time spent palpating at 30°C is significantly greater than at any other temperature ($P < 0.01$) and significantly greater at 26°C than at 22°C ($P < 0.01$) or 18°C ($P < 0.05$). A significantly greater proportion of time is spent palpating on an infested leaf than on a

clean one at both 26°C and 30°C ($P < 0.05$).

There are no significant effects of temperature or infestation on the proportion of time spent preening.

There are no significant effects of temperature or infestation on the proportion of time spent resting or on the time handling hosts.

As with the other two species there are differences in the way in which time is apportioned between the various activities at different temperatures.

Clean leaf

At 18°C the proportion of time walking is significantly greater than that spent preening or resting ($P < 0.05$). There is no significant difference in the proportion of time spent in the other activities.

At 22°C there are no significant differences in the way that time is apportioned between the activities.

At 26°C a significantly greater proportion of time spent walking than preening ($P < 0.01$) or resting ($P < 0.05$ at 26°C).

At 30°C a significantly greater proportion of time is spent walking than preening ($P < 0.01$), or resting ($P < 0.01$).

Figure 18. Histogram showing proportion of time spent in various activities by female Leptomastix dactylopii at different constant temperatures, whilst searching on clean and host infested leaf surfaces.

The behaviour of the parasitoids was observed during a five minute period whilst confined on the leaf in an inverted, 10 cm diameter, glass petri dish.

A Clean leaf B Infested leaf

The columns of the histogram represent the mean proportion of the time spent in the various activities by six individuals, amounting to a total of 30 minutes observation time per treatment.

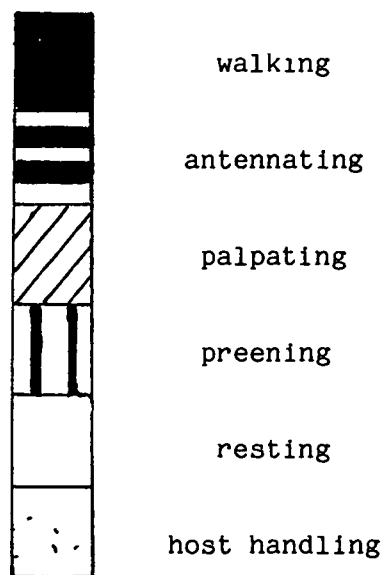
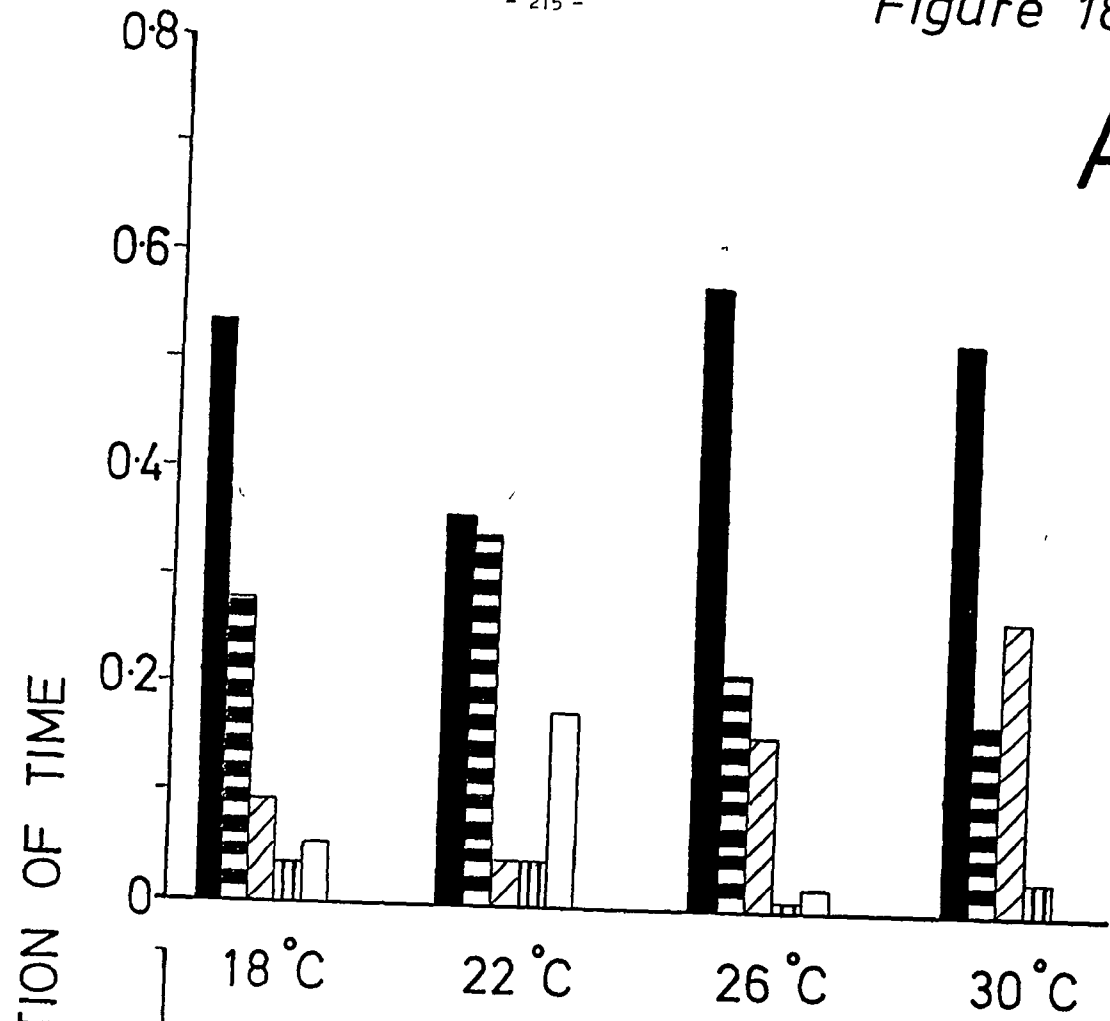
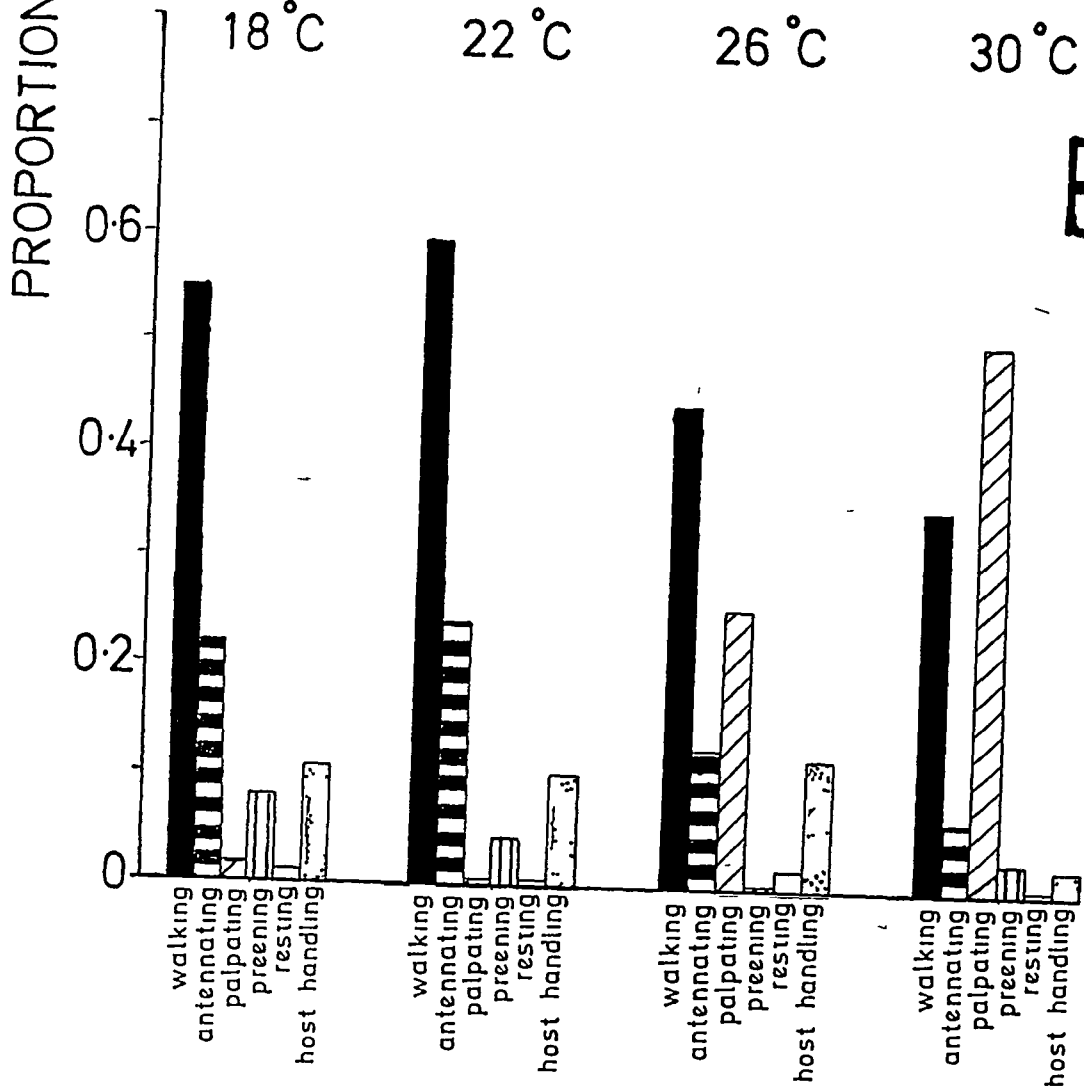


Figure 18

A



B



Infested leaf

The patterns of activity seem to change in a different manner on an infested leaf. At 18°C there are no significant differences in the time spent in the different activities.

At 22°C a significantly greater proportion of time is spent walking than preening ($P < 0.05$), palpating or resting ($P < 0.01$).

At 26°C the proportion of time spent walking is significantly greater than that spent preening or resting ($P < 0.05$).

At 30°C a significantly greater proportion of time is spent palpating than preening, resting ($P < 0.05$) or host handling ($P < 0.01$).

L. abnormis

As with A. pseudococci there appears to be a shift in the pattern of activities with increasing temperature (see figure 19).

Analysis of variance shows that temperature has no significant effect on the proportion of time spent walking on a clean leaf, but that on an infested leaf a significantly greater proportion of time is spent walking at 18°C than at 26°C ($P < 0.05$). The overall trend is a linear one, the proportion of time spent walking decreasing with increasing temperature. A significantly greater proportion of time is spent walking on a clean than on an infested leaf at 22°C, 26°C and 30°C ($P < 0.01$).

The proportion of time spent antennating is independent of temperature on both clean and infested leaves. Analysis of variance identifies an effect of infestation, with a significantly greater proportion of time spent

antennating in the presence of hosts than on a clean leaf ($P < 0.05$).

The proportion of time spent palpating does not change with temperature, but is influenced by infestation, with a greater proportion of time spent palpating on an infested leaf than on a clean one ($P < 0.001$).

The proportion of time spent preening is not affected by either temperature or infestation.

The proportion of time spent resting is not affected by temperature, but a significantly greater proportion of time is spent resting on a clean leaf than on an infested one ($P < 0.05$).

There is no significant effect of temperature on the proportion of time spent in host handling.

The way in which time is apportioned between the various activities appears to be affected by both temperature and infestation.

Clean leaf

At 18°C there is a significant difference between the proportion of time spent walking and that spent palpating ($P < 0.05$). Otherwise there are no significant differences in the way that time is apportioned between the various activities.

At 22°C a significantly greater proportion of the time is spent walking than in any other activity (antennating and resting. $P < 0.01$, palpating, and preening $P < 0.001$).

Figure 19. Histogram showing proportion of time spent in various activities by female Leptomastidea abnormis at different constant temperatures, whilst searching on clean and host infested leaf surfaces.

The behaviour of the parasitoids was observed during a five minute period whilst confined on the leaf in an inverted, 10 cm diameter, glass petri dish.

A Clean leaf B Infested leaf

The columns of the histogram represent the mean proportion of the time spent in the various activities by six individuals, amounting to a total of 30 minutes observation time per treatment.

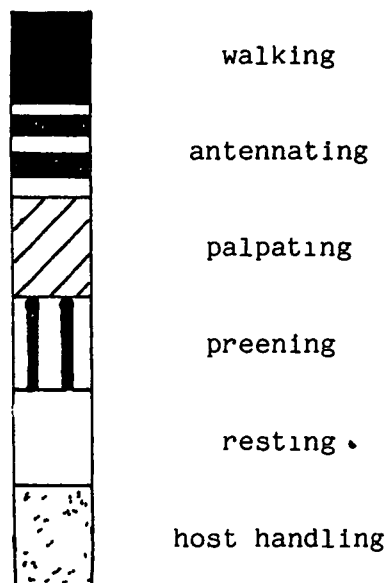
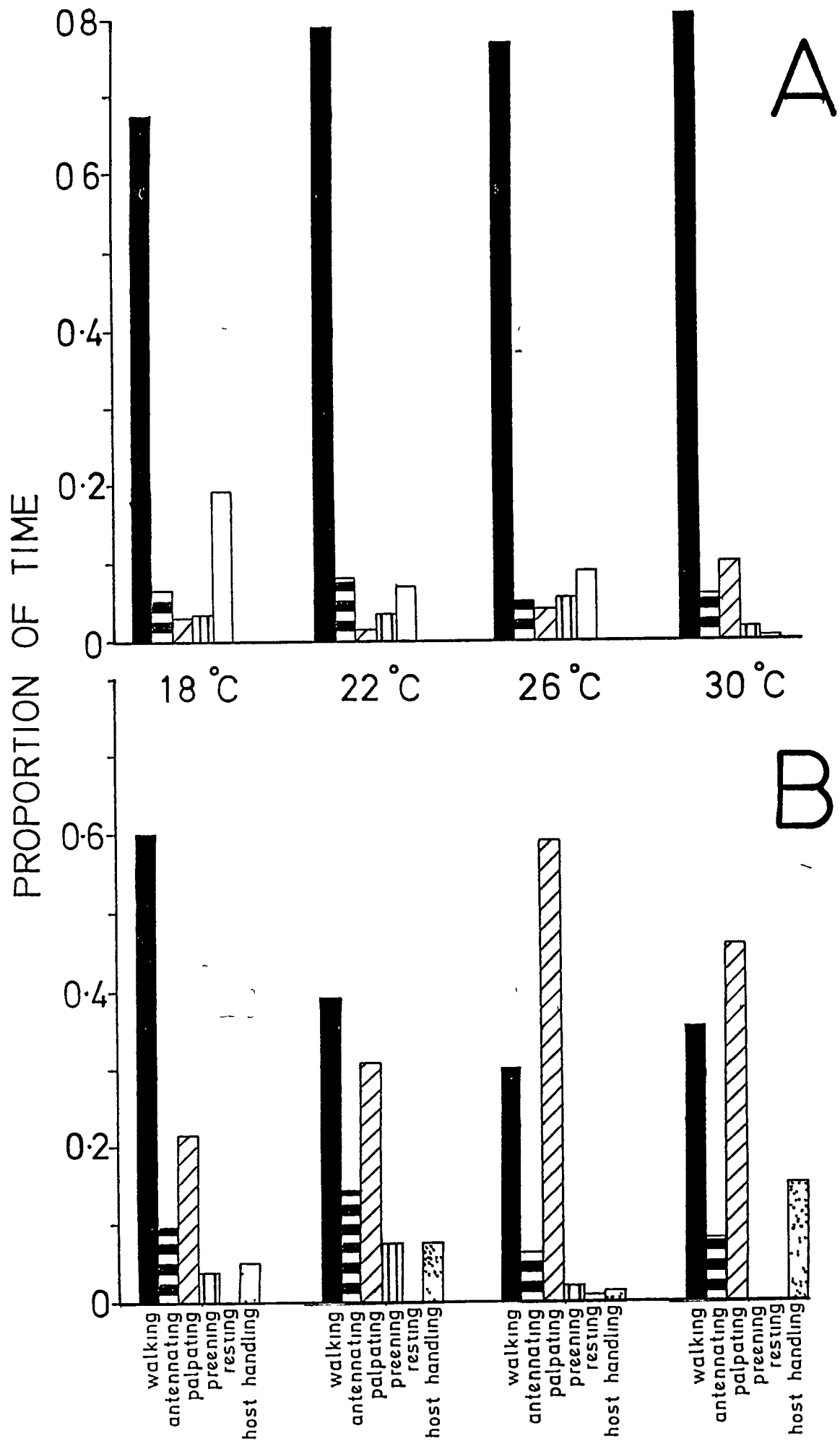


Figure 19

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At 26°C the pattern is similar, a significantly greater proportion of the time being spent walking than antennating, preening, resting ($P < 0.01$), or palpating ($P < 0.001$), with no significant differences between the other activities.

Similarly at 30°C, the proportion of time spent walking is significantly greater than that palpating ($P < 0.05$), antennating ($P < 0.01$), preening or resting ($P < 0.001$).

Infested leaf

The way time is apportioned between the various activities is very different on an infested leaf.

At 18°C a significantly greater proportion of time is spent walking than preening or resting ($P < 0.05$).

At 22°C there are no significant differences in the way that time is apportioned between the activities.

At 26°C a significantly greater proportion of time is spent palpating than preening, resting or host handling ($P < 0.01$), but there are no differences between the other activities.

The same is true at 30°C (although the level of probability shows a less highly significant effect, $P < 0.05$).

Discussion

From these results it is apparent that the overall effect of temperature on searching behaviour is the resultant of several component parts, which all have different thermal responses. The combined effect leads to the differences in searching pattern which are so apparent in figures 11, 12 and 13. In all three cases the differences in searching patterns at 18°C and 30°C, on clean and host infested leaves are obvious. This indicates that for a given temperature, infestation changes the area covered by the parasitoid and that temperature has a pronounced effect on the area of a clean leaf that can be searched, but little influence on searching on an infested leaf. Thus at 18°C the distance walked is not markedly different on a clean and an infested leaf, but in the latter case searching is far less extensive, being restricted within a small portion of the total leaf area. At 30°C the distance walked and the proportion of the leaf surface covered is evidently greater than at 18°C on a clean leaf. However, the distance walked and area covered on an infested leaf does not appear to be very different. These differences come about due to the influence of temperature on a number of aspects of the parasitoid's behaviour.

The observed increase in the walking speed with temperature is consistent with results found using other insects (Biever, 1972). The tendency for walking speed to approach a maximum at high temperature, as seen with L. abnormis and L. dactylopii is also to be expected. Reduction in walking speed on host contaminated surfaces has also been frequently reported (Waage, 1978, Yano, 1978), but the influence of temperature on this phenomenon has not been previously studied. The evidence is that the presence of host cues "damps" the effects of temperature, resulting in slight but generally insignificant changes in walking speed with increased temperature.

Another feature of intensive searching behaviour within patches is increased turning movements leading to more thorough coverage of a relatively small area (Murdie and Hassell, 1973, Vinson et al., 1978, Waage, 1978; Yano, 1978). This feature is also evident in the observations made on A. pseudococci, L. dactylopii and L. abnormis, all of which showed a greater number of turns per metre walked in the presence of hosts than in their absence. However, it is impossible to determine from this experiment whether a higher rate of turning is elicited by the presence of host stimuli (in the form of honeydews, etc.) ie. klinokinesis or due to moving out of a contaminated patch ie. klinotaxis.

Whether hosts are present or not, the rate of turning is largely independent of temperature, the number of turns per metre being constant despite increased walking speed. This is due to the rate of turning per second spent walking increasing with temperature, the effect being damped by the presence of host cues. Intensive searching within patches is thus largely unaffected by temperature.

It appears from the data that the parasitoids show a greater tendency to jump at higher temperatures and on clean rather than infested leaf surfaces. However, none of the trends were significant, except that L. dactylopii jumped more at 22°C than at 18°C. Jumping frequency is likely to be an indication of the parasitoids "desire" to disperse from a given area. This is probably influenced by both temperature and infestation, and it may be that the five minute observation periods were too short to allow sufficient jumping, for a significant effect to be detected.

In the experiments on a clean leaf the parasitoids made more frequent contacts with the edge of the arena as the temperature (and correspondingly

their walking speed) rose. However, only L. abnormis abandoned the leaf more frequently at higher temperatures, indicating that the frequency of edge contact is not directly proportional to the rate of leaf abandonment. The lower frequency of abandonment on an infested leaf cannot however be attributed directly to any influence of host cues, as the frequency of edge contact was so much lower than on clean leaves, particularly at high temperatures. The tendency shown by the parasitoids (except L. dactylopii) to abandon the leaf more frequently at 30°C, despite making less contacts with the arena edge, may be indicative of a weakening in the arrestment responses to the host patch. However, there is insufficient data from this investigation to make any definitive statements and further work would be necessary to show any such effect.

A. pseudococci showed an increase in both the number of host encounters and the number of ovipositions made, with rising temperature. At low temperatures the number of ovipositions and the number of host encounters were similar, but at higher temperatures more encounters occurred which did not result in oviposition. This phenomenon was noted in the aphid parasitoid, Praon exoletum, which showed a reduction in ovipositional strikes at temperature extremes. As a result of this and changes in other behavioural traits, the functional response of this parasitoid was also found to vary with temperature (Messenger, 1968). Neither L. dactylopii nor L. abnormis showed differences in host encounter or oviposition rates with increasing temperature.

All three parasitoid species studied here show a marked difference in behaviour when hosts are present from when they are absent. These two distinct phases of parasitoid searching behaviour: searching between host patches and searching within host patches have been shown for other parasitoids (Waage, 1979). For A. pseudococci, L. dactylopii, and

L. abnormis, both must still be regarded as random processes, as there is no evidence of directional responses in the location of host patches, and observations made in this study indicate that host location within a patch is an entirely random event. However, the behavioural switch resulting from the entry into a host patch means that overall searching strategy has non-random elements.

The effects of temperature on the two different patterns of behaviour may be summarised as follows

A. pseudococci

Patch location

At low temperature the proportion of the population which is active is very small. Those that are active do not apportion significantly more time to walking than to antennating, preening or resting, walk slowly and with few major changes in direction. Thus their efficiency at locating patches of hosts at 18°C is likely to be very low.

As the temperature rises to 22°C, the proportion of the population which is active increases dramatically, and significantly more time is apportioned to walking than to any other activity except antennating. Thus one would expect that the parasitoid's ability to locate host patches would be greater at 22°C than at 18°C.

At 26°C the proportion of the population which is active is no higher than at 22°C, and there is no significant increase in the proportion of time spent walking over that at 22°C. The walking speed though is higher than at 18°C and the number of turns made per second spent walking is higher

than at either 22°C or 18°C. Again the result would be increased patch location efficiency with the higher temperature.

As the temperature rises still further to 30°C, the proportion of the population which is active increases, the walking speed is higher than at any other temperature, but the proportion of time spent walking is not significantly greater. Increased patch location efficiency is still probable.

Patch arrestment

At 18°C only a small proportion of the population is active, and there is no more time apportioned to walking than to any other activity. More time is apportioned to resting than any activity, which may be adaptive in that it means that whilst temperatures are unfavourable the parasitoid will remain within the host patch. The walking speed is low, and a very large number of turns made per metre walked. These effects combined together would produce a high level of arrestment in host patches, although the number of hosts encountered is likely to be very low.

At 22°C the proportion of the population active is much higher than at 18°C. A significantly smaller proportion of time is spent resting than at 18°C, but there is no more time apportioned to walking than to any other activity, with feeding becoming increasingly important. The walking speed remains very low and the rate of turning per metre high. Thus despite an overall rise in the level of activity, the degree of arrestment within a host patch is still high.

At 26°C the situation is very similar to that at 22°C, but with the number of hosts encountered increasing, the time spent in handling hosts begins to

contribute to arrestment within the patch. Thus patch arrestment is still high, and the probability of finding hosts increasing.

With a further rise in temperature and a greater proportion of the population active, the only changes are in the proportion of time spent with hosts, which is greater than at any of the lower temperatures. This is then contributing increasingly to parasitoid arrestment within host patches, which remains at a high level.

L. dactylopii

Patch location

The behaviour of L. dactylopii appears slightly different from that of the other two species. The amount of time apportioned to walking does not follow a consistent trend with temperature, and the proportion of time spent resting does not decrease above 26°C. Palpating takes up a major proportion of the time at higher temperatures. The effect of temperature on walking speed does not show such distinct linearity as it does with the other species. The overall effect of these factors is to lower the searching efficiency at 22°C, although it is still likely to be more efficient than at 18°C.

Patch arrestment

Again L. dactylopii shows differences from the other species, with a greater proportion of time spent walking at low temperatures. The proportion of time spent with hosts seems relatively constant across temperature, except at 30°C where it declines. The walking speed on an infested area increases much more with temperature than for the other

species. These factors may reduce the patch arrestment response at higher temperatures, although feeding becomes an important activity and may compensate to some degree for the increased walking speed and reduced tendency to be arrested by hosts.

L. abnormis

Patch location

The behaviour patterns of L. abnormis on a clean leaf are basically very similar to those of A. pseudococci, and change in a similar way with temperature, except that this parasitoid never walks as fast and tends to have a higher rate of turning per metre.

Patch arrestment

Again very similar to A. pseudococci except that the proportion of time spent feeding contributes more heavily to arrestment within host patches at higher temperatures.

These factors have great significance in terms of the population dynamics of the host/parasitoid system, as arrestment within patches can contribute to stability of the relationship (Hassell, 1982; Waage and Hassell, 1982). Thus higher temperatures may be a stabilising factor in parasitoid/host population dynamics. It appears that higher temperatures increase the likelihood of the parasitoids locating host patches, without reducing arrestment within patches, which will tend to lead to aggregation of parasitoids within host patches. This in turn is likely to produce a clumped distribution of attacks, depending on the host density within the patches, leading to population stability if parasitism per patch is

density-dependant. Aggregation of parasitoids does not necessarily lead to density-dependant parasitism, if relatively more superparasitism occurs, or if time is wasted in activities other than searching. However, observations of actual parasitoid foraging in the glasshouse would be necessary in order to confirm that density-dependant or independant parasitism was occurring (Waage, 1983).

3.4 THE EFFECT OF TEMPERATURE ON OVIPOSITION

3.4.1 Oviposition by *P. citri* at various constant temperatures

Introduction

The number of eggs laid by individual females is a major factor determining the birth rate into an insect population and has a marked influence on population dynamics. Female *P. citri* are relatively sedentary and once they have settled down to feed as second instar nymphs, they will not generally move, unless disturbed. As the female does not have to locate any specific oviposition site, the number of eggs laid depends solely on the level of egg production.

The effects of temperature on egg laying have been widely documented for a number of insects. Generally the number of eggs laid increases with rising temperature up to an optimum, declining rapidly as temperatures increase still further (Bursell, 1964).

The number of eggs laid by individual *P. citri* under fluctuating conditions has been recorded by several authors, and reports vary considerably (Clausen, 1915, Myers, 1932, Quayle, 1938; Khoo, 1974). Little information is available on the effects of constant temperatures on egg laying by the mealybug, but in one study the mean number of eggs laid per female was found to increase with temperature from 217.89 at 15.5°C up to 342.1 at 27°C (Whitcomb, 1940).

The aim of this study was to investigate the effect of a series of constant temperatures on the number of eggs laid by female *P. citri*.

Materials and methods

The experiments were carried out at four constant temperatures in either C.T. rooms or incubators. These were $18 \pm 0.5^{\circ}\text{C}$, $22 \pm 0.5^{\circ}\text{C}$, $26 \pm 0.5^{\circ}\text{C}$ and $30 \pm 0.5^{\circ}\text{C}$. Light was supplied for an 18 h photoperiod by a combination of fluorescent tubes. Relative humidity varied between 45% and 60%.

Two full sized egg masses were transferred onto a clean, sprouted potato tuber in a ventilated, plastic box and placed at the experimental temperature. Three replicates were set up at each temperature. The mealybugs were allowed to develop through their entire life cycle until mature egg masses were produced by the F1 generation. Egg masses were then dissected in a drop of paraffin on a glass slide and a count made of the number of eggs that each contained. Paraffin was used as this is a solvent for wax, and facilitated egg mass dissection. 20 egg masses were used from each temperature treatment and the mean number of eggs per egg mass calculated.

Results

The mean number of eggs laid per female at each temperature is shown in table 30.

Analysis of variance shows that temperature has a significant effect on the number of eggs laid by female P. citri. The number of eggs laid at 30°C is significantly less than at any other temperature (l.s.d. at the 0.01% level).

TABLE 30. Effect of various constant temperatures on egg laying by Planococcus citri

TEMP (°C)	MEAN NUMBER OF EGGS/EGG MASS		
	n	$\bar{x}$	s.d.
18	20	304.4	53.69
22	20	244.05	58.44
26	20	287.9	93.3
30	20	83.6	26.06

n = number of egg masses dissected

Discussion

In this study P. citri laid approximately 244 eggs at 22°C and 288 at 26°C. These figures are slightly lower than those determined by Whitcomb (1940) as 269 at 21°C and 342.1 at 27°C. This may have been due to the use of different host plants, as Whitcomb reared the mealybugs on Coleus sp. and gardenia. Host plant has been shown to have a marked effect on the fecundity of the whitefly Trialeurodes vaporariorum (van Lenteren and Woets, 1976) and the use of different host plants may well explain the previously variable reports of mealybug fecundity. The trend towards larger egg masses at higher temperature apparent in Whitcomb's work is not shown here. No significant difference was found between the number of eggs laid at 18°C, 22°C and 26°C in this study.

The implications of these results are that at temperatures within the normal range, egg laying will not vary greatly. High temperatures reduce the numbers of eggs laid by P. citri, but experiments under fluctuating conditions would be necessary to determine the effects of short periods of high temperature, as would occur in the glasshouse. The fact that

oviposition by P. citri is impaired at 30°C, indicates that high temperatures will tend to slow down the population growth of the pest.

3.4.2 Oviposition by parasitoids at various constant temperatures

Introduction

For insect parasitoids, the number of eggs laid depends on the egg production capacity of the female and on the location of suitable oviposition sites. As the ability to find hosts is involved, egg production rates and oviposition rates may be very different. It is the latter which is more important in determining the number of offspring produced by a given individual or generation.

Egg production, host finding and the number of eggs laid are all affected by temperature, and these factors have been widely studied for a number of insects. Work on the effects of different constant temperatures on progeny production by A. pseudococci, showed that high temperatures stimulate egg laying by this parasitoid (Niyazov, 1968). Avidov et al., (1967) found that fertilised female A. pseudococci produced an average total of 33 offspring during their lives, at 26°C. They also investigated the effects of variable temperatures on egg laying, and found that the number of offspring per female generally increased with rising temperature.

The aim of this study was to investigate the effect of various constant temperatures on the number of progeny produced by female A. pseudococci, L. abnormis and L. dactylopii.

Materials and methods

The parasitoids used in this study were from cultures maintained at 26°C and 45-60% r.h. The experimental procedure was the same for all three species, unless otherwise stated, and the same for each temperature regime.

The temperatures used were $18 \pm 0.5^{\circ}\text{C}$, $20 \pm 0.5^{\circ}\text{C}$, $22 \pm 0.5^{\circ}\text{C}$ (L.dactylopii only), $24 \pm 0.5^{\circ}\text{C}$ (A. pseudococci and L. abnormis only), $26 \pm 0.5^{\circ}\text{C}$, $30 \pm 0.5^{\circ}\text{C}$ and $34 \pm 1^{\circ}\text{C}$. All (except the last) were achieved using constant temperature rooms with relative humidity between 45% and 60% and lighting, on a 18/6 h light/dark cycle, was provided throughout the experimental period by a combination of 30 W, 40 W and 65-80 W fluorescent tubes. The 34°C regime was achieved using a Gallenkamp compenstat illuminated cooled incubator (130 mm x 50 mm x 50 mm) with constant lighting provided by 4 x 8 W fluorescent lamps.

15 female parasitoids were removed from cultures containing adults aged between 1-7 days, and 5 were placed, under CO_2 induced anaesthesia, in each of three ventilated, plastic boxes (173 mm x 115 mm x 65 mm) streaked with honey solution (approx 50:50 honey.water) at either end. These were then left at the experimental temperature for approximately 17 h to recover from the anaesthetic and acclimatise to the temperature. Three sprouted potatoes, heavily infested with mealybug (at least 200/potato) of the appropriate size for each parasitoid species, were then introduced into the boxes and left undisturbed for 5 h. After this period of time the female parasitoids were removed under CO_2 , and the boxes incubated at $26 \pm 0.5^{\circ}\text{C}$. Emergence of offspring was monitored twice daily.

Results

For each of the three parasitoid species, progeny production by 5 females over a 5 h period at 6 different constant temperatures are shown in table 31.

TABLE 31. Effect of various constant temperatures on progeny production by Anagyrus pseudococci, Leptomastix dactylopii and Leptomastidea abnormis

MEAN NUMBER OF OFFSPRING/FEMALE/5 HOURS						
TEMP (°C)	<u>A. pseudococci</u>		<u>L. dactylopii</u>		<u>L. abnormis</u>	
	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.
18	2.17 a	1.25	0.67 a	0.91	2.27 ac	1.26
20	4.43 b	1.52	-	-	1.97 a	1.05
22	-	-	1.53 ad	1.37	-	-
24	2.93 ab	1.69	4.27 b	1.97	5.43 b	3.53
26	4.23 b	1.92	3.7 bd	1.0	6.73 b	2.42
30	7.37 c	2.05	6.2 c	1.69	6.37 b	2.81
34	2.47 a	0.83	2.6 d	0.4	4.93 bc	1.29

Means based on offspring from 30 individuals in 6 replicates. Means followed by the same letter are not significantly different (l.s.d. at the 5% level). Comparisons may be made across temperature for a given species, but not between species.

A. pseudococci

Analysis of variance shows that temperature has a highly significant effect on progeny production ($P < 0.01$). Generally the number of offspring produced increases with temperature up to 30°C and declines thereafter. Progeny production is significantly greater at 30°C than at 18°C ($P < 0.001$), 20°C ($P < 0.01$), 24°C ($P < 0.001$), 26°C ($P < 0.01$) and 34°C ($P < 0.001$), greater at 26°C than at 18°C ($P < 0.05$) and greater at 20°C than at 18°C and 34°C ($P < 0.05$).

L. dactylopii

Analysis of variance shows a very highly significant effect of temperature on progeny production ($P < 0.001$), the number of offspring produced rising to a peak at 30°C. Progeny production is significantly greater at 30°C than at 18°C, 22°C ($P < 0.001$), 24°C ($P < 0.05$), 26°C ($P < 0.01$) and 34°C ($P < 0.001$), greater at 26°C than at 18°C ($P < 0.001$) or 22°C ($P < 0.05$),

greater at 24°C than at 18°C ($P < 0.001$), 22°C ($P < 0.01$) or 34°C ($P < 0.05$); and greater at 34°C than at 18°C ($P < 0.05$).

L. abnormis

Analysis of variance shows a highly significant effect of temperature on progeny production ($P < 0.01$). The number of offspring produced rises to a peak between 26°C and 30°C. Progeny production is significantly greater at 26°C and 30°C than at 18°C or 20°C ($P < 0.01$); greater at 24°C than at 18°C or 20°C ($P < 0.05$) and greater at 34°C than at 20°C ($P < 0.05$).

Discussion

In these experiments, the parasitoids were supplied with large numbers of mealybug, so that the need to search for hosts was virtually eliminated. The results thus represent the maximum egg laying potential of the parasitoids at the different temperatures.

The results for A. pseudococci are in good agreement with those of Niyazov, (1968), who found that in a 4-5 day period, female A. pseudococci laid less than one egg at 15°C, 1.1-1.5 eggs at 20°C, 3.1-4.4 eggs at 25°C and 14.3 eggs at 30°C. A large increase in progeny production at 30°C over that at 26°C was also observed in this study.

The results show that the effect of temperature on progeny production by the three parasitoid species is broadly similar, the number of eggs laid increasing with rising temperature up to an optimum and then declining. However, some differences between the species are apparent, which may have a significant influence on their population dynamics.

The number of eggs laid by L. abnormis increases enormously between 20°C and 24°C, but above this temperature there are no significant differences in progeny production. This parasitoid thus reaches its maximum oviposition rate at moderate temperatures and maintains it over a wide temperature range. Both A. pseudococci and L. dactylopii show significantly higher progeny production at 26°C than at 18°C, and a further significant increase as the temperature rises to 30°C. However, at 34°C the number of eggs laid declines for both species. These two species require much higher temperatures than L. abnormis to attain their maximum oviposition rate.

These results suggest that L. abnormis will be able to attain a high birth rate into the population (given adequate hosts) at lower temperatures than will A. pseudococci or L. dactylopii.

3.4.3 Oviposition by the parasitoid *Leptomastix dactylopii* under cycling temperature regimes

Introduction

The use of a series of constant temperatures to evaluate the influence of temperature on a given aspect of an insect's biology is an extremely convenient way of standardizing data in attempting to understand a complex phenomenon. However, it is doubtful that the results of such studies can always be directly applied to natural, fluctuating conditions. The behavioural element in oviposition is liable to make it particularly difficult to use constant temperature data to predict how fluctuating conditions will affect egg laying.

It has been reported that the fecundity of some species of insect increases in fluctuating conditions as compared to constant temperatures (Messenger, 1964a). Data on A. pseudococci suggest the opposite, with fertilised females producing fewer progeny on average under a fluctuating regime than at a constant temperature, equivalent to the mean of the fluctuating regime (Avidov et al., 1967).

Within the glasshouse winter temperatures impair the efficiency of tropical natural enemies like those under study in this project. Reduced progeny production at low temperatures is one of the major factors contributing to this. However, relatively short periods of higher temperature may be enough to ensure a significant amount of oviposition, and increase the parasitoid's effectiveness.

The aim of this study was to use specific cycling temperature regimes in an attempt to determine the period of higher temperature necessary to ensure a

reasonable level of progeny production by L. dactylopii, and to investigate the relationship between fecundity at constant temperature and that under fluctuating conditions. The other two parasitoids were not used in this experiment.

Materials and methods

The parasitoids in this study were allowed to oviposit for a total of 4 days, the first day at a constant $26 \pm 0.5^{\circ}\text{C}$ and the following three days under one of the experimental regimes. These were as follows

- 1) control - constant 26°C .
- 2) 5 h 26°C /19 h 18°C .
- 3) 12 h 26°C /12 h 18°C .

Under these cycling regimes each temperature was controlled to within $\pm 0.5^{\circ}\text{C}$.

Fifteen female parasitoids were removed from culture, anaesthetised using CO_2 and placed in three ventilated plastic boxes (5 in each) streaked with honey solution. These were then left for approximately 17 h at $26 \pm 0.5^{\circ}\text{C}$. Three heavily infested, sprouted potatoes were introduced into each box the following morning and left for 24 h at 26°C . Three similar boxes were then prepared, each containing another three heavily mealybug infested potatoes and the female parasitoids were transferred to these fresh boxes under CO_2 anaesthetic. These were then placed at one of the experimental temperature regimes, and subsequently incubated at 26°C . The following day the females were again transferred to fresh boxes and similarly on the fourth day. After 96 h the parasitoids were dissected to determine their egg complements. Emergence of offspring in the experimental boxes was

monitored twice daily.

Results

It is apparent from the data collected over four days at a constant 26°C that progeny production is higher on day 1 than on subsequent days. Analysis of variance fails to show any significant difference in the number of offspring produced on days 2, 3 and 4 at a constant 26°C. Similarly, there are no significant differences in progeny production over the three days at 12 h 26°C/12 h 18°C or 5 h 26°C/19 h 18°C.

Progeny production during day 1 at a constant 26°C was also consistent for all experiments, and thus results were pooled and the mean values are presented in table 32.

Analysis of variance shows a very highly significant effect of the regime used, significantly more offspring being produced at a constant 26 than at either of the other regimes ($P < 0.001$). There is no significant difference between the progeny produced in 5 hours at 26°C and 12 hours at 26°C.

There is a significant difference between the mean progeny production on day 1 at 26°C when the parasitoids were previously deprived of hosts for 12-15 hours, and the mean of subsequent days at 26°C, when they were not previously host starved ($P < 0.01$). The ratio of offspring produced by host starved as compared with non host starved adults is almost 2 1.

Table 33 shows the mean egg complement per female after three days at each regime. Analysis of variance shows that there are no significant differences in the mean number of eggs per female at the different regimes.

TABLE 32. Effect of various cycling temperature regimes on progeny production by Leptomastix dactylopi

MEAN NUMBER OFFSPRING/5 FEMALES/DAY									
TEMPERATURE REGIME (°C) Days 2-4	Day 1 Constant 26°C		Day 2		Day 3		Day 4		MEAN Days 2-4 $\bar{x}$ s.d.
	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	
Constant 26	43.33	17.6	20.0	7.21	20.67	8.39	32.0	4.36	24.22 8.33
12 h 26/12 h 18	46.33	2.08	13.67	4.6	9.33	4.04	13.0	7.81	12.0 5.27
5 h 26/19 h 18	39.67	21.78	9.0	1.73	6.0	1.0	9.33	4.51	8.11 2.93
MEAN Day 1	43.11	14.34							

In each case means are based on offspring from a total of 15 females in 3 replicates

TABLE 33. Effect of various cycling temperature regimes on egg complements of female Leptomastix dactylopii

TEMP REGIME	MEAN NUMBER EGGS/FEMALE		
	n	$\bar{x}$	s.d.
constant 26°C	15	15.27	3.79
12 h 26°C/12 h 18°C	15	18.33	4.32
5 h 26°C/19 h 18°C	15	15.67	3.81

n = number of females dissected at end of experiment

Discussion

In each treatment, the first 24 hours was at a constant 26°C. Comparison of the progeny production during this standardizing period showed no significant differences between treatments. It is thus valid to compare progeny production between treatments on subsequent days, when they were subjected to the experimental regimes. The results show that there is no significant difference in the number of progeny produced in 5 h at 26°C and 12 h at 26°C, when the rest of the day is spent at 18°C. Although females are capable of laying more eggs in 24 h at 26°C, the evidence suggests that they are most efficient in their first 5 h at this temperature.

The results from the control, at a constant 26°C, show that on the first day after a period in the absence of hosts significantly more offspring are produced than on days following a period when hosts are freely available. When they had been deprived of hosts for a 17 h period, females laid almost twice as many eggs in a day as they did when hosts were constantly available. Being deprived of hosts clearly has a major influence on egg laying. Further evidence for this can be gleaned from the results of constant temperature studies on oviposition, where the parasitoids were

left for a five hour period at the experimental temperatures after a period of 17 h in the absence of hosts. The number of eggs laid in 5 h at 26°C was only 25% less than in 24 h at the same temperature, whereas when hosts were continuously available progeny production in a 5 h period was 67% less than in 24 h at the same temperature. It is apparent that after a period in the absence of hosts, the parasitoids tend to lay a large number of eggs within a relatively short period of time. The implications of this are that providing there are periods deprived of hosts (which will almost certainly occur in the glasshouse), relatively short periods of time at high temperatures will favour high oviposition rates.

The mean egg complements of the female parasitoids after three days under the various regimes were not significantly different, the overall average being 16.422 ± 4.126 eggs per female. Thus it appears that egg production is not limited by longer periods at low temperature. However, at each regime there is a large discrepancy between the potential fecundity as measured by the number of mature eggs present within the female and the number of offspring actually produced. For example at a constant 26°C fewer than 5 progeny were produced per female, less than 1/3rd of the potential. The relationship between egg production and egg laying at different temperatures may be a complex one, and requires further investigation, but it is apparent that the number of eggs laid is not directly proportional to the egg complement of the female.

Predictions of progeny production under fluctuating conditions from constant temperature data will be inaccurate unless adjusted to account for the increased oviposition rate after being deprived of hosts and the tendency to lay more eggs in the initial period at a higher temperature than in subsequent hours. For example predictions from constant temperature data would suggest that L. dactylopi would lay 31.15 ± 22.3

eggs under a 5 h 26°C/19 h 18°C regime. This is approximately four times the actual figure observed.

3.5 THE EFFECT OF TEMPERATURE ON ADULT LONGEVITY

Introduction

The longevity of individuals is one of the major factors influencing the abundance of an insect, and its population dynamics. For insect parasitoids the overall searching capacity and fecundity of individuals and hence the number of hosts parasitized, is affected by the adult longevity.

The effects of temperature on adult longevity have been studied for many species of insects. Generally an inverse relationship between temperature and length of life has been described within the optimum temperature range (Raney et al., 1971; Siddiqui et al., 1973; Greenfield and Karandinos, 1976, Allsopp, 1981; Browning and Oatman, 1981), but over a wider range the relationship has been found to be bell shaped (Karandinos and Axtell, 1967).

Data on adult longevity has commonly been presented as a mean value with its standard deviation (Force and Messenger, 1964, Greenfield and Karandinos, 1976, Browning and Oatman, 1981). Others have presented the data as age specific survival curves, which are graphical representations of the probability of surviving to age t , as a function of t (Messenger, 1964b; Tanigoshi et al., 1975; Barfield, Bottrell and Smith, 1977, Siddiqui et al., 1977). A third method of representing longevity data using the Weibull distribution was put forward by Pinder et al., (1978) and used along with survival curves by Allsopp, (1981).

The aim of this study was to determine how adult longevity of the three mealybug parasitoids A. pseudococci, L. dactylopii and L. abnormis varies at different constant temperatures, when fed and kept at high relative humidity.

Materials and methods

The experimental procedure used was identical for each species. Mummified mealybugs were removed from cultures of the parasitoids maintained at $26 \pm 0.5^{\circ}\text{C}$ and 45-60% r.h., and isolated individually in 50 mm x 25 mm glass vials, streaked with honey solution (about 50:50 honey:water). The glass vials were inverted over polythene caps containing distilled water to maintain a high relative humidity, with a 40 mesh/cm nylon gauze covering the water. The vials were kept at 26°C and monitored twice daily for emergence of adult parasitoids. After emergence of the adults, vials were placed at one of 4 experimental temperatures.

Constant temperatures of $26 \pm 0.5^{\circ}\text{C}$ and $18 \pm 0.5^{\circ}\text{C}$ were achieved in constant temperature rooms, and Gallenkamp compenstat illuminated cooled incubators were set at $22 \pm 0.5^{\circ}\text{C}$ and $30 \pm 0.5^{\circ}\text{C}$ respectively for the other two temperatures. Lighting was continuous in the incubators, and on a 18/6 h light/dark cycle in the C.T. rooms. Mortality was recorded twice daily.

Adult longevity data is summarized by means of the Weibull frequency distribution, where the proportion surviving at time t is given by.

$$F_t = 1 - \exp(-[(t/b)^c])$$

where b and c are the scale and shape parameters of the distribution (Pinder et al., 1978).

These parameters were estimated by linear regression (Hahn and Shapiro, 1967) and LT_{50} values were calculated from the fitted Weibull curves.

Results

The survival curves for males and females of each species are shown in figs. 20, 21 and 22. The proportion surviving at each time interval is shown, along with the fitted Weibull curve. Tables 34, 35, 36, 37, 38 and 39 show the value of the two Weibull parameters and the calculated LT_{50} for both sexes of each species at the four constant temperatures.

Tests of the equality of the b and c parameters across temperature show different results for the three species.

A. pseudococci

Females. The value of the c parameter is not significantly different at 18°C, 22°C and 26°C, but is smaller at 30°C ($P < 0.05$) than at any of the other temperatures. The value of b generally decreases with rising temperature being significantly greater ($P < 0.05$) at 18°C than at any other temperature and greater at 22°C and 26°C than at 30°C ($P < 0.05$).

TABLE 34. Adult longevity of female Anagyrus pseudococci: Weibull parameters for survival curves at various constant temperatures.

	TEMPERATURE (°C)			
	18	22	26	30
n	40	39	40	51
b	23.101 z	12.101 y	11.468 y	3.256 x
c	2.065 e	2.001 e	1.971 e	1.121 f
LT ₅₀	19.344	10.076	9.522	2.341

n = number of individuals observed. Numbers followed by the same letter not significantly different at the 5% level (tables of Thoman and Bain, 1969).

Males: As with females the value of c decreases at 30°C, but is only significantly different from that at 18°C ($P < 0.05$). The value of the b parameter also decreases with rising temperature being smaller at 26°C than at 18°C or 22°C ($P < 0.05$) and smaller at 30°C than at any other temperature ($P < 0.05$).

TABLE 35. Adult longevity of male Anagyrus pseudococci. Weibull parameters for survival curves at various constant temperatures.

	TEMPERATURE (°C)			
	18	22	26	30
n	19	20	29	14
b	20.190 z	14.403 z	8.048 y	3.843 x
c	1.695 e	1.418 ef	1.352 ef	0.989 f
LT ₅₀	16.263	11.123	6.137	2.652

n = number of individuals observed. Numbers followed by the same letter not significantly different at the 5% level (tables of Thoman and Bain, 1969).

Figure 20

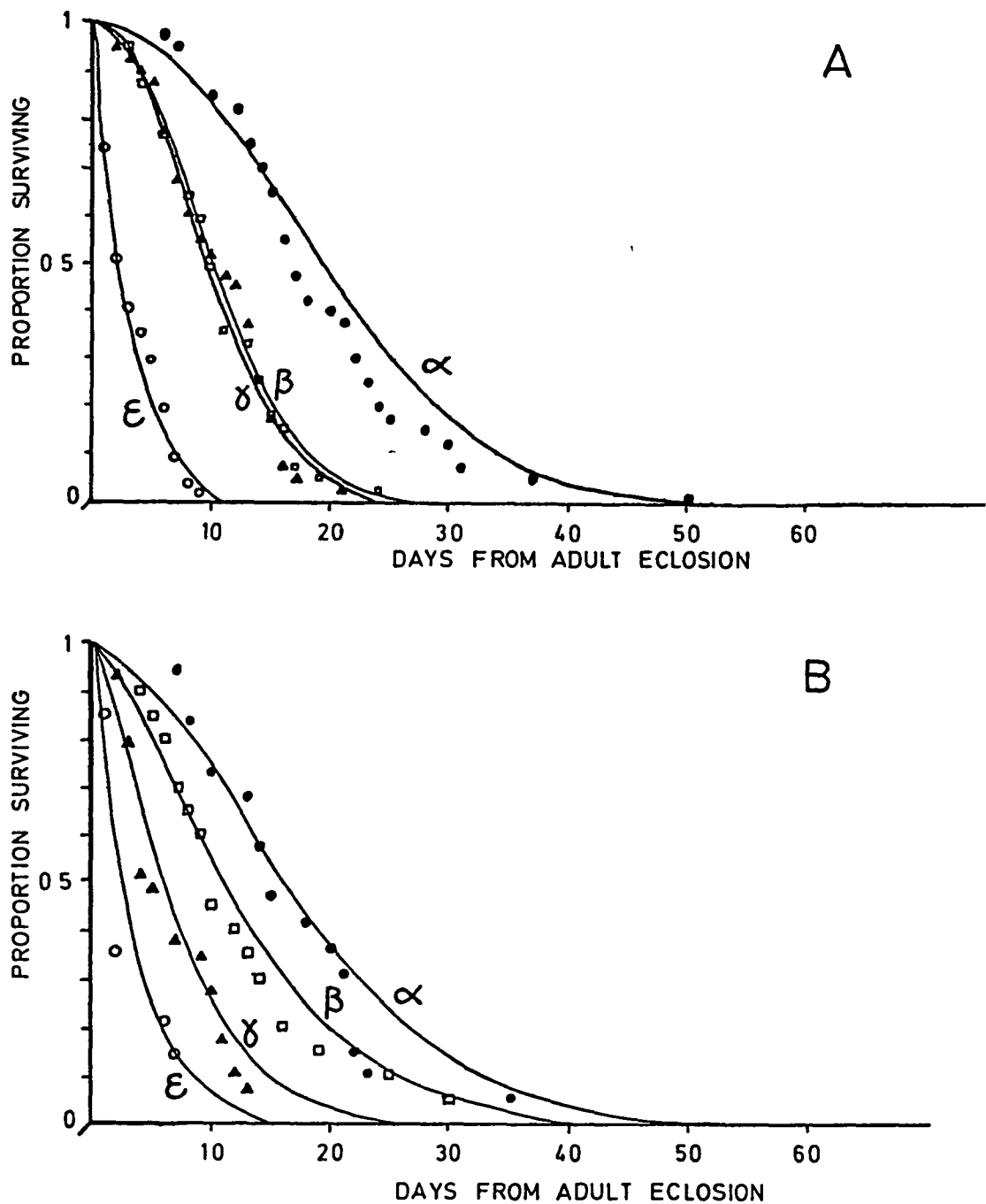


Figure 20 Survival curves for adult *Anagyrus pseudococci* at various constant temperatures A females, B males

Temperatures ● 18°C, □ 22°C, ▲ 26°C, ○ 30°C

B Fitted Weibull curves α (18°C) $F(t) = 1 - \exp[-(t/20.1901)^{1.6946}]$
 β (22°C) $F(t) = 1 - \exp[-(t/14.4031)^{1.4181}]$
 γ (26°C) $F(t) = 1 - \exp[-(t/8.048)^{1.352}]$
 ε (30°C) $F(t) = 1 - \exp[-(t/3.8428)^{0.98873}]$

A Fitted Weibull curves α (18°C) $F(t) = 1 - \exp[-(t/23.1008)^{2.0648}]$
 β (22°C) $F(t) = 1 - \exp[-(t/12.1009)^{2.0011}]$
 γ (26°C) $F(t) = 1 - \exp[-(t/11.4676)^{1.9712}]$
 ε (30°C) $F(t) = 1 - \exp[-(t/3.2456)^{1.1212}]$

L. dactylopii

Females: The trends for this parasitoid are somewhat different, as the value of the c parameter is significantly greater at 26°C than at 18°C or 22°C ($P < 0.05$). Similarly the value of the b parameter increases at 26°C, and is greatest at this temperature ($P < 0.05$) and smallest at 30°C ($P < 0.05$).

TABLE 36. Adult longevity of female Leptomastix dactylopii: Weibull parameters for survival curves at various constant temperatures.

	TEMPERATURE (°C)			
	18	22	26	30
n	40	42	43	49
b	21.398 z	18.729 z	32.616 y	4.50 x
c	1.424 e	1.544 e	2.131 f	1.666 ef
LT ₅₀	16.542	14.771	27.462	3.611

TABLE 37. Adult longevity of male Leptomastix dactylopii: Weibull parameters for survival curves at various constant temperatures.

	TEMPERATURE (°C)			
	18	22	26	30
n	24	26	20	19
b	18.291 z	14.186 z	28.386 y	5.811 x
c	1.581 ef	2.187 e	2.162 e	1.039 f
LT ₅₀	14.507	11.998	23.959	4.084

n = number of individuals observed. Numbers followed by the same letter not significantly different at the 5% level (tables of Thoman and Bain, 1969).

Figure 21

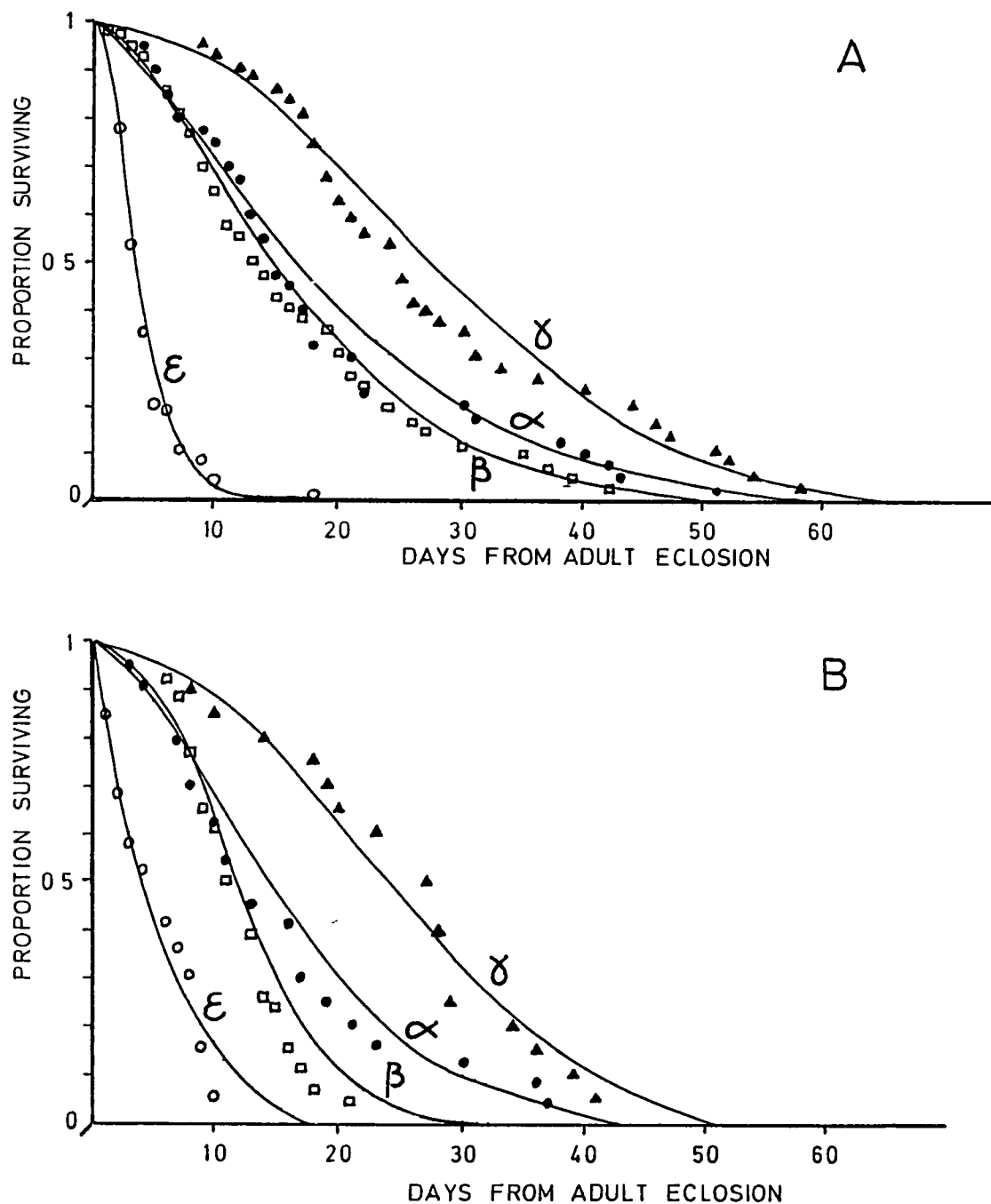


Figure 21 Survival curves for adult *Leptomastix dactylopii* at various constant temperatures A females, B males

Temperatures ● 18°C, □ 22°C, ▲ 26°C, ○ 30°C

B Fitted Weibull curves α (18°C) $F(t) = 1 - \exp[-(t/18\ 291)^{1.5812}]$
 β (22°C) $F(t) = 1 - \exp[-(t/14\ 1864)^{2.1874}]$
 γ (26°C) $F(t) = 1 - \exp[-(t/28\ 3856)^{2.162}]$
 ϵ (30°C) $F(t) = 1 - \exp[-(t/5\ 8111)^{1.0389}]$

A Fitted Weibull curves α (18°C) $F(t) = 1 - \exp[-(t/21\ 3976)^{1.4239}]$
 β (22°C) $F(t) = 1 - \exp[-(t/18\ 7295)^{1.5436}]$
 γ (26°C) $F(t) = 1 - \exp[-(t/32\ 6158)^{2.1312}]$
 ϵ (30°C) $F(t) = 1 - \exp[-(t/4\ 5000)^{1.666}]$

Males: The value of the c parameter decreases at high temperature, and is significantly smaller at 30°C than at 22°C or 26°C ($P < 0.05$). As with females of this species, b is significantly larger at 26°C than at any other temperature ($P < 0.05$) and significantly smaller at 30°C than at any other temperature ($P < 0.05$).

L. abnormis

Females: The shape of the survival curve is not affected by temperature within the range tested for this parasitoid. However, as with

A. pseudococci the b value generally decreases with rising temperature, being significantly greater at 18°C than at any other temperature ($P < 0.05$) and greater at 22°C and 26°C than at 30°C ($P < 0.05$).

TABLE 38. Adult longevity of female Leptomastidea abnormis: Weibull parameters for survival curves at various constant temperatures.

	TEMPERATURE (°C)			
	18	22	26	30
n	40	40	40	35
b	29.105 z	22.064 y	19.72 y	8.07 x
c	1.514 e	1.559 e	1.556 e	1.302 e
LT ₅₀	22.846	17.44	15.58	6.09

n = number of individuals observed. Numbers followed by the same letter not significantly different at the 5% level (tables of Thoman and Bain, 1969).

Males: The value of the c parameter decreases with rising temperature, being significantly greater at 18°C than at 22°C or 30°C ($P < 0.05$). The b value increases between 18°C and 22°C ($P < 0.05$), but decreases again at 30°C ($P < 0.05$).

Figure 22

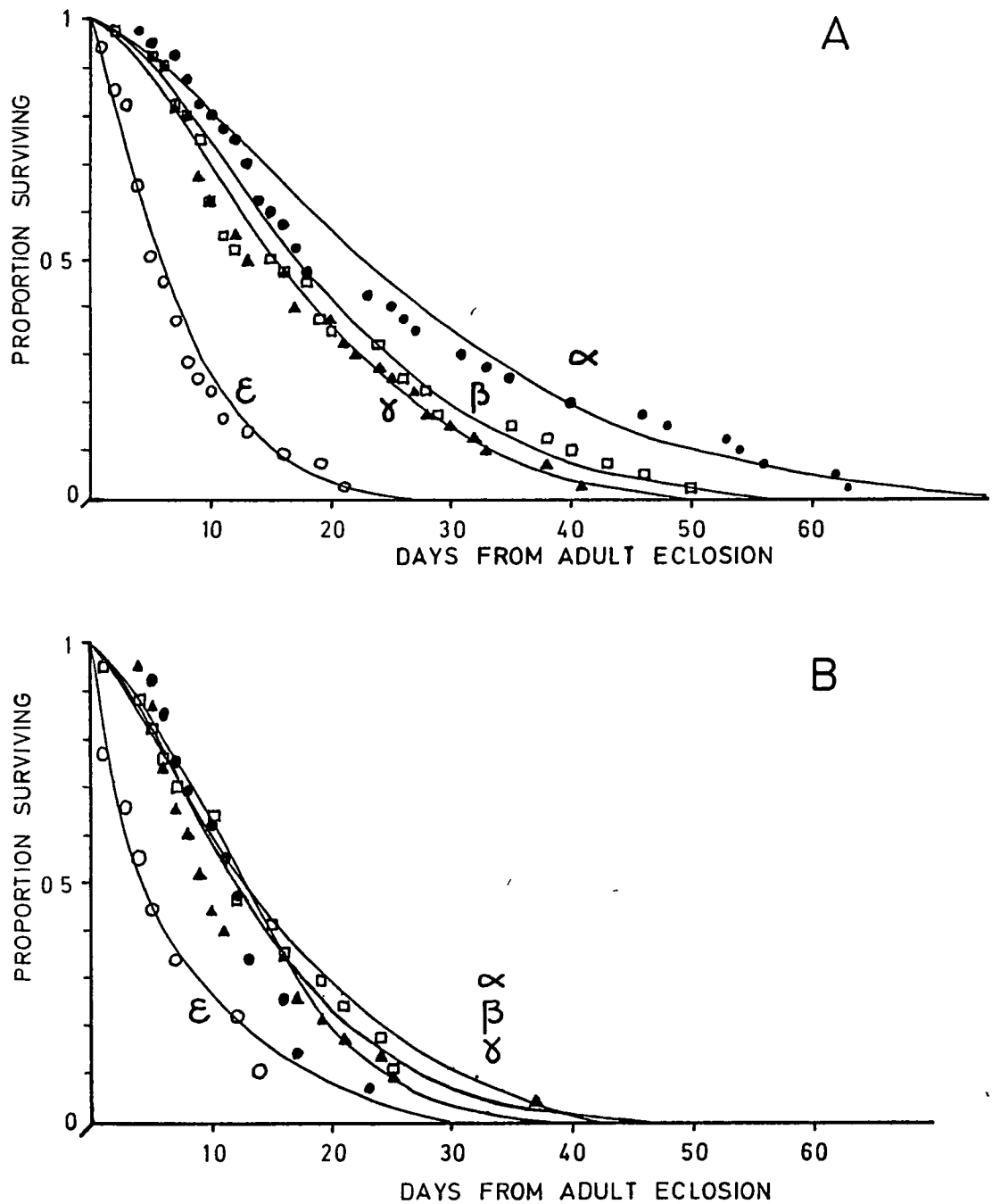


Figure 22 Survival curves for adult *Leptomastidea abnormis* at various constant temperatures A females, B males

Temperatures ● 18°C, □ 22°C, ▲ 26°C, ○ 30°C

B Fitted Weibull curves α (18°C) $F(t) = 1 - \exp[-(t/12\ 9975)^{2\ 2661}]$
 β (22°C) $F(t) = 1 - \exp[-(t/16\ 672)^{1\ 2863}]$
 γ (26°C) $F(t) = 1 - \exp[-(t/15\ 2709)^{1\ 4333}]$
 ϵ (30°C) $F(t) = 1 - \exp[-(t/7\ 2589)^{0\ 85706}]$

A Fitted Weibull curves α (18°C) $F(t) = 1 - \exp[-(t/29\ 8456)^{1\ 5136}]$
 β (22°C) $F(t) = 1 - \exp[-(t/22\ 0636)^{1\ 5586}]$
 γ (26°C) $F(t) = 1 - \exp[-(t/19\ 7196)^{1\ 5556}]$
 ϵ (30°C) $F(t) = 1 - \exp[-(t/8\ 0703)^{1\ 3017}]$

TABLE 39. Adult longevity of male *Leptomastidea abnormis*: Weibull parameters for survival curves at various constant temperatures.

	TEMPERATURE (°C)			
	18	22	26	30
n	18	17	23	9
b	12.997 z	16.672 y	15.271 yz	7.259 x
c	2.266 e	1.286 f	1.433 ef	0.857 f
LT ₅₀	11.613	12.538	11.825	4.733

n = number of individuals observed. Numbers followed by the same letter not significantly different at the 5% level (tables of Thoman and Bain, 1969).

Discussion

It can be clearly seen from figures 20, 21 and 22 that temperature affects the survival curves of the parasitoids. Survival curves can be classified as type I, II or III depending on their shape (Pinder et al., 1978). In general, the curves for all three parasitoids are of type II, where the age specific survival rate is constant, but at high temperature there is a shift to type III, where age specific mortality decreases with increasing time. Temperature also affects the scale of the curve, and the survival period of the population decreases with rising temperature. At low temperatures a large number of individuals survive for relatively long periods of time and the death rate is fairly constant. As the temperature rises the survival period decreases, but the death rate remains fairly constant. At high temperature the shape of the survival curve changes, and the majority of the parasitoids die over a short period of time. However, the probability of surviving increases with time. Thus those few individuals surviving the initial period stand a greater chance of survival.

There are some notable exceptions to the above generalization, particularly with regard to L. dactylopii, which lives longer at 26°C than at any other temperature.

These results are similar to those observed for other parasitoids, with the survival curves changing with temperature, and resulting in reduced longevity at higher temperatures (Force and Messenger, 1964; Hofsvang and Hagvar, 1975a; Barfield, Bottrell and Smith, 1977, Gol'berg, 1982).

The Weibull distribution describes the survival curves fairly well and provides a useful tool for summarizing longevity data. Using the mean longevity as a description of life-span of a population is an oversimplification, as it takes no account of the shape of the survival curve. It is the shape of the curve which is important in terms of the population dynamics of insects. A population with a survival curve which is approximately linear has potentially very different dynamics from one with the same mean longevity or LT_{50} , but with a sigmoidal survival curve. Merely representing longevity with a mean and standard error will not provide the information necessary to illustrate this. Although the fitted Weibull curve does not provide an exact fit to actual data, the parameters provide absolute values for the size and shape of the curve, which can be statistically analysed. This gives a standardizing method which allows the differences between survival curves to be tested.

Several distinct trends are apparent with regard to the effect of temperature on the Weibull parameters. For female A. pseudococci and L. abnormis the value of b declines with rising temperature, whilst the c value remains relatively constant until the temperature reaches 30°C at which point it declines markedly. L. dactylopii shows a peak in both b and c values at 26°C. Again there are similarities with previous work.

Allsopp, (1981), found that the b values for survival of false wireworms also declined fairly evenly with temperature, but that c values were less consistent, although they were always low at high temperature.

The results for A. pseudococci are quite different from those of Avidov et al., (1967) for the same species. These authors quote LT₅₀ values (for males and females combined) of 40 days at 15°C, 17 days at 28°C and 11 days at 32°C, which indicate that the parasitoid was much longer lived than is suggested from the work presented herein. However, they also present results for adults fed sucrose solutions rather than honey, and these appear to be much closer to those in this study. It may be that the nutritional value of the diluted honey used here is similar to that of sucrose and that this accounts for the discrepancy. Certainly nutritional status is very important in determining longevity in parasitoids (Hofsvang and Hagvar, 1975b; Browning and Oatman, 1981).

♀ L. abnormis have the longest life-span of the three species studied at low temperatures, and is least susceptible to high temperatures.

A. pseudococci has generally the shortest life-span throughout the temperature range, and can only survive for very short periods at high temperature. Although L. dactylopii is the longest lived at 26°C, it appears that L. abnormis may be best adapted to the wide range of temperatures, which occur in temperate glasshouses.

The relationship between longevity under constant and fluctuating conditions is one which has received little attention. Barfield, Bottrell and Smith, (1977) reported that longevity of the parasitoid Bracon mellitor decreased under fluctuating conditions as compared with constant, although they did not state which parameter was used to summarize longevity. They appear to mean that the time over which the population

survives is reduced i.e. b decreases. The evidence presented by these authors is that the shape of the survival curve is altered under fluctuating conditions, tending towards type I curves, where the age specific mortality increases with increasing time. At constant temperatures the indications are that the curves were of type II, as has been found for the parasitoids used in this study. This suggests that c may increase under fluctuating conditions. If this is the case, a larger proportion of the population will remain alive in the initial period although the overall life-span of the population will be reduced. This would result in a greater probability of the parasitoids finding and parasitizing hosts.

Whatever the relationship between constant and fluctuating temperature it is apparent that high temperatures will tend to decrease parasitoid longevity. The LT_{50} is shorter at 30°C than at lower temperatures, for all three species and it is probable that it would not be favourable to the population dynamics of the parasitoids to be subjected to lengthy periods at high temperature.

3.6 DISCUSSION: THE EFFECTS OF TEMPERATURE ON THE BIOLOGY AND POPULATION DYNAMICS OF P. citri AND ITS PARASITOIDS

Temperature has a pronounced effect on the biology of both P. citri and its parasitoids A. pseudococci, L. dactylopii and L. abnormis. In the field the differences in response to temperature will be one of the factors determining which parasitoids are most effective against the mealybug and to what extent populations of the pest are restrained. However, within the controlled environment of the glasshouse manipulation of the temperature may be possible to favour the parasitoids and enhance their ability to control otherwise damaging populations of the pest.

The laboratory studies of the effects of temperature on the biology of the insects give some insight into the how this type of manipulation might be important. Control will be brought about if the ratio of recruitment to losses in the parasitoid population exceeds that in the pest population.

Adult female P. citri lay eggs within a cottony egg mass, shrinking and eventually dying as the eggs are laid. The longevity of the mealybug is thus synonymous with its developmental period, which becomes shorter with increasing temperature. The most pronounced effects of temperature on mealybug development occur at relatively low temperatures, with the mean developmental period being reduced from 80.667 days at 18°C to 46 days at 22°C. The number of eggs laid is also influenced by temperatures in this range with an average of 304.4 [±] eggs being laid per female at 18°C and 244.05 at 22°C. However, as P. citri will complete almost 2 life cycles at 22°C in the space of one at the lower temperature, a much larger number of eggs are produced at 22°C than at 18°C in the same space of time. Thus between these temperatures the birth rate increases with increased temperature. This trend continues up to 26°C, with the rate of development

increasing and no significant difference in egg production. At still higher temperatures however, the trend is reversed as the developmental period does not change significantly, and egg production drops off markedly. It is apparent from this that rapid increase in mealybug numbers is favoured by moderate temperatures. High temperatures are detrimental to mealybug population growth.

The effects of temperature on the parasitoid populations are more complex as more factors are involved in determining the birth rate into the population. Female parasitoids lay eggs throughout their adult life, and thus adult longevity plays an important part in the population dynamics. Temperature does have an influence on adult longevity, the life span of the parasitoids being reduced at high temperature. At low temperatures the parasitoids generally live for longer, although there are inter-specific differences which will be discussed below. This increases the period available for egg laying, but several other factors also have an influence on this. Firstly, the female parasitoids generally lay fewer eggs per day at low temperatures and those which are laid will develop more slowly. Also the searching efficiency of the parasitoids is lower, as they are less active and able to cover less area in search of hosts than at higher temperatures. Appendices 2, 3 and 4 show estimates of the effective area searched by the three parasitoids at different temperatures, when searching for host patches on clean surfaces, and searching within host patches. These can be used as a rough guide to the effect of temperature on the area searched, but should not be taken as constituting actual data. At low temperatures (18°C), host patch location efficiency is low, and within host patches there is less likelihood of them contacting hosts. At higher temperatures, particularly around 26°C the balances in these factors change. Longevity at this temperature is slightly reduced, but this is more than compensated for by the increase in developmental rate, egg laying

capacity and searching efficiency. At higher temperatures still longevity is severely reduced, and although host patch location is more effective, the increase in the developmental rate is small, and egg laying is also not greatly affected. Generally, increases in birth rate of the parasitoids are favoured by higher temperatures than are favourable for the mealybugs, however the individual parasitoid species do differ in their temperature responses and this may be significant in determining which is the most effective control agent for P. citri.

High temperatures appear to be most favourable to A. pseudococci, due to its high searching efficiency, high egg laying capacity and rapid developmental rate. Longevity is reduced at 30°C, the LT_{50} being 2.34 days for females, but the females are capable of laying about 15 eggs per day provided hosts are available. Host searching is efficient at this temperature, the parasitoid being able to cover an area of approximately 8.68 mm²/s in search of host patches, almost twice the area covered at 26°C (see appendix 2a). Arrestment within patches is high, due to reduced walking speed, high rate of turning and frequency^{of} host encounters. Once hosts are found and eggs laid, development through to adulthood is rapid, taking an average of 11.567 days. At 26°C the life span of adults is prolonged, the LT_{50} being 9.52 days, but host searching is less efficient as the parasitoids can only cover an area of 4.235 mm²/s on a clean leaf surface and within host patches encounters are less frequent. The females are capable of laying about 9 eggs per day, and development through to the adult takes 14.05 days on average. At 22°C there is no great change, but at 18°C the parasitoids efficacy is severely reduced. The longevity of A. pseudococci at this temperature is increased, the LT_{50} being 19.34 days. However, searching efficiency is very low, as the parasitoids can only cover an area of about 0.65 mm²/s in search of host patches and they spend a great deal of time resting. Even if hosts are located, oviposition rate

is depressed at 18°C and development from egg to adult takes 40.5¹/₂ days on average. In addition the depressed searching efficiency may impair mate location, which could result in a smaller proportion of mated females in the population and a resultant switch in the sex ratio, towards males.

High temperatures are also favourable to L. dactylopii, although the optimum for this parasitoid appears to be around 26°C, as this is the temperature at which it is longest lived, whilst having almost as high a searching capacity as at 30°C and almost as rapid rate of development. Fewer eggs are laid at 26°C than at 30°C, but this is probably more than compensated for by the other factors. Lower temperatures appear to be rather detrimental to L. dactylopii, its patch location efficiency being very low at 22°C (see appendix 3a), its longevity reduced over that at 26°C and its fecundity also low.

L. abnormis is the longest lived of the three parasitoids, and its life span is not markedly changed at temperatures from 26°C down to 18°C. It also reaches its maximum egg laying capacity at slightly lower temperatures than the other two species, laying about 11 eggs per day at 24°C. Searching efficiency in terms of the area covered per unit time is lower than either A. pseudococci or L. dactylopii at high temperatures, but is not so different at moderate temperatures (see appendix 4). The developmental rate is also slower than either of the other parasitoids, but the difference is less pronounced at 18°C than at 26°C.

It appears that L. abnormis is the best adapted of the three parasitoid species to its mealybug host in that it operates effectively at temperatures favourable to P. citri, whereas the other two species tend to become more efficient at higher temperatures.

Experiments under cycling conditions suggest that the effect of 1 hour at a given temperature on the development is constant, whatever the insect's previous thermal history. Thus the development time under fluctuating conditions can be predicted with a high degree of accuracy from constant temperature work, provided that temperatures are regularly and accurately recorded. The most consistently accurate and reliable method of carrying out such predictions uses high order polynomial regression to describe the temperature/development relationship and then sums the rates for each hour at a given temperature until development is complete.

The effects of fluctuating conditions on oviposition are not additive. Relatively short periods of time at high temperatures are adequate to ensure that a female will lay a high proportion of its egg complement, provided that ample hosts are available. Periods in the absence of hosts will greatly stimulate oviposition by the parasitoids, almost doubling the number of eggs laid in a given time.

The effects of fluctuating temperatures on longevity were not studied, but other workers have reported that alternating temperatures tend to reduce longevity as compared with equivalent constant temperatures (Siddiqui et al., 1973; Barfield, Bottrell and Smith, 1977). However, there is also some evidence to suggest that the shape of the survival curve of parasitoids may change under fluctuating conditions, tending more towards type I curves. In this case, a larger proportion of the population survive initially, even if the overall longevity is reduced. Most insects reach peak fecundity, laying the most eggs per day, in the early part of their lives (after an initial pre-oviposition period). This has been shown for A. pseudococci (Avidov et al., 1967). Thus the fact that a larger proportion of a given population survives initially may be extremely important in determining the rate of increase, as most females may be

capable of laying the bulk of their eggs in this period.

The behavioural responses of the parasitoids to temperature will probably be relatively immediate, and thus it is likely that the searching capacity under fluctuating conditions can be extrapolated directly from that expected at each of the constituent temperatures.

The inferences of this are that short periods at relatively high temperature may be highly advantageous to the efficiency of the parasitoids. In addition this might act as a stabilising factor on the population dynamics of the host/parasitoid system. Low temperatures result in inefficient searching by the parasitoids, and a consequent reduction in the probability of their locating host patches. This will tend to destabilize the relationship. However, even short periods at high temperature would lead to increased host patch location by the parasitoids. As high temperatures do not seem to decrease arrestment in patches, the patches will still be exploited to the maximum extent. A return to lower temperatures will tend to lead to continued arrestment in patches and overall, it is likely that these influences will lead to aggregation of the parasitoid in host patches, so stabilizing the relationship.

3.7 THE EFFECT OF LIGHT ON THE BEHAVIOUR OF PARASITIDS

3.7.1 Phototaxis

Introduction

The response to light is an important behavioural characteristic in natural enemies which are to be used in biological control. Natural enemies which have a strong phototactic response, may be less effective than those which do not, particularly in the glasshouse. Any insect attracted to artificial lights or towards the roof of the glasshouse will have a reduced searching efficiency and may be lost altogether.

Fisher, (1963) reported that L. dactylopii shows positive phototaxis, and used this response in collection of the parasitoid from mass cultures.

The aim of this study was to determine whether the three mealybug parasitoids exhibit a strong phototactic response.

Materials and methods

Female and male parasitoids aged between 1 and 7 days were collected from a culture under carbon dioxide anaesthetic, into a 75 mm x 25 mm glass vial, approximately 5 of each sex per tube. This tube was then attached to an identical one by means of a two-way cap, with a ^{large} hole in the middle. There was thus free access from one tube into the other. Once the parasitoids had recovered from the anaesthetic, a black, plastic cover was placed over the end containing the parasitoids. The tubes were then placed, lying horizontal, in a well lit constant temperature room, held at $26 \pm 0.5^{\circ}\text{C}$, and left for 1 h. Light was provided by a combination of fluorescent

tubes, at approximately $8.55 \pm 0.02 \text{ W/m}^2$. After this time the number of parasitoids in each of the tubes was counted. This procedure was replicated 10 times for each parasitoid species.

Controls were treated in exactly the same manner, except that the tubes were placed inside a black plastic sack for one hour in the same C.T. room, held at the same temperature.

Each replicate was set up at 1000h to avoid any effect of diurnal activity levels.

Significance of results was tested using a d-test for comparison of two percentages based on samples > 30 (Bailey, 1959).

Results

The proportion of individuals moving from the darkened vial to the light are shown, along with controls, for each species of parasitoid in table 40.

The results of the d-tests show that for both male and female

A. pseudococci and L. abnormis, a significantly greater proportion are found to have moved from one tube to the other in the light than in the dark ($P < 0.001$).

There are no significant differences between the proportion of male or female L. dactylopii moving from one tube to the other in darkness or light.

TABLE 40. Phototactic response of the parasitoids Anagyrus pseudococci, Leptomastix dactylopii and Leptomastidea abnormis.

SPECIES	SEX	PROPORTION MOVING FROM DARK TO LIGHT					
		CONTROL			DARK/LIGHT		
		n	p	s.e.	n	p	s.e.
<u>A. pseudococci</u>	male	49	0.20	0.057	49	0.82	0.056
	female	52	0.058	0.033	52	0.81	0.055
<u>L. dactylopii</u>	male	36	0.42	0.082	46	0.41	0.073
	female	49	0.39	0.069	45	0.49	0.075
<u>L. abnormis</u>	male	45	0.067	0.037	56	0.39	0.065
	female	32	0.094	0.052	43	0.70	0.07

n = number of parasitoids used; p = proportion moving from darkened tube towards light (or in the case of controls from one tube to the other) with standard error.

Discussion

The results show up several behavioural characteristics of the parasitoids. Firstly, A. pseudococci and L. abnormis show a photokinetic response, with an inhibition of movement in the darkness. These two insects also show a strong phototactic response, a large proportion of the individuals moving towards the light. This was particularly pronounced in the case of A. pseudococci and female L. abnormis, but less so for male L. abnormis. L. dactylopii shows a moderate phototactic response, with almost half the individuals moving towards the light. However, almost as many moved from one tube to the other in the control, where the parasitoids were in darkness. This suggests that L. dactylopii does not show a photokinetic response.

3.7.2 The effect of darkness on parasitoid activity levels

Introduction

The period during which parasitoids are active will influence their searching capacity and thus their ability to locate a given host population. The indications from the previous experiment were that A. pseudococci and L. abnormis exhibit photokinesis and move less in the dark than in the light, whereas such behaviour was less apparent in L. dactylopii. The aim of this experiment was to determine the effect of darkness on the activity levels of the three mealybug parasitoids.

Materials and methods

Parasitoids aged between one and seven days were taken from cultures and 5 females and 5 males of each species were isolated in clean, glass Petri dishes (100 mm in diameter). These were then left to acclimatize for 2 hours in a constant temperature room held at $26 \pm 0.5^{\circ}\text{C}$, 45-60% r.h. lit by 5 fluorescent strip lamps, at approximately $8.55 \pm 0.02 \text{ W/m}^2$.

2 minute observations were then made of each parasitoid, during which time the number of periods of activity and their duration was noted.

Observations were made at 3 different times during the day, the first commencing at 1100^h, the second at 1400^h and the third at 1600h.

This procedure was then repeated in exactly the same way, except that the room was in darkness. Observations were made under 3 x 60 W red light bulbs, the lights being completely extinguished between observation periods.

Significance of results was tested using a d-test for comparison of two percentages based on samples > 30 (Bailey, 1959).

Results

The proportion of individuals active in the light and in the dark is shown in table 41. The results are similar for all three species, with a significantly greater proportion of individuals active in the light than in the dark ($P < 0.01$).

TABLE 41. The effect of darkness on the activity levels of the parasitoids Anagyrus pseudococci, Leptomastix dactylopii and Leptomastidea abnormis.

SPECIES	PROPORTION SHOWING ACTIVITY							
	n	DARK			n	LIGHT		
		p		s.e.		p		s.e.
<u>A. pseudococci</u>	30	0.23	a	0.077	40	0.85	b	0.056
<u>L. dactylopii</u>	30	0.10	a	0.055	40	0.55	b	0.079
<u>L. abnormis</u>	30	0.20	a	0.073	40	0.77	b	0.066

Parasitoids were observed for two minutes each. n = number of parasitoids observed, p = proportion of total time active with standard error (s.e.). Numbers followed by the same letter are not significantly different at the 0.01% level. N.B. comparisons may not be made between species.

Discussion

All three parasitoid species are much more active in the light than in the dark. L. dactylopii shows slightly less activity in the light than the other two species, which was indicated from the results of the previous experiment. Generally the proportion of the sample showing activity in the

light, tallies well with the proportion moving into the light in the phototaxis experiment. In the dark a rather smaller proportion of the sample were active than was suggested from the previous experiment, where the parasitoid showed little evidence of a photokinetic reaction. However, in the previous experiment up to 10 individuals were confined together in a single vial, whereas in this experiment the individuals were isolated. It may be that L. dactylopii is sensitive to interference, and is highly active when in contact with other members of its own species.

The results for the other two species are fairly consistent with those of the previous experiment. If only about 20% of individuals are active, then the proportion moving from one vial to the other would be expected to be very low. These results support the hypothesis that both A. pseudococci and L. abnormis show a strong photokinetic effect.

These results are significant as they suggests that the area which the parasitoids will search in a day, will be dependent on the number of hours of light available. If a parasitoid is inactive then it will not lay any eggs and thus the period available for egg laying will also depend on photoperiod. This adds another dimension to the effect of season on the efficiency of the parasitoids as biological control agents. During the winter when the photoperiod is shorter, the parasitoids are likely to be less effective, as the proportion of time spent searching will be considerably less.

SECTION 4: GLASSHOUSE TRIALS

SECTION 4: GLASSHOUSE TRIALS

INTRODUCTION

Glasshouse trials play an essential part in the development of a biological control programme for use in protected cultivation. No amount of pure laboratory study can compensate for delay in attempting use of a natural enemy in the situation in which it will eventually be needed (Debach and Huffaker, 1971). The release of the natural enemy into the glasshouse, gives an opportunity to test biological control in practice. Any problems or drawbacks that then become apparent will provide a focus for laboratory research, and potential for improving control can be evaluated. Without glasshouse trials much time and effort may be wasted in following the wrong lines of research, but used in conjunction, laboratory and glasshouse trials provide the most effective means of evaluating natural enemies for use in protective cultivation (van Lenteren, 1980).

This section outlines experiments carried out under glasshouse conditions, designed to provide information on the more practical aspects of developing a biological control programme for P. citri in protected cultivation. Investigations were aimed at providing information on the relative value of single versus multiple species releases, the factors governing release levels and the performance of the parasitoids working along with the predator Cryptolaemus montrouzieri in the glasshouse.

4.1 BIOLOGICAL CONTROL OF P. citri ON ORNAMENTAL PLANTS IN CAGES UNDER GLASSHOUSE CONDITIONS

Introduction

Several attempts have been made to suppress infestations of the mealybug P. citri in glasshouses using biological control. A number of natural enemies have been tried including the predator C. montrouzieri alone (Whitcomb, 1940; Panis and Brun, 1971), and in combination with the parasitoids L. abnormis (Pritchard, 1949, Douth, 1951) and L. dactylopii (Douth, 1952). The highest degree of success was reported in the latter case, where virtual eradication of the mealybug was achieved on previously heavily infested Stephanotis. In this case the use of a combination of a predator and a parasitoid demonstrated just how effective biological control can be.

The number of natural enemies that must be released in order to achieve control has not been studied in detail. Douth, (1952) released 19,360 L. dactylopii and 600 C. montrouzieri to control mealybug on 528 plants, but he makes no mention of the size of the glasshouse involved. Steiner and Elliott (1983) recommend releasing these two natural enemies at the rate of 5/m² of planted area or 2-5/plant for the predator and 5/plant for the parasitoid. However, they made no mention of the extent of the pest infestation, the plant type or the size of the plant involved.

The dispute as to whether natural enemies should be used singly or in combination in classical biological control projects has continued for many years. Much of the evidence from practice and theoretical modelling points to a higher degree of success being achieved with a combination of natural enemies (May and Hassell, 1981). However, there is also seemingly

conflicting evidence which suggests that although the level of biological control will usually increase as the number of natural enemy species present increases, the greater the number of natural enemies the more difficult it may be to establish additional species and the more species released simultaneously, the lower the rate of establishment (Ehler and Hall, 1982).

This trial was designed as a preliminary one to compare the biological control of P. citri given by various combinations of the parasitoids and the predator C. montrouzieri; and to determine the relative effects of two different release levels, within cages under glasshouse conditions.

Materials and methods

The ornamental plant Pilea cadierei was used as the host for P. citri. The plants were grown from cuttings in 100 mm diameter, plastic plant pots and used when they had reached a height of 100-150 mm. Six plants were placed in each of 5 small, perspex cages (410 mm x 610 mm x 535 mm), with metal trays as bases. These bases were lined with capillary matting and raised up on wooden blocks in plant-pot saucers (100 mm diameter), coated on the inside with the sticky material PLA 3002 to prevent cross contamination of cages by mealybug crawlers. The cages were supplied with ventilation by a 12 W fan turning at 2500 r.p.m. Air passed down 80 mm Osma R, plastic tubing and into the cages through a 100 mm diameter, gauze covered aperture at the back.

The Pilea plants were infested with mealybug using nettle (Urtica dioecia) shoots as transfer plants. Shoots about 150 mm long were placed in a mealybug culture cage containing hatching crawlers, and another containing mainly 3rd instar P. citri. The shoots were left approximately 15 hours

and then removed and placed on the experimental plants. In this way the Pilea were infested with a mixed age population of the mealybug, with a predominance of 1st instar nymphs. The plants were left several days to allow the mealybugs to settle down to feed, before the population per plant was estimated.

The parasitoids and predator were collected from their individual cultures under carbon dioxide anaesthetic and released into the experimental cages ^{introduction of the mealybugs.} four days after ~~the~~. The following combinations of natural enemies were used:

Cage 1. control - no natural enemies released.

Cage 2. 5 pairs each of A. pseudococci, L. dactylopii, L. abnormis and C. peregrinus.

Cage 3: 25 pairs each of A. pseudococci, L. dactylopii, L. abnormis and C. peregrinus

Cage 4: 25 pairs L. dactylopii.

Cage 5 25 pairs each of A. pseudococci, L. dactylopii, L. abnormis and C. peregrinus and 2 pairs of adult C. montrouzieri.

This trial was carried out at an early stage, when C. peregrinus was also being evaluated as a biological control agent for P. citri. It was therefore included in this trial. Subsequently, this parasitoid was dropped from the investigations, as it showed little promise and became a contaminant of mealybug cultures (see section 2). It continued to be used in glasshouse releases along with the other species (see section 4.3).

Throughout the experiment the capillary matting was kept moist and once weekly it was drenched with a solution containing "Bio 7" (a high nitrogen, high potash, liquid fertiliser). Plant condition was assessed after 2 weeks and 5 weeks and the experiment was terminated after 2 months, when

plant condition was again assessed and mealybug populations per plant estimated. The plant condition was assessed using the scoring system outlined below. This allowed some quantification of the effect of the mealybugs on the plant and gave a greater degree of objectivity in the interpretation of results.

<u>SCORE</u>	<u>PLANT CONDITION</u>
5	Plant healthy
4	Up to 50% of leaves discoloured and drooping
3	All leaves discoloured and drooping
2	All leaves discoloured, some leaf drop apparent
1	Plant defoliated
0	Plant defoliated and original stem dead

Results

The results are summarized in tables 42a, 42b and 43. The first two tables show the estimated mealybug populations in each cage at the beginning of the experiment and after the experiment had run for 2 months.

Analysis of variance shows that at the beginning of the experiment, there were no significant differences between cages in the populations of any of the mealybug stages. However, at the end of the experiment there were significantly more mid-grown mealybugs on the control plants than on those in cages 2, 3 or 5 (l.s.d. at 5% level), and significantly more crawlers on the control plants than on those in any other cage (l.s.d. at 0.1% level).

TABLE 42. Biological control of Planococcus citri on ornamental plants using various combinations of natural enemies.

a Total mealybug populations in each treatment before release of natural enemies.

NUMBER OF MEALYBUGS OF EACH STAGE					
TREATMENT	1	2	3	4	5
Adults	0	7	2	2	7
Mid-grown	470	410	405	355	415
Crawlers	5310	6040	5180	5630	8180
Egg masses	0	1	2	2	1

b Total mealybug populations in each treatment 2 months after release of natural enemies.

NUMBER OF MEALYBUGS OF EACH STAGE					
TREATMENT	1	2	3	4	5
Adults	1	0	0	0	0
Mid grown	11	3	0	8	0
Crawlers	137	0	0	4	0
Egg masses	1	0	0	1	0

Figures represent total number of mealybugs on the six Pilea plants in each treatment.

Table 43 shows the condition of the plants in each treatment throughout the course of the experiment. The plant condition was scored according to the parameters given in the key, so that some comparison was possible between treatments. The plates 18, 19, 20, 21, 22, 23 and 24 will help in interpreting the scoring system.

TABLE 43. Plant condition scored throughout trial of Biological Control of mealybug on Pilea cadierei in cages.

TREATMENT	MEAN SCORE							
	After 2 weeks		After 5 weeks		After 2 months		Mean plant height	
	$\bar{x}$	$\pm$ s.d.	$\bar{x}$	$\pm$ s.d.	$\bar{x}$	$\pm$ s.d.	$\bar{x}$	$\pm$ s.d.
1	3.0	0.63	1.17	0.41	0.0	0.0	22.5	13.32
2	2.5	0.84	1.17	0.41	0.5	1.22	73.3	42.86
3	3.0	0.63	2.17	0.75	3.8	1.79	147.0	40.71
4	2.0	0.89	1.83	1.17	2.67	2.58	120.8	54.26
5	1.67	1.03	1.67	1.03	2.2	2.59	118.0	54.43

Treatment 1 - no natural enemies

Treatment 2 - 5 pairs of each species of parasitoid

Treatment 3 - 25 pairs of each species of parasitoid

Treatment 4 - 25 pairs of L. dactylopi

Treatment 5 - 25 pairs of each species of parasitoid + 2 pairs adult
C. montrouzieri

Discussion

The results suggest that any of the combinations of control agents used can reduce P. citri populations under glasshouse conditions, and also reduce the level of damage done to ornamental plants by heavy infestations of the mealybug. It is apparent that in all treatments the mealybug population declined dramatically during the two months' duration of the experiment, even where no control agents were introduced. This can be attributed to the rapid loss of condition and defoliation which the plants suffered soon after being infested, leading to a much reduced food source for the second generation of mealybug. The rapidity with which the plants lost condition correlated well with initial mealybug levels - those with higher infestations succumbing more quickly. Pilea cadierei seemed to be particularly sensitive to the attack, the control plants dropping all their leaves and in one case dying. However, where a combination of parasitoids was released in large numbers, or a combination of parasitoids and the predator C. montrouzieri, the plants were saved from severe damage and regained strong healthy growth.

PLATES 19 - 21. Condition of Pilea cadieri plants at end of cage trial of biological control of Planococcus citri using a variety of natural enemies.

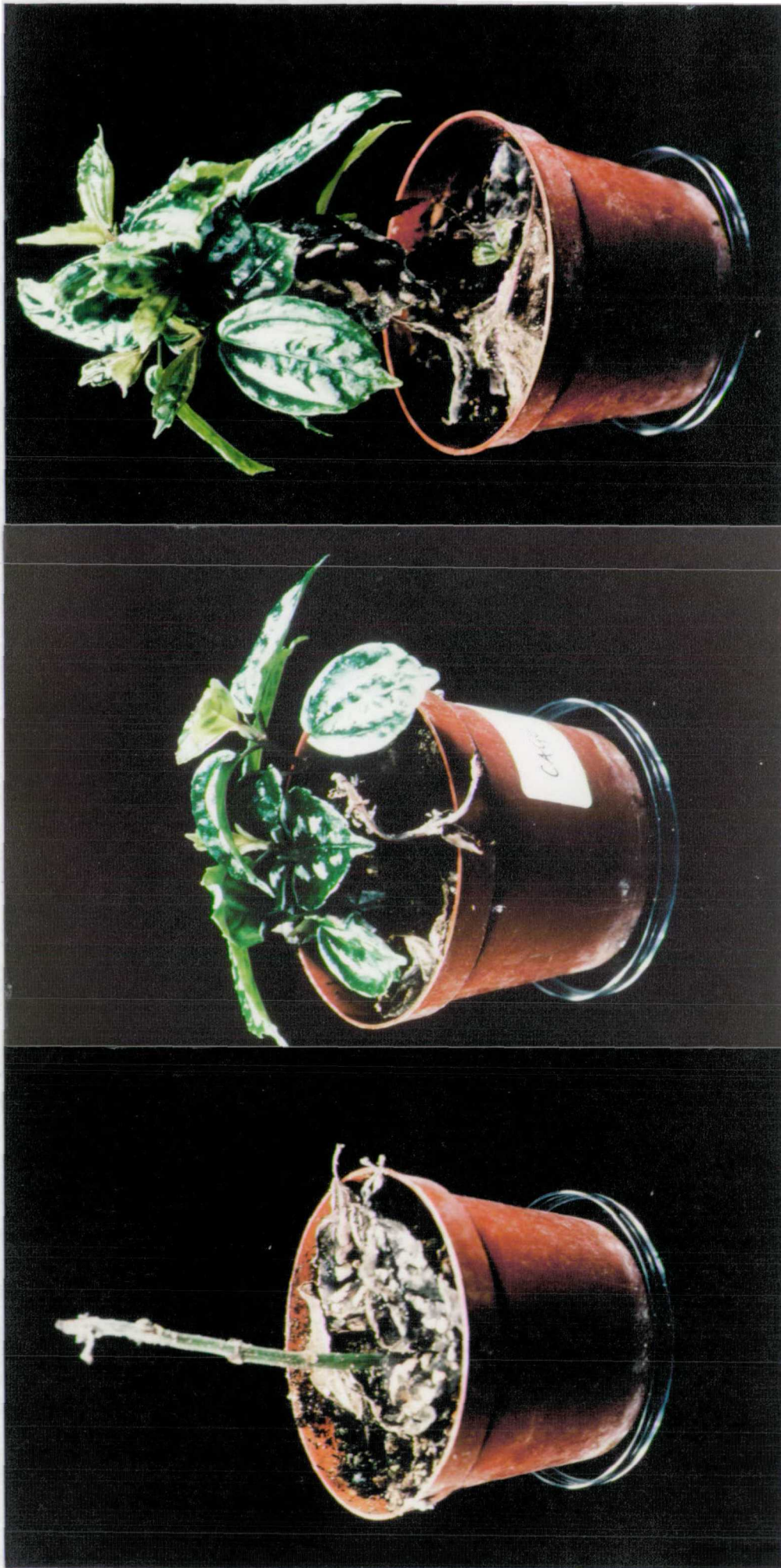


PLATE 19. Treatment 1 - no natural enemies.

Note. Plant defoliated
Score = 1

PLATE 20. Treatment 2 - five pairs of each parasitoid species.

Note. Original stem defoliated and dead - healthy shoots appearing from the base.

PLATE 21. Treatment 3 - 25 pairs of each parasitoid species.

Note. Some discoloured and drooping foliage, healthy growth with shortened internodes.
Score = 4

PLATES 22 - 24. Condition of *Pilea cadieri* plants at end of cage trial of biological control of *Planococcus citri* using a variety of natural enemies.



PLATE 22. Treatment 4 - 25 pairs of *Leptomastix dactylopii*.

Note. Plant defoliated but resprouting from original stem.

PLATE 23. Treatment 4 - 25 pairs of *Leptomastix dactylopii*.

Note. Drooping and discoloured leaves with healthy new growth.
Score = 4

PLATE 24. Treatment 5 - 25 pairs of each parasitoid + 2 pairs adult *Cryptolaemus montrouzieri*.

Note. Plant healthy.

Score = 5

The combination of parasitoids at the lower initial level was slow to exert control over the mealybug, and was unable to prevent all but one plant from becoming defoliated. However, successful control was eventually achieved allowing healthy new shoots to appear and flourish. The final mealybug population was very low, probably because the different parasitoids attacked different host stages and were thus effective at dealing with the mixed-age mealybug infestation.

At the higher initial level the combination of parasitoids was largely effective in preventing the defoliation of the plants, and allowing more rapid and full recovery.

L. dactylopii alone was unable to exert such a high level of control initially, although they were apparently more successful than the combination of parasitoids at the equivalent level of introduction. This may be an indication that their preferred host stages are the most damaging. The fact that more mealybug remained at the end of the experiment in the L. dactylopii cage than in any of the other cages supplied with natural enemies, may be due to the fact that the single species could not deal so well with the mixed age mealybug population. This is to be expected, as having a preference for 3rd instars and adults, some of the younger pest individuals would escape parasitism.

The combination of predator and parasitoids produced excellent results on the most heavily infested plants, totally eradicating the pest and allowing the plants to produce healthy new growth. However, during the course of the experiment relatively few parasitoids were seen, unlike all the other cages (except the control). It appears that C. montrouzieri is rather indiscriminate in its predation, eating parasitized as well as healthy mealybugs. This could present problems in using the parasitoids in

combination with this predator. However, in a full sized glasshouse it is unlikely that C. montrouzieri would manage to eliminate its host, as it did in this more confined space, as it is known to be inefficient in its searching at low host population densities (Whitcomb, 1940). Thus it may be expected to die out leaving a low level of the pest, which the parasitoids may then be able to contain.

Taking mealybug population as the criterion for measuring the success of the various combinations of natural enemies, it appears that the combination of all four parasitoids at the higher initial level, and the parasitoid combination plus C. montrouzieri were the best (see table 42 a and b). Second most successful was the combination of parasitoids at the lower initial level, and the least successful was L. dactylopii alone. However, none of these could be separated statistically.

Using plant condition at the end of the experiment as the criterion for judging success of control, the best appears to be the combination of parasitoids at the higher initial level, second L. dactylopii alone; then the combination of parasitoids and predator, and least successful was the combination of parasitoids at the lower initial level (see table 43).

It is interesting that using the different criteria to assess the success of control gives different results. In both cases, the combination of the parasitoids, released at the higher initial level is best. The addition of the predator does not affect success in terms of the effect on mealybug numbers, but the indications are that control takes longer to come about, thus allowing the plants to be more severely affected.

The results of this trial must be seen in perspective. Firstly they were carried out on an initially high level of mealybug infestation - a

situation which the use of parasitoids as control agents would aim to avoid. Secondly, the physical limitation to dispersal imposed upon the natural enemies in being caged, will have had a considerable effect on the rate and degree of parasitism and predation achieved. Dispersal is impossible under these conditions and thus insects flying up to the top of the cage have no alternative but to return to the same plants in the long run. This will inevitably lead to higher levels of parasitism and predation than would occur with freely dispersing populations of natural enemies. This effect is increased by allowing several generations of the parasitoids to act on the same host population. However, recognising these limitations, the evidence from this trial is that in the short term a combination of parasitoids provided excellent control and that the individual species did not appear to impair each other's parasitic abilities. In the longer term the situation may be different, as competitive displacement may occur.

The cages used covered an area of approximately 0.25 m^2 and contained six plants. Thus the numbers released, at the lower level, correspond to about 4 pairs/plant or 100 pairs/ m^2 , to control approximately 6000 mealybugs of mixed age. The higher level was five times greater. The results obtained with the lower numbers of parasitoids were adequate in terms of reducing the mealybug population under cage conditions, and with a less sensitive host plant damage may not have been so extensive. However, better control was undoubtedly achieved with larger numbers of natural enemies. In the glasshouse the parasitoids will be free to disperse widely and host density will tend to be lower, it is therefore likely that the higher release rate may be more realistic in practice.

The indications from this trial are that it is important to take into account the plant type when deciding on the numbers of natural enemies

which will be necessary to control a given pest. A sensitive plant may require the release of larger numbers of natural enemies, if damage is to be minimized, and the numbers of pest present will also have an effect. It would appear from this that Steiner and Elliott's guidelines, of releasing 5 parasitoids/plant or 5/m² of planted area, may be over simplistic. Further information is necessary on the relationship between number of hosts, number and size of plant, area occupied by the plants and the number of parasitoids to be released.

4.2 AN INVESTIGATION OF HOST FINDING ABILITY OF THE PARASITIDS IN VARIOUS SIZED ARENAS

Introduction

Despite previous use of the parasitoids L. dactylopii and L. abnormis as biological control agents in glasshouses, little data has been published on the numbers which need to be released in order to achieve control. It is likely that this will vary with the size of the mealybug population, the number of plants, plant type and the dimensions of the glasshouse. Steiner and Elliott (1983) suggest^{ed} releasing 5 L. dactylopii per plant or 5 per m², regardless of the mealybug population, but gave no data to back this up. The indications from the previous trial were that good control could be achieved by the parasitoids, released at a rate of 4 pairs/plant, when confined in a cage. However, in the glasshouse, such confinement in the vicinity of hosts would not occur, and the number of parasitoids required may be much greater.

The aim of this experiment was to determine the effect of the area to be searched, on the host finding ability of the three parasitoids

A. pseudococci, L. dactylopii and L. abnormis, by measuring the percentage parasitism achieved with a constant number of hosts at different densities.

Materials and methods

Twenty female parasitoids of each species were released with a standard number of mealybugs in each of three "arenas" of different volume, namely a ventilated, plastic box (173 mm x 115 mm x 65 mm), a ventilated, perspex cage (410 mm x 610 mm x 535 mm) and a glasshouse cubicle (4.5 m x 3.66 m x 2.25 m). These represent volumes in the ratio 1 100 30,000 (approx).

The same number of mealybugs was used in each case distributed on 2 heavily infested, sprouted potatoes in the plastic box, on four sprouted potatoes in the cage, and spread over ten sprouted potatoes evenly distributed around the glasshouse cubicle. Three glasshouse cubicles were used, one for each species, and the plastic boxes and cages were placed on staging in the cubicles. This ensured similar conditions in each arena. All cubicles were set to the same temperature regime in advance.

The number and stage of mealybugs used was different for each species.

L. abnormis was given approximately 200, first and second instar mealybugs in each arena. A. pseudococci and L. dactylopii were each given approximately 150 third and final instar hosts in each arena. Sixty female parasitoids of each species were removed from their respective cultures under carbon dioxide anaesthetic, using an aspirator, and placed in 3 glass vials (20 females per vial) streaked with honey solution. They were left in these vials for 24 h, giving a period deprived of hosts.

The infested potatoes were placed in plant pot saucers and distributed as evenly as possible in each of the three different sized arenas. The parasitoids were released at the end of the afternoon (about 1600h), one vial being placed in each of the arenas and opened. They were then left undisturbed for 5 days. At the end of this period the infested potatoes were collected up from the cubicles and cages, placed in plastic boxes and taken, with the experimental plastic boxes, to a constant temperature room set at $26 \pm 0.5^{\circ}\text{C}$. Carbon dioxide was passed into each box and all female parasitoids present were removed, the boxes then being left for the parasitized hosts to develop. Emergence of parasitoids was monitored daily, the adults being removed under carbon dioxide and counted.

Three replicates were carried out, the first during April, the second during May and the last during June, 1984. For the second replicate more hosts were supplied to each species in each arena; approximately 400 to L. abnormis, and approximately 250 to A. pseudococci and L. dactylopii. For the third replicate L. abnormis was given approximately 600 hosts, and A. pseudococci and L. dactylopii approximately 150 hosts.

Results and Discussion

TABLE 44. Percentage parasitism achieved by the parasitoids Anagyrus pseudococci, Leptomastix dactylopii and Leptomastidea abnormis attacking Planococcus citri in different sized arenas.

	MEAN PERCENT PARASITISM					
	<u>A. pseudococci</u>		<u>L. dactylopii</u>		<u>L. abnormis</u>	
	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.
Plastic box	58.1	22.06	81.83	16.85	87.67	16.82
Perspex cage	41.8	33.66	35.17	10.42	50.73	4.67
Glasshouse cubicle	1.8	2.54	3.1	3.0	9.17	13.29

Means with standard deviations based on 3 replicates, except A. pseudococci plastic box and perspex cage, 2 replicates only.

The results are shown in table 44. Analysis of variance shows that there are significant differences in the percentage parasitism achieved, by all three species of parasitoid in the different arenas.

A. pseudococci

Percentage parasitism was greater in the cage than in the cubicle (l.s.d. at the 5% level). Unequal replication does not allow comparisons to be made between the other treatments using the l.s.d.

L. dactylopii

Percentage parasitism was higher in the cage than in the glasshouse cubicle (l.s.d. at the 5% level) and significantly greater in the plastic box than in the cage (l.s.d. at the 1% level) or in the cubicle (l.s.d. at the 0.1% level).

L. abnormis

The percentage parasitism was significantly greater in the cage than in the glasshouse cubicle (l.s.d. at the 1% level), and significantly greater in the sandwich box than in the cage (l.s.d. at the 5% level) or the cubicle (l.s.d. at the 0.1% level).

Discussion

L. abnormis achieved a higher percentage parasitism in all three arenas than did either of the other two species, with A. pseudococci being probably the least successful in this respect. There was a great deal of variability in the results for A. pseudococci, whereas L. abnormis showed fairly consistent results in the two smaller arenas, and only showed high variability in the percentage parasitism achieved in the glasshouse cubicle. This is to be expected as the probability of finding so few hosts, in such a large area are small. These differences in variability between species may reflect the differences in their response to temperature. The three replicates were carried out at different times of year, the first in April, when night time temperatures were low, and daily peaks relatively small, whilst the other two were in May and June, when average temperatures were higher and maximum temperatures were showing high peaks. In general L. abnormis appears less sensitive to changes in temperature within the normal range (18°C-30°C), than either of the other species (see section 3), and thus it might be expected to show less

variation in the percentage parasitism it achieves, at different times of year.

The introductions were made at a rate of approximately 1000 female parasitoids/m² in the plastic box, 80/m² in the cage, and <1/m² in the cubicle. In both the perspex cage and the plastic box the percentage parasitism achieved was high enough to ensure that there would have been many more parasitoids in the second generation, than were initially released. Thus at these very high rates of release, where minimal searching by the parasitoids was necessary in order to locate hosts, they could be expected to overwhelm the host population fairly rapidly. However, in the glasshouse cubicle the percentage parasitism achieved was low and would have resulted in around 4 individuals in the second generation, for A. pseudococci, 8 for L. dactylopii, and 37 for L. abnormis. In order for the parasitoids to become "established" i.e. capable of maintaining their own population, they must parasitize a large enough proportion of the host population to ensure that the second generation has a similar ratio of parasitoids to hosts, as the first. If this is not the case then the parasitoid population will dwindle and become extinct. The indications are that for all species the release rate used in the glasshouse cubicle was too low to ensure establishment, as at least 40 offspring would be necessary in order to give 20 females in the second generation (assuming a sex ratio of 1:1). It is remarkable that L. abnormis appears almost capable of achieving this at such a low release rate. This infers that this parasitoid has a quite astonishing host finding ability.

The 100 fold volume increase from the sandwich box to the cage resulted in a decrease in percentage parasitism of less than half, for each species. The almost 300 fold increase in volume from the cage to the cubicle still

only resulted in a 22 fold decrease in percentage parasitism by A. pseudococci, less than 12 fold decrease for L. dactylopii and less than 6 fold decrease for L. abnormis.

These results suggest that the volume or area in which the parasitoids are expected to work, does have an effect on the percentage parasitism achieved, but that the number of parasitoids required to ensure establishment does not increase in direct proportion to the volume. The indications are that Steiner and Elliott's guideline of 5 parasitoids per m² of planted area may be a reasonable one, but that the number required per unit planted area will vary with the overall size of the glasshouse involved.



PLATE 25. The ornamental plants section of the Wye College conservatory.

4.3 SEASONAL MEALYBUG POPULATIONS AND THE EFFECT OF NATURAL ENEMIES IN THE WYE COLLEGE CONSERVATORY

Introduction

Biological control programmes in glasshouses have generally aimed at establishing natural enemies in synchrony with the pest, to obtain a stable host/predator/parasitoid relationship. Where successful, this results in the pest population being reduced below the economic threshold for damage, but not eliminated, the pest remaining at a low level. This has been the aim in using Phytoseulis persimilis against red spider mite, and Encarsia formosa against whitefly on tomatoes and other crops of protected cultivation (Hussey and Bravenboer, 1971; Wyatt, 1974).

Attempts to control mealybugs on ornamental plants have not specifically adopted this approach. These crops are of high value, and as they are grown for their aesthetic qualities, the level of pest which can be tolerated is very low. It was found that C. montrouzieri and L. dactylopii could virtually eradicate P. citri on Stephanotis plants, providing temperatures remained favourable. However, the natural enemies then died out themselves and thus had to be reintroduced when the pest reappeared (Doutt, 1952). The disadvantage of the instability of this approach may be outweighed by the fact that plants can be maintained virtually pest free for long periods of time.

At Wye College a large conservatory is kept, stocked with a wide variety of tropical crops and ornamental plants (see plate 25). Many of these plants suffer attack by the mealybug P. citri, scale insects and other pests. Chemical control of these insects has been largely unsatisfactory, and in 1978 the use of pesticides was stopped completely, with the aim of

attempting biological control of the major pests. The predator C. montrouzieri was released as a control agent for mealybugs and was partially successful over several years. Mealybug populations were reduced during the summer, but lower winter temperatures appeared to impair the predators' activity and they died out allowing mealybug levels to increase again, often to unacceptable levels. This parallels the experiences of Whitcomb (1940) with this predator, who found that temperatures below 20°C, and low host population levels prevented successful pest control. The tendency of predators to be inefficient at low host levels is well established. Parasitoids, however, tend to be suited to maintaining low and relatively stable host populations (Samways, 1981).

The aim of this study was to determine whether a combination of parasitoids used along with the predator could improve control of P. citri in the conservatory, whether the parasitoids could be established or whether inoculative releases would be necessary to maintain control; and to monitor the effects of the parasitoids on the seasonal mealybug populations and the levels of parasitism achieved.

Materials and methods

The Wye College conservatory is a large free standing glasshouse 9 m x 30 m. It is divided into three sections of approximately equal size, two tropical and one temperate. The first of the hot house sections is devoted to tropical crop plants including Musa sp., Coffea arabica, Guava psidium guajava and others. The second hot house section has a central plantation including Ficus benjamina, Aristolochia sp., Passiflora quadrangularis, Passiflora sp. and a wide variety of pot plants on staging around the walls. The third temperate section is dominated by cacti and other mediterranean plants eg. Citrus sp., and Olea sp.

P. citri occasionally occurred in the temperate section, but was troublesome mainly in the 2 hot house sections and it was in these that attempts at biological control were made. An examination was made of all the plants in the tropical crop and ornamental sections, and those suffering the most severe P. citri infestations were identified. Five areas were selected for regular sampling: The Coffea arabica, the Guava, the Aristolochia, the Calliandra and the area of pot plants consisting of Saintpaulia sp. and Streptocarpus sp. Unfortunately the Calliandra was removed after the first sampling and thus the Passiflora quadrangularis was sampled in its place. This however was also removed at the beginning of 1984 and another species of Passiflora was then used in the monitoring scheme. For this reason the data on the Passiflora sps. are less satisfactory.

Monitoring mealybug population For all except the pot plants the sampling procedure was as follows. 5 x 300 mm shoots or sections of shoots were selected at random from the bottom, middle and top of the plant. These shoots were carefully examined and the number of adults, egg masses, mid-grown nymphs (ie. 2nd and 3rd instars) and crawlers (1st instars) recorded separately.

The pot plants occupied an area of about 1 m², containing about 30 plants in all (see plate 26). For sampling, 3 Saintpaulia and 3 Streptocarpus were selected at random and the number of mealybugs on them counted, using the same 6 categories as above.

In order to allow comparisons to be made between the different plants the total number of mealybugs (adults, nymphs and parasitized) was calculated per unit leaf area. A typical leaf from each of the sample plants was measured and the number of leaves sampled counted giving the total leaf

area sampled. The number of mealybugs per unit leaf area was then determined by dividing the total number by the calculated leaf area.

The data showed large differences in variance in many of the samples and thus it was deemed invalid to use standard statistical tests, based on assumptions which could not be met. For this reason non-parametric statistical analyses were employed.

The changes in the number of mealybugs per unit leaf area with time were analysed using the Kruskal-Wallis one-way analysis of variance test (Siegel, 1956). Differences in the numbers of mealybugs per unit leaf area on the different plants were detected using the Kruskal-Wallis test and the Mann-Witney U-test was used to determine statistical differences between infestations on individual plants. The Mann-Witney U-test was also used to show differences in the numbers of mealybugs per unit leaf area before and after parasitoid release.

Parasitoid releases. The parasitoids were released at a number of sites, near to the plants with the heaviest mealybug infestations. The parasitoids were collected from cultures containing newly emerged adults and placed in an empty plastic box. During the late afternoon the box (or boxes) were taken to the conservatory and opened near the most heavily infested plants, allowing the parasitoids to escape. Releases were made during the late afternoon, as temperature and light conditions in the middle of the day tend to encourage excessive dispersal (Steiner and Elliott, 1983).



PLATE 26. Pot plants, Streptocarpus sp. and Saintpaulia sp. in the Wye
College conservatory.

Monitoring of parasitoid presence Two methods were used. At first attempts were made to monitor the presence of parasitoids using trap plants. Potato tubers were sprouted and grown to a height of about 150 mm in potting compost. They were then removed from the compost, cleaned, and their roots trimmed off. Each week, 2 were placed in a mealybug culture containing 2nd and 3rd instar nymphs for 24 hours and then removed to the conservatory, where they were placed on a free standing plinth, one in the tropical crops section near the Guava bush, and one in the ornamentals section near the Aristolochia plant. Trap plants were left one week before being removed to incubate in plastic boxes at 26°C, 2 more being put out to replace them. Any parasitoids emerging from the trap plants after incubation in the constant temperature room were collected and counted.

When regular bi-monthly monitoring of the mealybug population on the selected sample plants started in October 1982, any parasitized, mummified mealybugs were recorded along with the mealybug counts. In order to allow comparisons to be made between plants, the number of parasitized individuals per unit leaf area was calculated as described above for the total number of mealybugs. Percentage parasitism per sample shoot was also calculated.

Non-parametric statistical procedures were used to analyse the data on parasitism, for the same reasons as were outlined above for mealybug populations. The number of parasitized mealybugs per unit leaf area and the percentage parasitism over time were both analysed using the Kruskal-Wallis one-way analysis of variance.

Figure 23. Seasonal populations of mealybugs and levels of parasitism on different plants in the Wye College conservatory.

A plot of seasonal temperatures is shown: continuous black line represents weekly mean temperatures, with dots showing minimum and maximum temperatures for each week.

Plots of numbers of mealybugs and numbers of parasitized mealybugs on the five sample plants are shown separately.

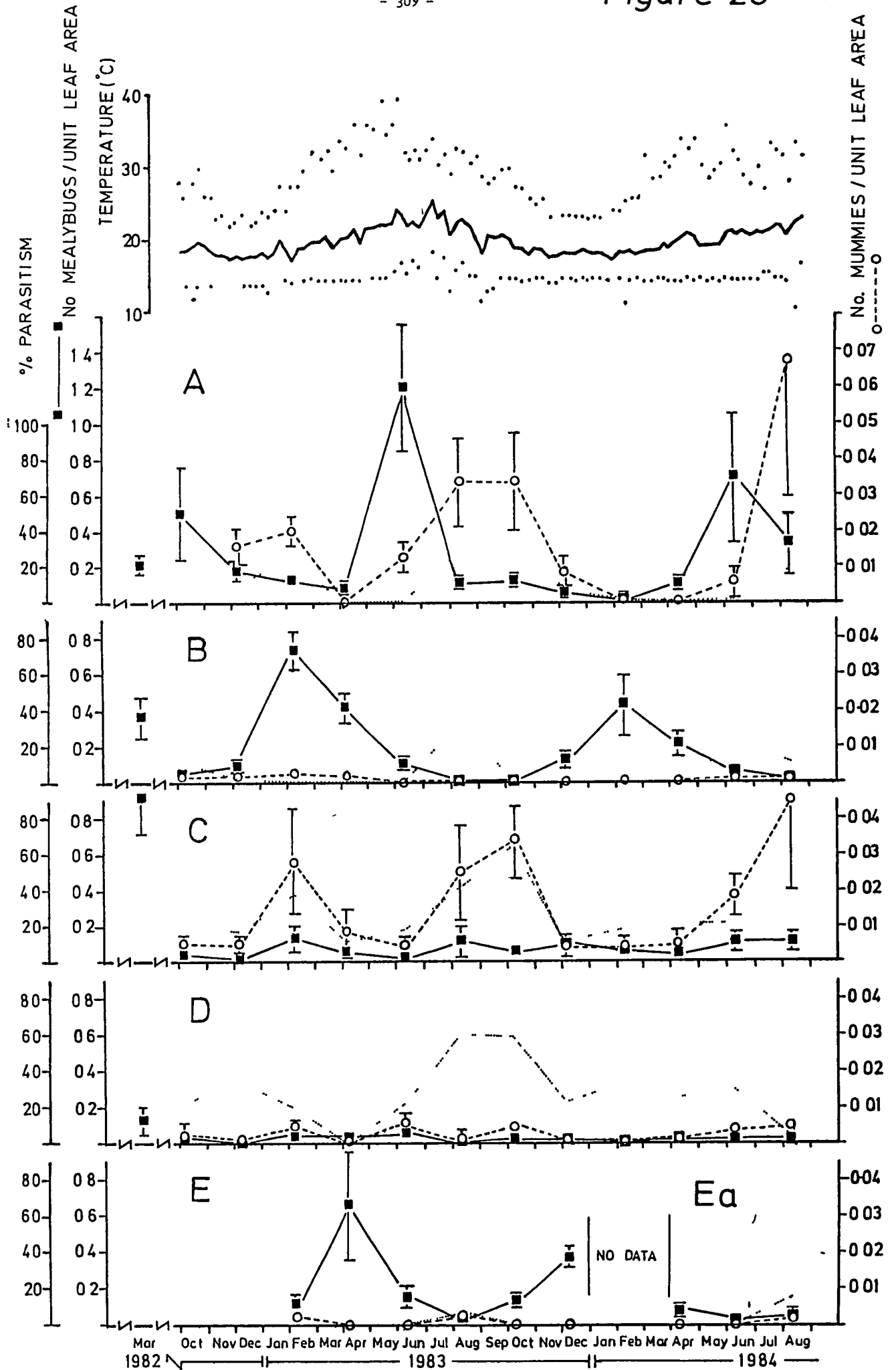
Points represent mean number of individuals per cm² of leaf area, with standard error bars.

■ Number of mealybugs/cm² ○ Number of mummified mealybugs/cm²

Dotted line shows mean percent parasitism.

- A Pot plants - Streptocarpus sp. and Saintpaulia sp.
- B Aristolochia sp.
- C Psidium guava
- D Coffea arabica
- E Passiflora quadrangularis
- Ea Passiflora sp.

Figure 23



Results

Mealybug population monitoring An initial estimate of mealybug populations on the five sample plants was made in March 1982 before any parasitoids were introduced. Thereafter, regular monitoring was undertaken every two months from October 1982, until August 1984.

The results for each of the plants sampled are presented in figure 23, along with a plot of mean, minimum and maximum weekly temperatures.

The data from the first sample on the 15th March 1982 (taken before parasitoids were released) were subjected to Kruskal-Wallis one way analysis of variance. This showed up significant differences in the mealybug infestations on the different plants ($P < 0.01$). The Guava was worst affected, with significantly more mealybugs than on any of the other plants ($P < 0.05$, Mann-Witney U-test). Significantly more mealybugs were found on the Aristolochia and the pot plants than on the Coffea.

Parasitoid releases: The first release was made shortly after the first mealybug population assessment in March 1982, when 50 pairs of each of the parasitoids A. pseudococci, L. abnormis, L. dactylopii and C. peregrinus were liberated near to the Guava bush (site 1), and near to the Aristolochia (site 2). Thereafter, inoculative releases were made when and where necessary, according to mealybug levels. The numbers and place of release are shown in table 45.

Parasitoid presence: The use of trap plants to monitor the presence of parasitoids was continued for 4 months and the results are shown in table 46.

TABLE 45. Releases of natural enemies into the Wye College conservatory during 1982, 1983 and 1984.

		NUMBERS RELEASED				
Date	Site	<u>A. pseudococci</u>	<u>L. dactylopii</u>	<u>L. abnormis</u>	<u>C. peregrinus</u>	<u>C. montrouzieri</u>
3/82	1	100		100	50	
	2	100		100	50	
4/82	1	200	200	200	100	
	2	200	200	200	100	
5/82	1	200	200	200	100	
	2	200	200	200	100	
6/82						
7/82	1			100		
	2			100		
	3	200	100	400	150	
	4			100		
8/82	3	150	100	200	200	
	4	50	50	50	50	
9/82						
10/82						
11/82						
12/82						

NUMBERS RELEASED

TABLE 48. (cont.)

Date	Site	<u>A. pseudococci</u>	<u>L. dactylopii</u>	<u>L. abnormis</u>	<u>C. peregrinus</u>	<u>C. montrouzieri</u>
1/83						
2/83	1			250		
	2			800	100	
	5			300		
3/83	1				100	
	2	50	100	30	300	
	5	40	250	50	250	
	6	50	50	20	20	10
4/83	3	30	30			
	5			1100	100	
	6		50	350	100	
5/83	3	250	50	500	400	50
	4	70	50			
	5	80	50	150	200	20
	6	100	50	650	1200	120
6/83	2				200	
	3	100	100	70	200	
	4	100	130	150	200	
	5	120	200	250	200	
	6				200	
7/83	1	30	50	250		
8/83						
9/83	1	150		50		100

TABLE 48. (cont.)

NUMBERS RELEASED

Date	Site	<u>A. pseudococci</u>	<u>L. dactylopii</u>	<u>L. abnormis</u>	<u>C. peregrinus</u>	<u>C. montrouzieri</u>
10/83	5		100			50
11/83	1	30	30	30		15
	2					350
	5	100	300	30		150
	6	150	100			
12/83						
1/84						
2/84	2					50
3/84	2			100		50
	3					50
4/84	2			70		40
5/84	2	30		300		30
	4	50		300		25
6/84	1	60		300		
	2	50	30	170		
	3			100		
	4			350		
	6			200		50
7/84	1		150	300		80
	6					50

Site 1 - Guava, Site 2 - Aristolochia, Site 3 - Coffea, Site 4 - Pot plants; Site 5 - Passiflora quadrangularis;
 Site 6 - Passiflora sp.

The data for some weeks are missing, due to the fact that the trap plants were occasionally found by C. montrouzieri, and all (or most) of the mealybugs eaten by the predator. Adult L. dactylopii and A. pseudococci often emerged from the trap plants, during this period and L. abnormis were sometimes present. The indications from this are that the parasitoids were locating mealybug hosts and succeeding in producing offspring. The trap plants continued to show evidence of parasitoid presence during a two month period in which no releases were made. The original adults released could not possibly have remained alive and ovipositing over such an extended period.

TABLE 46. Emergence of parasitoids from conservatory trap plants.

DATE	<u>A. pseudococci</u>	<u>L. dactylopii</u>	<u>L. abnormis</u>
5/4/82	-	-	-
13/4/82	-	-	-
23/4/82	+	+	+
3/5/82	+	+	+
21/5/82	+	+	-
27/5/82	-	+	-
4/6/82	-	+	-
18/6/82	+	+	-
25/6/82	+	+	+
2/7/82	+	+	-
9/7/82	-	+	-
16/7/82	-	+	-
23/7/82	+	+	+
6/8/82	+	+	+

Trap plants left in the conservatory for the week ending at date shown.
+ indicates parasitoids emerging from trap plant - no parasitoids emerging.

The use of trap plants was discontinued for two reasons:

- 1) They gave an indication of parasitoid presence, but no information could be gained from the trap plant results about the level of parasitism being achieved in the host population on the plants.

2) The data for a number of weeks are missing, because the trap plants were frequently found by C. montrouzieri adults or larvae, which were capable of eating virtually all the mealybugs present, so that it could no longer be used as a guide to the presence of parasitoids.

Parasitism assessed during mealybug population monitoring The vast majority of the mummified mealybugs seen during sampling were those parasitized by L. abnormis, although occasionally L. dactylopii mummies were also found. During the sampling, adult L. abnormis were regularly seen on the Guava and adult L. abnormis and L. dactylopii were occasionally seen on the Coffea and the pot plants.

The level of parasitism observed varied greatly from plant to plant, but generally the peaks in the number of parasitized mealybug per unit leaf area occurred at the same times of year on the different plants.

In order to compare mealybug infestations on the different plants after parasitoid release, it was deemed most valid to use data for the same period in each year. No data were available for March 1983 or 1984 and thus data on mealybug numbers in the months of February and April of these two years were used. In all cases there were significant differences between plants ($P < 0.05$). Aristolochia was always the worst affected, with significantly higher mealybug populations than any of the other plants ($P < 0.05$). In April 1983 and February 1984 there were no significant differences in the mealybug populations on the other plants, but in February 1983 and April 1984 the mealybug numbers were significantly higher on the pot plants than on the Coffea, but not the Guava ($P < 0.05$).

Pot plants. Mealybug numbers on these plants varied significantly with time ($P < 0.05$) and showed regular annual fluctuations with peaks occurring

in the summer (see figure 23). March populations in 1982 (before the parasitoids were released) were not significantly different from those in February and April in either 1983 or 1984.

The number of parasitized individuals did not differ significantly over time, but showed a great deal of variability. Peaks in percentage parasitism tended to occur in the months following heavy mealybug infestations, percentage parasitism rising as the total number of mealybugs present was falling. The differences over time were significant ($P < 0.001$). The differences in percentage parasitism did not result merely from a decline in mealybug numbers, as the number of parasitized mealybug present increased at the same time.

Aristolochia Mealybug numbers per unit leaf area on this plant showed significant variation with time ($P < 0.001$). A regular annual trend is apparent with numbers peaking in February, both in 1983 and 1984, but falling to very low levels during the summer months.

The number of mealybugs present was not significantly different before and after parasitoid release at corresponding times of year, except that numbers were significantly higher in February 1983 than in March 1982 ($P < 0.05$), despite the release of the parasitoids.

The number of parasitized mealybug was very low at all times and frequently no parasitism was recorded at all during sampling. However, there were significant differences in the number of parasitized mealybugs per unit leaf area over time ($P < 0.05$). Percentage parasitism also remained low and did not vary significantly.

Guava: The mealybug population on this plant was significantly lower at all times during 1983 and 1984, than in March 1982 before the parasitoids were released ($P < 0.01$). Having been the most heavily infested plant at the time of the first sample, it carried significantly lower mealybug populations than the Aristolochia during the corresponding period in subsequent years ($P < 0.05$). The number of mealybugs present remained relatively constant and showed no significant variation over time. The number of parasitized mealybugs did vary ($P < 0.05$), but peaks tended to correspond with periods of high mealybug counts. Percentage parasitism, though not varying significantly, also tended to increase at times when the mealybug population was highest.

Coffea This plant was the least severely infested with mealybug at the time of the first sample, and remained so after the release of the parasitoids. The number of mealybugs present was generally lower after release of the parasitoids and spring populations were lower in 1984 than in 1982 ($P < 0.05$), although there was no significant difference in the spring levels in 1983 as compared with 1982. Mealybug numbers did not vary significantly with time, remaining at a constantly low level. The number of parasitized mealybugs was also low and remained relatively constant, but percentage parasitism did vary significantly over time ($P < 0.05$).

Passiflora: Unfortunately rather less data was collected from this plant than from the others. Sampling did not begin until January 1983 and was disrupted by the removal of the plant in January 1984. However, it was at times, one of the most severely infested plants and one on which the parasitoids seemed to be least effective. Frequently no parasitism was recorded and highest parasitism, in terms of both actual numbers of parasitized mealybugs and percentage parasitism, coincided with periods of low mealybug presence.

Discussion

Generally the extent of the mealybug problem in the Wye College conservatory declined markedly after the introduction of the parasitoids. The amount of visible mealybug damage and presence was noticeably less from 1982 to 1983 and from 1983 to 1984 (see plates 27 and 28). This trend is reflected in the data presented in figure 23. However, it is apparent that the effects from plant to plant were very different, and it is likely that this was a result of a number of interacting factors.

Several of the plants suffered cyclical outbreaks of the pest, but peak mealybug populations did not occur at the same times of year on the different plants. Environmental factors cannot therefore be implicated, and it seems more likely that host plant quality was the major influence in inducing outbreaks. Periods of high mealybug infestation on the Aristolochia coincide with the spring flush of new growth which starts during January, whilst peak infestations on the pot plants occur at flowering times in June. It is possible that the mealybugs take advantage of the mobilisation of resources by the plant, using the higher nutrient quality of the sap to increase their own fecundity. This has been shown for the Sycamore aphid (Drepanosiphum palantoides), which exhibits high levels of reproductive activity during the spring and autumn when phloem nitrogen is high, but low levels during the summer when there is little movement of nutrients around the plant (Strong, Lawton and Southwood, 1984). Similarly, the Psyllid Acizzia russellae shows peak populations at times when its host plant produces a spring flush of growth. No increase in phloem nitrogen was detected in this case, but host plant quality was deemed the major factor regulating numbers of the herbivore (Webb and Moran, 1978).



PLATE 27. Heavily infested
Coffea arabica in
the Wye College
conservatory.

PLATE 28. Clean, healthy Coffea
arabica after release of
parasitoids.



Although temperature will undoubtedly have had an effect on the rate of increase of the mealybugs through its influence on the development and oviposition rate, it does not appear to be the major factor inducing outbreaks.

The fact that outbreaks occur demonstrates an inability of the natural enemies to regulate pest populations under certain circumstances. However, mealybug numbers on both the Guava and Coffea did not vary significantly and remained relatively low after the introduction of the parasitoids. Both these plants also produce flushes of new growth and indeed have periods of flowering and fruiting, when the phloem nitrogen is likely to be high. However, it can be seen from figure 23 that in general a rise in mealybug numbers is accompanied by an increase in percentage parasitism. Peaks and troughs in the numbers of the pest tend to be mirrored in the levels of parasitism, which suggests that there is a degree of density dependence in operation. More detailed work would be necessary to demonstrate this phenomenon unequivocally. However, the constant presence of the parasitoids on these plants, and the marked reduction in the levels of mealybug on the Guava after release of the parasitoids, are indications that regulation of the pest by the parasitoids was occurring.

The inability of the parasitoids to control pest populations on the Aristolochia and Passiflora may be due to a number of factors. These plants have a climbing habit, whereas the Coffea and Guava are bushes. This may effect the searching efficiency of the parasitoids. Both the Aristolochia and the Passiflora contain toxic secondary plant substances, aristolochic acid in the former and cyanogenic glycosides in the latter, which make them unpalatable to most herbivores. The first stage in host finding for most parasitoids is to locate the host habitat (Doutt, 1959). If these plants are repellent to the parasitoids due to their secondary

plant substances, they may not be recognised as host plants and hence parasitism would be low. There are also many examples of herbivorous insects developing an ability to sequester toxins from the plants on which they feed, so rendering themselves unpalatable to predators. Oviposition by the parasitoid Comperiella bifasciata is retarded when its host the California red scale, Aonidiella aurantii, is feeding on sago palm rather than Citrus sp. Percentage mortality of immature stages of the parasitoid is also greater when the host is feeding on this plant (Smith, 1957). Similarly the ichneumonid parasitoid Hyposoter exiguae shows prolonged larval period, reduced pupal eclosion, smaller size and shortened adult longevity when parasitising hosts fed a diet containing high levels of the alkaloid α -tomatine (Campbell and Duffey, 1979). Little work has been done in this field with mealybugs, but it was found that Pseudococcus adonidum feeding on Nerium oleander did not sequester the toxic cardenolides present (Rothschild, 1973).

It may be that the inability of the parasitoids to regulate mealybug numbers on the Aristolochia and Passiflora is due to a combination of direct and indirect effects of the secondary plant substances they contain, but no evidence is available to support this hypothesis, and more work on the subject is warranted.

The pot plants, Streptocarpus sp. and Saintpaulia sp. do not contain toxic secondary plant substances, and another explanation must be sought for the ability of the mealybug populations to outstrip control by the parasitoids. It can be seen from figure 23 that outbreaks of the pest were severe, but short lived. It can also be seen from the figure that percentage parasitism is rising as the mealybug populations decline. This may be an indication of a delayed density dependant response, indicating that the parasitoids are contributing to the decline in pest infestation, but that

the response is not immediate (Hassell, 1985). The inability of the parasitoids to establish a stable equilibrium with their host on these plants may be due to the fact that both plants have hairy leaves. These hairs may impair the progress of the parasitoids searching the leaf surface, so reducing the area which they can cover and making them unable to locate hosts at low densities (van Lenteren and Woets, 1976). At high pest density, extensive searching would be unnecessary and the parasitoids may be expected to locate hosts more easily. If this is the case then one would expect a delayed density dependent response, the parasitoids only succeeding in finding and parasitizing the hosts when they are present in large numbers. Again further work would be necessary to confirm this, but it is a possible explanation. Another possibility is that the stability of the relationship was disrupted by a regular turn-over of plants. This occurred because the plants were removed, for sale, and replaced by others, which may have been contaminated with mealybugs. The removal of parasitized mealybugs and their replacement with healthy ones, would have a destabilizing influence on the system.

The effect of the inoculative releases of parasitoids made throughout the study period are difficult to determine, but evidently varied from plant to plant. The release of large numbers of parasitoids on both the Aristolochia and Passiflora at times when hosts were abundant, did not result in any increase in parasitism. The slight increases in parasitism seen when mealybug populations were at low levels were not sustained. L. abnormis established a relatively stable population on the Guava and the effect of additional releases of parasitoids seems to have been negligible. After some releases, parasitism increased but after others it decreased, and it must be concluded that the established parasitoid population was the major factor regulating the levels of parasitism of the mealybug on this plant. The series of releases made over a monthly period, near the Coffea

did coincide with an increase in parasitism. Mealybug levels were relatively low, and the increased percentage parasitism may have been a direct result of the introductions made. After this series of releases a stable host/parasitoid relationship seems to have been established. Releases of parasitoids on the pot plants also coincided with increases in percentage parasitism, but a stable host/parasitoid relationship did not result.

The effect of the predator Cryptolaemus montrouzieri on mealybug populations also varied from plant to plant. No quantitative data are available, but observations of the predators' activity were made during sampling. It did not show any evidence of avoiding mealybugs feeding on either Aristolochia or Passiflora and predator numbers reached high levels on both these plants and almost certainly contributed to the eventual decline in pest numbers. It was also in evidence on the pot plants once mealybug infestation had reached a high level. Again it probably made some contribution to the rapid decline of the pest.

Very few C. montrouzieri were ever seen on the Guava bush, although some larvae were to be found throughout the warmer parts of the year and particularly at fruiting time. It is difficult to assess their effect on the mealybug populations, but despite their indiscriminate predation of parasitized and healthy mealybugs, they did not appear to disrupt the stability of the host/parasitoid relationship.

The Coffea did support an apparently fairly stable population of the coccinellid, possibly due to the presence of the hemispherical scale (Sassetia coffeae) as an alternative food source.

The data from this study support the evidence previously available that the

predator C. montrouzieri, though very effective when temperatures are favourable and host populations abundant, is incapable of preventing build up of P. citri on Passiflora plants (Murray, 1978). This is probably due to inefficient searching at low host density and the subsequent delay in population build up. When host plant quality is favourable, allowing increased fecundity of the mealybugs the predator cannot respond quickly enough to prevent heavy infestations of the pest developing. However, given adequate hosts and a favourable environment it can rapidly decimate heavy infestations of P. citri almost to the point of eradication in a short period of time.

There is sufficient evidence to suggest that the parasitoids are capable of preventing build up of P. citri under certain conditions. Where a stable relationship can be built up, parasitism becomes density dependent and any increase in mealybug numbers results in an increase in percentage parasitism thus preventing host outbreaks. There is an indication that temperature may have an effect on the levels of parasitism achieved by the parasitoids. Although the fluctuations in the mealybug population do not necessarily appear to correlate with periods of higher temperature, peaks in the number of mealybugs parasitized and percentage parasitism do seem to occur during the summer and autumn (see figure 23). Mealybug population control on certain plants, for example those containing toxic secondary plant substances, may be difficult to achieve with these parasitoids, due to their seeming inability to establish on mealybugs feeding on this type of plant. More work is necessary on the plant/pest/parasitoid interaction on plants of this type, in order to explain the population dynamics and develop control practices accordingly. The period between samples in this study was too long to allow detailed interpretation of the population dynamics of the system under investigation. Far more information could have been gained if weekly samples had been taken, and if more samples had

been included from each plant.

If innoculative releases are to be made then timing is critical and the parasitoids should be liberated before any sign of host population build up.

4.4 BIOLOGICAL CONTROL OF P. citri IN THE GLASSHOUSE USING PARASITOIDS AND A PREDATOR

Introduction

Mealybugs had posed problems for some time in two small glasshouses used for rearing the host plant of a number of species of butterfly at Wye College. The use of insecticides against the pest was impossible, and attempts to remove the mealybugs using methylated spirit applied by paint brush was only partially successful and extremely time consuming. As low levels of mealybug could be tolerated the house was an ideal candidate for attempting biological control. Thus the predator C. montrouzieri and the parasitoids A. pseudococci, L. abnormis and L. dactylopi were released in an attempt to reduce the mealybug to non-pest levels in one of the houses, the other being kept as a "control".

Materials and methods

The two glasshouses were small, aluminium framed houses (5.65 m x 2.20 m x 2.20 m), heated during the winter. The plants were kept in pots on wooden staging and ranged in size from 200 mm in height to over 600 mm. They were predominantly Passiflora sp. and Citrus sp. both of which are common hosts for the citrus mealybug P. citri. The number of plants present varied, as plants were regularly removed from the houses to be used as food plants for the butterflies. Different plants which had been fed to the caterpillars and subsequently cut back to encourage resprouting, were brought in to replace them. This made assessment of mealybug populations on individual sampling plants impossible, and thus a scoring system was developed in order to allow monitoring of the severity of the mealybug infestation.

<u>SCORE</u>		<u>LEVEL OF INFESTATION</u>
0	-	No mealybug present.
1	-	Scattered, individual mealybugs present.
2	-	Egg masses present.
3	-	Colonies of mealybug present.
4	-	Many colonies, up to 50% of plant infested.
5	-	Severe infestation, over 50% of plant affected.

Forty plants were selected at random in each house and "scored" for mealybug infestation using the above scale. This was done at monthly intervals in order to monitor the seasonal variations.

Parasitoids and the predator were collected from culture cages into plastic boxes and introduced into one of the glasshouse, being released in the late afternoon when temperatures were subsiding and light intensity falling. The first release was made on the 20th April 1983. The number and timing of releases are shown in figure 24.

After six months sampling in the control house had to stop, as it was needed for different work. The plants were moved out and placed in the experimental house, and sampling continued as before.

A constant record was kept of the temperature in the glasshouse using a thermograph recorder.

Results and discussion

The mean scores of mealybug infestation in the two houses are shown in figure 24.

Figure 24

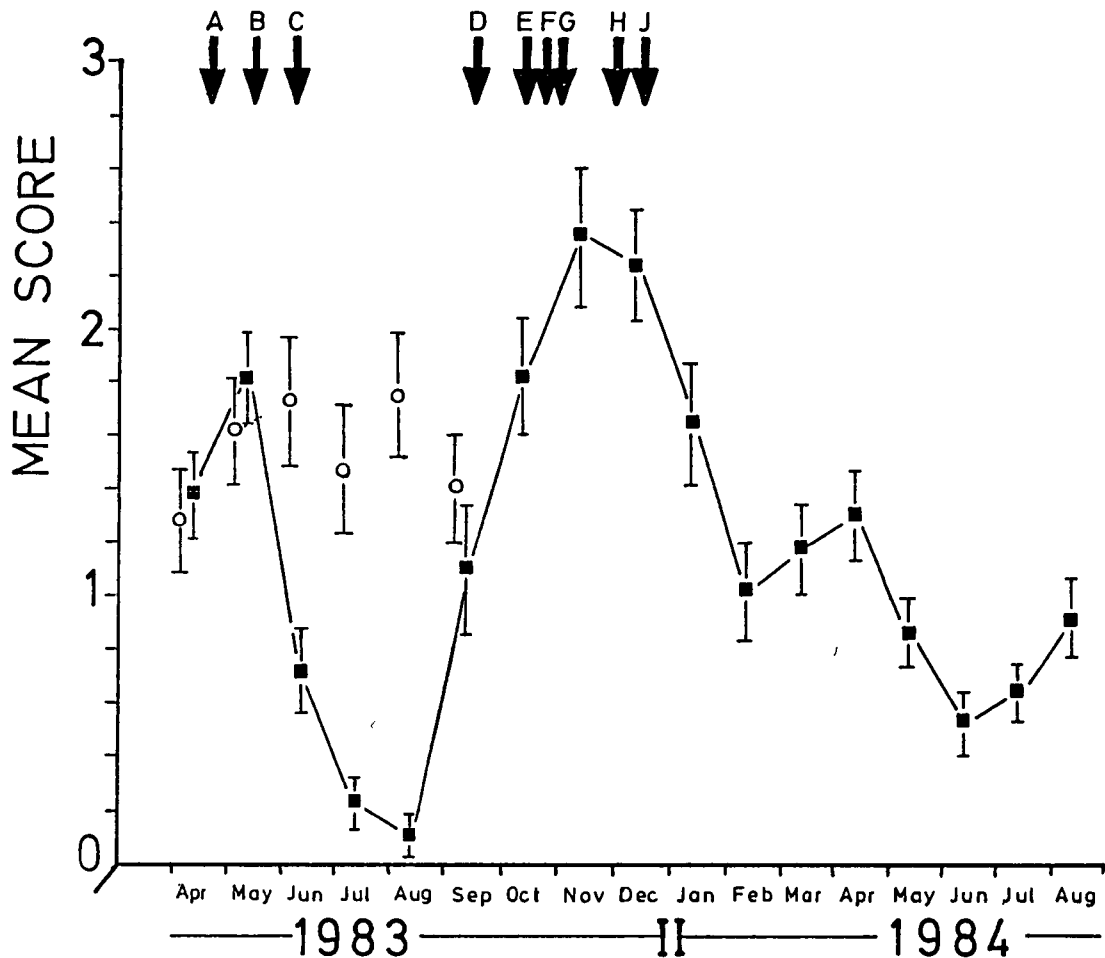


Figure 24 Monthly mealybug populations on plants in a small glasshouse, and the effect of liberations of natural enemies.

Points represent mean monthly scores (with standard errors) of mealybug populations on 40 plants in two small glasshouses

○ without natural enemies ■ natural enemies introduced

Plants were scored on a scale of 1-5, depending on the severity of the infestation (see text)

Timing of releases into the second glasshouse indicated by arrows Composition of releases as follows

- | | | | | | | | | | | | | |
|---|-----|---|---------------------|-----|---|---------------------|-----|---|---------------------|----|---|---------------------|
| A | 100 | L | <u>dactylopii</u> | 100 | L | <u>abnormis</u> | 30 | C | <u>montrouzieri</u> | | | |
| B | 100 | L | <u>dactylopii</u> | 200 | L | <u>abnormis</u> | 25 | C | <u>montrouzieri</u> | | | |
| C | 75 | L | <u>dactylopii</u> | 120 | L | <u>abnormis</u> | 20 | C | <u>montrouzieri</u> | | | |
| D | 150 | A | <u>pseudococci</u> | 50 | L | <u>abnormis</u> | 80 | C | <u>montrouzieri</u> | | | |
| E | 30 | A | <u>pseudococci</u> | 300 | L | <u>dactylopii</u> | 80 | L | <u>abnormis</u> | 25 | C | <u>montrouzieri</u> |
| F | 300 | L | <u>dactylopii</u> | 25 | C | <u>montrouzieri</u> | | | | | | |
| G | 150 | A | <u>pseudococci</u> | 200 | L | <u>dactylopii</u> | 50 | L | <u>abnormis</u> | 30 | C | <u>montrouzieri</u> |
| H | 150 | A | <u>pseudococci</u> | 150 | L | <u>dactylopii</u> | 150 | C | <u>montrouzieri</u> | | | |
| J | 15 | C | <u>montrouzieri</u> | | | | | | | | | |

Initially the mean scores in the two houses were similar, but in June 1983 (approximately 2 months after the first release of natural enemies) the mean score in the experimental house declined substantially.

Mann-Witney U-tests showed that there was no significant difference between the scores of mealybug infestation in the two houses for the first two months, but thereafter the scores in the control house were significantly greater than those in the experimental house each month, up until monitoring ceased in the control house in October 1983.

This decline in the level of infestation in the experimental house was due almost entirely to the activity of the predator C. montrouzieri. During the June sampling many larvae were seen feeding on the mealybugs. Some adult parasitoids were also recorded.

The mealybug infestations remained low in the experimental house until September. At this time a batch of mealybug infested plants were brought in from elsewhere, thus boosting the mean scores. This coincided with a period when the numbers of predators had declined due to vastly reduced prey presence. No predators or parasitoids were seen during the September sample. The mean scores the following month were increased still further by the introduction of the plants from the control house. Mean scores continued to rise until November 1983 and then began to decline once more.

During the November sample there was evidence of C. montrouzieri larvae feeding on some of the more heavily infested plants, and by January 1984 predation was widespread. These larvae were the offspring of adults introduced from September onwards. It took a long time for them to start to reduce mealybug numbers, probably due to lower winter temperatures lengthening the developmental period of the beetles and reducing searching

efficiency and fecundity. There was no evidence of parasitoid activity at this time and the reduction in mealybug infestation can be attributed entirely to the activity of the predator. The failure of the parasitoids to exert any controlling influence on the mealybugs may have been due to the host plant. The majority of plants in the house were Passiflora sp., which contain toxic cyanogenic glycosides. These plants may not be recognised during host habitat location by the parasitoids, or may make mealybugs feeding on them unpalatable to the parasitoids (see discussion in section 4.3).

It is apparent from this trial that the predator C. montrouzieri is extremely effective in reducing P. citri populations. However, there is a lag phase between pest numbers building up and the predator responding to it. This is probably temperature dependant, with the lag phase being shorter when temperatures are higher. With inoculative releases at times when prey numbers are low it may be possible to prevent serious outbreaks, provided temperatures are high enough to allow efficient searching by the predator.

GENERAL DISCUSSION

GENERAL DISCUSSION

As with many coccids, the mealybug Planococcus citri (Risso) has achieved pest status in situations where it is freed from the pressures normally exerted on its population growth by natural enemies. Thus outbreaks have occurred principally in glasshouses, which provide an ideal habitat for the mealybug not only free from natural enemies, but also protected from adverse environmental conditions (Wyatt, 1974), in other areas where the mealybug is an introduced species (Clausen, 1915), or where the use of broad spectrum insecticides has severely reduced natural enemy populations (Dean et al., 1971). It is thus an obvious candidate for classical biological control, a technique which has been used successfully against many other scale insects and mealybugs (DeBach et al., 1971, Caltagirone, 1981). This has now been attempted, both in the field and in the glasshouse, on many host plants, using a variety of natural enemies, over a period of almost a century. The degree of success achieved has been very variable, and it is apparent that merely introducing natural enemies does not necessarily provide a solution.

All attempts at the use of natural enemies to control P. citri to date, have been done with very little pre-introduction research, using parasitoids and/or predators whose biology has been little investigated and is therefore poorly understood. This is in contrast to the glasshouse biological control programmes for whitefly and red spider mite, which have involved extensive research into the biology of the pests and their natural enemies (Hussey and Huffaker, 1976, Vet et al., 1980). It is becoming increasingly apparent that an enormous number of factors interact in order to determine the population dynamics of a given insect, even within a relatively simple ecosystem, like a glasshouse. If these factors are not understood and taken into account, then trial and error introductions of

natural enemies will inevitably produce variable results. However, some knowledge of the factors affecting the success of control may allow manipulation of the system in order to favour natural enemies. The experiments carried out in this study have provided some information which may make the use of natural enemies more reliable as a method of managing P. citri populations.

More success has resulted from attempts to control P. citri using a combination of parasitoids and predators, than using predators alone, both in glasshouses and in the field (Bartlett and Lloyd, 1954, Douth, 1952). A higher degree of control was achieved using the predator Cryptolaemus montrouzieri and a single parasitoid species, Leptomastix dactylopii against P. citri on glasshouse grown Stephanotis, than when a number of predators and parasites were released together against mealybugs on gardenias (Douth, 1951, Douth, 1952). The use of multiple species releases is still a controversial area (Huffaker et al., 1976), and the two most successful examples of biological control in glasshouses to date have both relied on a single natural enemy, the parasitoid Encarsia formosa against whitefly, and the predator Phytoseiulus persimilis against red spider mite (Way, 1976). In this study preliminary cage trials performed under glasshouse conditions did not reveal any adverse competitive effects, when the parasitoids Anagyrus pseudococchi, Leptomastix dactylopii, Leptomastidea abnormis and Coccidoxenoides peregrinus were used in combination. When C. montrouzieri was also present, there was some indication that the predator did not discriminate between parasitised and unparasitized mealybugs, but under these artificial conditions, this did not reduce the level of control achieved.

The four species of parasitoid were therefore introduced into the Wye College conservatory, as a supplement to C. montrouzieri, which was already

present. Innoculative releases of all the parasitoid species were made throughout a 2 year period, but only L. abnormis became established, and persisted during long periods between releases. This parasitoid was regularly seen as an adult in the conservatory, and the vast majority of mummified mealybugs found during the regular 2 monthly samples, were those of L. abnormis. Thus although preliminary trials failed to show any evidence of competitive displacement between the parasitoids, it is apparent that in the glasshouse L. abnormis was the dominant species.

The success of L. abnormis over the other three species is probably the result of a number of factors. It has a rather wider host size range than the other species, and will attack 1st, 2nd, and 3rd instar mealybugs, thus providing itself with a more abundant supply of hosts than the more selective species. Secondly, it was considerably more successful in parasitizing hosts distributed at low density in a glasshouse cubicle than either A. pseudococc1 or L. dactylop11, suggesting that it may be more efficient at host location. Thirdly, results of laboratory studies on the effects of temperature on the biology of the parasitoids, suggest that L. abnormis may be better adapted to its host, under the conditions prevailing in the Wye College conservatory.

L. abnormis is generally the most fecund of the parasitoids and maintains a high level of progeny production over a wide temperature range. The other species require higher temperatures in order to maximize their progeny production, and can only maintain this high level over a relatively narrow range of high temperature. P. citri lays most eggs at relatively low and moderate temperatures, and thus L. abnormis is better adapted to respond to population growth of the mealybug at temperatures most favourable to its host.

L. abnormis is also generally the longest lived of the three species over a wide temperature range, and seems less susceptible to high temperatures. Both A. pseudococci and L. dactylopii show severely reduced adult longevity at a constant 30°C, whilst the effect is less pronounced with L. abnormis.

There do not appear to be any great differences between L. abnormis and the other two species in the various components of searching behaviour in relation to temperature. L. abnormis apparently spends a relatively larger proportion of its time walking in the absence of hosts than the other species, but its walking speed is generally rather slower (particularly at high temperature) and it shows a similar rate of turning. The relative contribution of these factors to the overall searching efficiency is difficult to determine, but no consistent differences across temperature can be found. All three species show reduced walking speed, increased rate of turning and spend a smaller proportion of their time walking, within host patches. These changes in the behavioural patterns result in arrestment within host patches, but there is insufficient evidence to demonstrate whether the parasitoid species differ greatly in this respect. However, as mentioned above L. abnormis did demonstrate a higher host finding ability than the other two parasitoids under glasshouse conditions, and thus in practice appears to have the highest searching efficiency.

L. abnormis has the longest developmental period of the three parasitoids over the entire temperature range from 18°C to 30°C and in this respect appears less efficient than either A. pseudococci or L. dactylopii. In comparing three Encarsia species and one Eretmocerus sp. in relation to their potential as control agents for whitefly at various temperatures, Vet and van Lenteren (1981) placed a high value on the length of the developmental period in determining the parasitoids' efficiency. The developmental period of the host (Trialeurodes vaporariorum) is similar to

that of the parasitoids and for successful restraint of the pest population it is critical that development of the parasitoid is faster than that of the host. However, all the mealybug parasitoids studied here have shorter developmental periods than the host at all temperatures tested, and thus the relative developmental rates are less important. Also, L. abnormis can develop over a wider temperature range than the other two species, as it has the lowest, lower developmental threshold and the highest upper threshold. Thus under natural fluctuating conditions this compensates to some degree for its slower developmental rate.

The indications are that these effects may combine to allow L. abnormis to respond better to changes in the host population, which tends to be favoured by low to moderate temperatures. High temperatures are most favourable to efficient searching, rapid development and high fecundity for the parasitoid A. pseudococci, but the mealybug's fecundity is much decreased at high temperatures, as is its developmental period and thus its longevity. Thus this parasitoid is likely to exert its maximum effect on P. citri under conditions which are themselves tending to regulate the mealybug's populations. Although L. dactylopi responds less well to high temperatures than A. pseudococci, it is even less efficient at low temperatures. L. abnormis, however, is fairly efficient over a wide range of temperatures and is thus probably the better adapted to its host.

In general the degree of damage and extent of the mealybug problem in the conservatory declined after the release of parasitoids. However, regular monitoring of mealybug and parasitoid populations revealed large differences in the pest problem and degree of parasitism on the five different sample plants. Pest numbers on the Guava declined after release of the parasitoids and remained relatively constant and at low levels, as did mealybug numbers on the Coffea. On these plants L. abnormis appears to

have established a stable relationship, maintaining P. citri at much lower levels than occurred before the parasitoids were released. Increases in the mealybug numbers on the Guava seem to coincide with increased percentage parasitism, suggesting that there may be a degree of density dependance in operation. However, the interpretation of population changes and the demonstration of density dependance are notoriously difficult, and more detailed information and analysis would be necessary to confirm a causal effect (Varley and Gradwell, 1963, Hassell, 1985). In contrast, the numbers of P. citri on the Aristolochia, Passiflora and pot plants (Streptocarpus sp. and Saintpaulia sp.) fluctuated enormously despite the presence of natural enemies, with peak populations reaching damaging levels. Similar differences have been found in whitefly populations on different host plants and the level of control which can be achieved by the parasitoid E. formosa. It has been shown that pests' fecundity, longevity and development are stimulated on aubergine (eggplant) and cucumber plants over that on tomato and sweet pepper (paprika) plants. The parasitoid is less effective on cucumbers and aubergine than on the other two plants, because it is hampered in its searching by the retiform venation and hairy leaves. These factors combine to make control of whitefly rather poor on cucumbers, less than satisfactory on aubergine, but good on tomatoes and paprika (Woets and van Lenteren, 1976). It is possible that similar stimulation of the mealybug's fecundity and longevity occur on the favoured host plants, but the parasitoids inability to respond to the host populations cannot be entirely accounted for by hampered searching on these plants. This may be the case on the pot plants, which have hairy leaves and extremely prominent venation, but the Passiflora has entirely smooth leaves. On these plants it may be that the toxic secondary plant substances they contain, deters the searching parasitoids from attacking mealybugs feeding on their leaves, or that the plants are not recognised as host habitat and thus not searched at all.

In the Wye College conservatory, peak populations of mealybug did not occur at the same time of year on all plants, but seemed to coincide with periods of new growth, fruiting or flowering on the individual plants. This suggests that changes in host plant quality may lead to increased fecundity of the pest and that this may be a more important factor in determining pest abundance than environmental conditions, which were the same for all plants. Similar changes have been noted in aphid populations in relation to host plant quality and in particular to periods of high phloem nitrogen content caused by new growth or senescence of old foliage (Wratten, 1974; Edwards and Wratten, 1980, Strong et al., 1984). Further studies are warranted on the effect of host plant on the biology of P. citri, to determine the factors leading to greater population increase.

Parasitoid populations on the Aristolochia and Passiflora were very low or non-existent at all times, fluctuated widely on the pot plants, rising several months after peaks in mealybug numbers, and were relatively stable on the Guava and Coffea. Peaks in parasitoid levels correlated much better with periods of rising or high ambient temperature, than did levels of P. citri, and thus although temperature cannot be implicated as a major factor inducing pest outbreaks, it may be important in determining the efficiency of the parasitoids in reducing host numbers.

There appear to be three factors interacting to determine mealybug population levels in the Wye College conservatory, host plant, natural enemies, and temperature. Changes in host plant quality can lead to massive increases in the numbers of P. citri on some host plants. On others the mealybug populations appear to be constrained by natural enemies and regulated around an equilibrium point in a relatively stable manner. Temperature can affect the extent of the mealybug outbreak on susceptible plants, with larger peak populations occurring in warmer years.

Temperature also influences the levels of parasitism and predation by the natural enemies. Little can be done to alter the effects of the host plant on the population growth of the pest, other than growing only those plants less favoured by the mealybug. However, the other two factors may be manipulated in attempts to reduce the extent of damage done by P. citri.

The evidence suggests that in general higher temperatures are more favourable to the parasitoids than the pest. However, energy conservation is an important factor, both from the point of view of economics, and resource usage. Few growers would be able to increase the overall heating levels in their glasshouses, and in any case temperatures must be geared primarily to the requirements of the crop plant. Temperature manipulation is also limited in amenity areas and offices, where temperatures must be set at levels which are comfortable for the people using them. However, it is plausible to contemplate boosting temperatures by several degrees during the warmer parts of the day, whilst allowing night time temperatures to sink a little lower to balance out the energy usage. This may be advantageous, as experiments under cycling temperatures showed that only relatively short periods at high temperature are required by the parasitoids in order to lay a high proportion of their available eggs. Such short periods of higher temperature are also liable to favour efficient searching. Insects generally react rapidly to changes in temperature, their activity levels responding to quite small changes (Barrass, 1970). Their searching behaviour can therefore be expected to change almost simultaneously with rising temperatures, resulting in larger areas being covered in the same period of time. Periods of higher temperature would thus lead to more efficient host patch location, without affecting aggregation, as intensive searching within patches appears to be largely temperature independent. The fact that periods of high temperature would be relatively short lived would further encourage aggregation in

patches, with lower temperatures decreasing the probability of leaving. The effects of temperature on interference between searching parasitoids, density responsiveness, and other factors which affect population stability, have not been studied. However, the indications are that manipulation of the temperature in this way may have a stabilizing effect on the population dynamics of the mealybug. The effects on the developmental period would also be favourable and work on other parasitoids suggests that cycling temperatures also favour adult longevity, through changing the shape of the survival curves leading to prolonged survival during the initial period, when egg laying is generally at a peak (Barfield, Bottrell and Smith, 1977).

During the winter, reduced photoperiod may also affect levels of parasitism. It has been shown that the parasitoids are almost totally inactive in the dark, and will thus not search for and parasitize hosts during periods of darkness. Similar results have been found for a number of other insects (Vet et al., 1980), although no difference was found in parasitoid distribution and levels of parasitism by E. formosa at differing light intensities (Hussey, Parr and Stacy, 1976). The reduced photoperiod during the winter will reduce the period available to the parasitoids for host location. This may be critical at a time when low temperature is also reducing efficiency. Thus it may be particularly advantageous to raise midday temperatures by several degrees during the winter months in order to favour the parasitoids over their host. The extent of the manipulation required would depend on the individual situation, but the aim would be to adjust temperatures to favour the factors mentioned above, with the overall effect of maximizing the reproductive potential of the natural enemies over that of their host.

Where manipulation of the temperature is impossible, it may be possible to improve control through the release of larger numbers of natural enemies. The feasibility of this will probably depend on economics and the cost of the biological control agents becomes the limiting factor. Preliminary trials under glasshouse conditions, showed that a five fold increase in the initial release level of a combination of the mealybug parasitoids, resulted in noticeably less damage to plants infested with high populations of P. citri, under the same conditions. Steiner and Elliott, (1983) suggest releasing 5 pairs/plant, for a free-flying situation in a glasshouse. This is of a similar order to suggested release rates for E. formosa against whitefly, which are used widely on a commercial basis (Woets, 1978). Given the higher value of ornamental plants, it may be viable for growers to release mealybug parasitoids at higher rates if the cost of natural enemies is similar. Steiner and Elliott's recommendation may provide a useful starting guide to releases, but there may be other factors which should also be taken into account.

The release programme for natural enemies of the greenhouse whitefly has been the subject of much intensive study, but no single successful method has been found, and it is suggested that the release programme should be determined by the situation in which it is to be used (Parr, Gould, Jessop and Ludlam, 1983). This is also likely to be the case for mealybug control using natural enemies and thus it is important to determine the criteria which will affect the numbers of natural enemies required. There is evidence from this study that plant type, mealybug infestation and glasshouse size must be taken into account when considering the release rate for natural enemies. Plant type is important, as a sensitive plant i.e. one which rapidly loses condition when attacked by mealybugs, will require an initial release of larger numbers of natural enemies, than a non-sensitive plant, if damage is to be avoided; the size of a mealybug

infestation will determine the rate at which a given plant suffers damage and loses condition; and the total area of the glasshouse also has an effect, as this influences the probability of the parasitoids finding hosts. It was found that an enormous increase in area did not result in a proportional decrease in percentage parasitism, but that it did have a definite effect on the number of hosts found. It was estimated that a 6, 12 and 22 fold increase in L. abnormis, L. dactylopii and A. pseudococci numbers, respectively, would be required to give the same degree of control of the same number of mealybug for a 300 fold increase in glasshouse volume.

Biological control of pests on ornamental plants has, until recently, been neglected due to the high degree of perfection expected in freedom from pests (van Lenteren et al., 1980). However, increasing problems of pesticide resistance and the now widespread use of foliage and other ornamental plants in offices, amenity areas and other public places have resulted in increased interest in biological and integrated control for these plants (Scopes, 1985). Integrated programmes are being actively researched and tested for chrysanthemum pests (Wyatt, 1974; Cross, Wardlow, Hall, Saynor and Bassett, 1983; Wardlow, 1985) and a complete integrated pest management programme is in operation for a variety of ornamental plants in interior landscapes in the U.K. This programme incorporates the use of A. pseudococci, L. dactylopii, and L. abnormis against P. citri and has now been in commercial operation for more than 2 years (Scopes, 1985).

Ornamental plantations offer a unique challenge as regards pest management. Biological and integrated control programmes have generally been directed against the pest complex of a single crop plant, be it tomatoes, cucumbers or chrysanthemums. However, interior landscapes and the collections within botanic gardens comprise a large number of unrelated plant species, where a given pest is likely to favour certain plants. In this situation natural

enemies will have to be selected which will efficiently regulate host populations on a range of host plants, and thus a combination of natural enemies may be most successful, some of which may respond better to hosts on one plant, whilst others prefer the host attacking a different host plant. Differences in the host plant may well account for the large variation in the success of attempts to control P. citri in glasshouses. In the future more reliable results may be attained following study of which natural enemies have been successful, on which host plants, in previous attempts at biological control of the mealybug.

Complete biological control of mealybugs on all types of ornamental plants may be impossible using the three parasitoid species studied here, and problems with plants of the Passiflora family and other plants which contain toxic secondary plant substances have been encountered. However, there are a large number of natural enemies of P. citri, some of which may be more successful on these plants. Integration with spot treatments of pesticide may be possible in some instances, providing the plants do not show toxic side effects.

Although this study has concentrated on the biological control of the glasshouse mealybug, it is recognised that integration with other methods for the control of other pests of ornamental plants will be necessary. The ability of the parasitoid L. abnormis to reduce mealybug populations and maintain them at a consistently low level on some host plants, provides a suitable biological agent which can be used in integrated programmes on ornamental plants. Biological agents are already widely used against other pests of foliage plants, particularly E. formosa against whitefly and P. persimilis against red spider mite. The fewer the chemicals required to control other pests, the greater will be the reduction in selection pressure on pests to develop resistance.

".....pest control cannot be analysed or developed as an exercise in control of a given pest alone; rather, it must be considered and applied in the context of the ecosystem in which a number of pest, or potential pest, populations exist and in which control actions are taken. Whatever the new technologies which may be developed for control or management of pest insects in the future, they must be applied with a knowledge of the operation of the pertinent ecosystem." (Doutt and Smith, 1971).

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APPENDICES

APPENDIX 1

APPENDIX 1a. Functional part of programme written in "BASIC" to predict developmental periods by accumulating day degrees.

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10 REM PART OF A PROGRAM TO CALCULATE ACCUMULATING DAY DEGREES
20 REM COMMODORE BASIC
30 INPUT" THERMAL CONSTANT";TC:REM E.G. 4000 HOUR DEGREES
40 INPUT" LOWER THRESHOLD",LT:REM E.G. 10°C
50 PRINT" INPUT STARTING RECORD POSITION":INPUT RP:HT=1
60 DOPEN#2,"GLASSDATA",L35:REM DISC FILE WITH GLASSHOUSE DATA
70 RECORD#2,(RP)
80 INPUT#2,MT:HT=HT+1:REM INPUT MONITORED TEMP FROM DISC E.G. 25°C
90 EH=(MT-LT):REM HOUR DEGREES ACCUMULATED IN ONE HOUR
100 ET=ET+EH:REM HOUR DEGREES TOTAL
110 IFET<TCTHEN80
120 DCLOSE#2
130 PRINT" LIFE CYCLE COMPLETE IN";HT/24;" DAYS":END
```

APPENDIX 1b. Functional part of programme in "BASIC" to predict developmental periods at different temperatures from polynomial regression fits to constant temperature data.

```
10 REM PART OF A PROGRAMME TO PREDICT LIFE CYCLE IN THE GLASSHOUSE
20 REM COMMODORE BASIC
30 DIM PR(12) REM DIMENSION POLYNOMIAL COEFFICIENTS
40 DOPEN#1,"BUGFILE",L10:REM OPEN DISC FILE CONTAINING POLYNOMIAL DATA
50 INPUT#1,PR(1) REM POLYNOMIAL ORDER E.G. 4
60 INPUT#1,PR(2):REM COEFFICIENT A E.G. 1945.6209
70 INPUT#1,PR(3):REM COEFFICIENT B E.G. -256.998855
80 INPUT#1,PR(4):REM COEFFICIENT C E.G. 13.0431528
90 INPUT#1,PR(5) REM COEFFICIENT D E.G. -.293230936
100 INPUT#1,PR(6) REM COEFFICIENT E E.G. 2.46151454E-03
110 INPUT#1,PR(7) REM COEFFICIENT F E.G. 0
120 INPUT#1,PR(8) REM COEFFICIENT G E.G. 0
130 INPUT#1,PR(9):REM COEFFICIENT H E.G. 0
140 INPUT#1,PR(10) REM COEFFICIENT I E.G. 0
150 INPUT#1,PR(11) REM LOWER TEMPERATURE LIMIT E.G. 15°C
160 INPUT#1,PR(12):REM UPPER TEMPERATURE LIMIT E.G. 35°C
170 DCLOSE#1
180 PRINT" INPUT STARTING RECORD POSITION":INPUT RP:HT=1
190 DOPEN#2,"GLASSDATA",L35:REM DISC FILE WITH GLASSHOUSE DATA
200 RECORD#2,(RP)
210 INPUT#2,MT:HT=HT+1:REM INPUT MONITORED TEMP FROM DISC E.G. 25°C
220 IFMT<PR(11)ORMT>PR(12)THENEH=0:GOTO250:REM SKIP IF OUTSIDE THRESHOLDS
230 FORK=PR(1)TOOSTEP-1.EH=EH+(PR(K+2)*MT+K):NEXTK:REM CALC REGRESSION
240 EH=(1/EH)*.0416 REM AMOUNT OF LIFE CYCLE IN ONE HOUR
250 ET=ET+EH:REM ACCUMULATING LIFE CYCLE
260 IFET<1THEN210
270 DCLOSE#2
280 PRINT" LIFE CYCLE COMPLETE IN";HT/24;" DAYS":END
```

Appendix 2. Calculation of effective area searched by Anagyrus pseudococci in patch location behaviour and intensive searching within host patches.

a. Patch location behaviour.

	TEMPERATURE			
	18	22	26	30
Mean proportion active.	0.3	0.8	0.68	0.91
Mean proportion time walking.	0.474	0.689	0.705	0.712
Mean walking speed (mm/s).	2.27	3.63	4.417	6.7
Mean swathe width of antennae (mm).	2.0	2.0	2.0	2.0
Effective area searched (mm ² /s).	0.646	4.002	4.235	8.68

b. Intensive searching within host patches.

	TEMPERATURE			
	18	22	26	30
Mean proportion active.	0.3	0.8	0.68	0.91
Mean proportion time walking.	0.139	0.215	0.381	0.358
Mean walking speed (mm/s).	1.05	1.06	1.337	1.93
Mean swathe width of antennae (mm).	2.0	2.0	2.0	2.0
Effective area searched (mm ² /s).	0.087	0.365	0.689	1.257

Appendix 3. Calculation of effective area searched by Leptomastix dactylopii in patch location behaviour and intensive searching within host patches.

a. Patch location behaviour.

	TEMPERATURE			
	18	22	26	30
Mean proportion active.	0.16	0.55	0.73	0.91
Mean proportion time walking.	0.536	0.362	0.588	0.531
Mean walking speed (mm/s).	2.04	2.61	5.717	5.78
Mean swathe width of antennae (mm).	3.0	3.0	3.0	3.0
Effective area searched (mm ² /s).	0.525	1.559	7.353	8.379

b. Intensive searching within host patches.

	TEMPERATURE			
	18	22	26	30
Mean proportion active.	0.16	0.55	0.73	0.91
Mean proportion time walking.	0.551	0.597	0.446	0.357
Mean walking speed (mm/s).	1.18	1.63	2.557	2.72
Mean swathe width of antennae (mm).	3.0	3.0	3.0	3.0
Effective area searched (mm ² /s).	0.312	1.606	2.491	2.651

Appendix 4. Calculation of effective area searched by Leptomastidea abnormis in patch location behaviour and intensive searching within host patches.

a. Patch location behaviour.

	TEMPERATURE			
	18	22	26	30
Mean proportion active.	0.22	0.66	0.81	0.74
Mean proportion time walking.	0.675	0.789	0.772	0.816
Mean walking speed (mm/s).	1.79	2.98	3.683	4.182
Mean swathe width of antennae (mm).	1.5	1.5	1.5	1.5
Effective area searched (mm ² /s).	0.399	2.328	3.455	3.788

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b. Intensive searching within host patches.

	TEMPERATURE			
	18	22	26	30
Mean proportion active.	0.22	0.66	0.81	0.74
Mean proportion time walking.	0.601	0.388	0.299	0.356
Mean walking speed (mm/s).	0.84	0.98	1.037	1.57
Mean swathe width of antennae (mm).	1.5	1.5	1.5	1.5
Effective area searched (mm ² /s).	0.166	0.376	0.374	0.62

"....there is never finished work, only work in progress...."

Pat McNeil, 1985.