Use of the Coccinellid, *Stethorus punctillum* Weise (Coleoptera: Coccinellidae) to Control the Glasshouse Red Spider Mite, *Tetranychus urticae* Koch (Acari: Tetranychidae).

By

Claire Victoria Beverley

Canterbury Christ Church University College

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ABSTRACT

Stethorus punctillum Weise (Coleoptera: Coccinellidae) is a predator of tetranychid mites. The aim of the study was to evaluate the coccinellid as a predator of two-spotted spider mites, *Tetranychus urticae* Koch (Acari: Tetranychidae) in glasshouses. Development of *S. punctillum* was evaluated under a range of constant temperatures from 18 to 34 ^{0}C and a cycling temperature regime at 10/25 ^{0}C. Results were analysed using polynomial regression analysis and a subsequent experiment evaluated this and the accumulated heat method as predictors of lifecycle duration.

Two temperatures typically tolerated by natural enemies in glasshouses were evaluated for effect on pre-oviposition period and fecundity in *S. punctillum*. Statistical analysis indicated that temperature did not have a significant effect on pre-oviposition period although there was a tendency for decreased pre-oviposition period with an increase in temperature from 20 to 26 ^{0}C. Beetles reared at 26 ^{0}C tended to oviposit at a significantly higher rate (eggs day $^{-1}$) compared with beetles reared at 20 ^{0}C. Adult longevity was not determined. However, the experiment was terminated on day 85 when the majority of adults remained alive.

Cold storage of *S. punctillum* adults for five, 10 and 15 days at 6 ^{0}C was evaluated for effect on the number of days to resume oviposition (end of storage to first egg), oviposition rate (eggs day $^{-1}$) and egg viability post storage. Storage was possible with no significant effect on mean oviposition rate when compared with the mean oviposition rate of beetles that were not stored. There was an indication that the physiological state of *S. punctillum* was an important factor affecting its ability to withstand cold storage. However, further research is indicated to evaluate this.

Effect of short day-length on juvenile development, pre-oviposition period and oviposition rate in *S. punctillum* was determined and compared with equivalent parameters for beetles incubated under long day-lengths. Analysis indicated that short day-length did not significantly effect juvenile development period in *S. punctillum*. Pre-oviposition period was significantly effected by photoperiod. A reduction in photoperiod resulted in a decrease in mean oviposition rate. However, results were variable and data for individual beetles were considered. Results are discussed with

reference to age of beetles and overall variability in oviposition rate of the test populations.

Olfactory responses of *S. punctillum* to odours from *Vicia faba* L. and *V. faba* plus *T. urticae* were evaluated in a four-arm olfactometer. Beetles showed directed movement towards host odours. The behaviour of *S. punctillum* was significantly modified in odour fields containing odours from *V. faba* plus *T. urticae*. The implications of these results to glasshouse pest management of *T. urticae* are discussed.

Semi-field trials were conducted to evaluate *S. punctillum* as a control agent of *T. urticae* in a glasshouse cucumber crop. Destructive leaf samples from the upper and lower crop canopies were taken on five assessment dates. While statistical analysis indicated no significant effect of crop level on densities of two-spotted spider mites, significant effects of crop level were recorded for densities of *S. punctillum*. Mean densities of two-spotted spider mites leaf $^{-1}$ were significantly lower on the final assessment date compared with the first. This contrasts with data for *S. punctillum* densities, where no significant differences were detected between assessment dates. Details of the inadvertent establishment of *Feltiella acarisuga* Vallot (Diptera: Cecidomyiidae) are also included in the results.

All experimental results are discussed with a view to the inclusion of *S. punctillum* as a commercially viable biological control agent in the management of *T. urticae* in glasshouses.

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1 INTRODUCTION

Spider mites, such as *Tetranychus urticae* Koch (Acari: Tetranychidae), are economically important pests on a number of glasshouse crops (e.g. tomatoes and cucumbers). Reports suggest that insecticide applications used to control these pests may actually aggravate the situation. This is possible by disrupting natural control by predators (e.g. Wilson *et al.*, 1998) or by encouraging the development of chemical resistance in spider mites (e.g. Hussey and Huffaker, 1976).

Biological control provides an alternative to chemical control and is largely concerned with encouraging a natural process to work in, in some instances, a rather unnatural setting, e.g. that of a monoculture in a glasshouse.

A number of insect groups contain natural enemies of spider mites e.g. Coleoptera, Thysanoptera, Heteroptera, Diptera and Neuroptera (Chazeau, 1985). In addition, *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) is a commercially available predatory mite used to control spider mites. Despite its widespread use against spider mites, there are a number of examples in the literature to support the search for alternative biocontrol agents. For example, Nihoul (1992) and Van Steekelenburg (1992) have reported breakdowns in control programmes implementing *P. persimilis* in glasshouse tomatoes. Hamamura *et al.* (1976) stated that *P. persimilis* larvae failed to develop at 35 ^{0}C. Further research has highlighted the inability of it to survive in the absence of two-spotted spider mites (El-Banhawy and El-Bagoury, 1991) and this was thought to be due to inefficiency in alternative food utilisation (McMurtry and Croft, 1997).

The predatory coccinellid, *Stethorus punctillum* Weise (Coleoptera: Coccinellidae) is an exclusive spider mite predator (e.g. Collyer, 1953; Putman, 1955), although its predation on aphids has been reported (e.g. Putman, 1955; Majerus, 1994; Hawkins, 2000). Putman (1955) observed larvae of the predator to feed on aphids but development to adulthood was poor (93 % mortality) and apparently oviposition was not possible on a diet of aphids. *S. punctillum* has been established in glasshouse pepper and cucumber crops (Elliot D., Applied BioNomics, personal communication; Raworth, 2001) but the majority of published work chronicling its value as a spider mite predator is based on research in strawberry plantations and fruit orchards (e.g. Collyer,

1953; Putman and Hearne, 1966; Injac and Dulič, 1992; García-Marí and González-Zamora, 1999). In these environments observations of the predatory coccinellid have mainly concentrated on its natural invasion and subsequent success in the control of spider mites together with other naturally occurring predators (e.g. García-Marí and González-Zamora, 1999; Roy *et al.*, 1999). Information relating to its performance in glasshouse culture, although promising, is limited (Rott and Ponsonby, 2000ab; Raworth, 2001). The reported merits of *S. punctillum* as a voracious and highly mobile spider mite predator (e.g. Readshaw, 1971) were used as a platform for the research documented here.

The aim of this study was to investigate the dynamics of *S. punctillum* more fully with a view to applying it in glasshouse situations. In glasshouses, *S. punctillum* would have to be incorporated into enclosed environments where temperature and humidity levels are maintained for optimum plant growth and yield for a range of crops. Therefore, the effect of a range of temperatures on the development of *S. punctillum* (Chapter 4) and the effect of temperatures typical to UK glasshouses on the fecundity and longevity of *S. punctillum* (Chapter 5) were assessed.

Commercial producers and suppliers of natural enemies use cold storage techniques to bridge gaps between supply and demand. A natural enemy able to withstand cold storage would make a more attractive investment when considering commercial production. Therefore, the effects of cold storage on *S. punctillum* were investigated by maintaining adults at 6 ^{0}C for five, 10 and 15 days and assessing fecundity post storage (Chapter 6). Furthermore, the effect of short day-length on *S. punctillum* was investigated (Chapter 7) to provide insight into the underlying mechanisms of diapause, part of the insect lifecycle often exploited in cold storage techniques.

Pivotal to successful pest control is the ability of a natural enemy to locate its host. When prey are scarce one of the essential characteristics of a predator is a high searching capacity (DeBach and Rosen, 1991). In order to evaluate possible chemical cues used by *S. punctillum* in host searching, a four-arm olfactometer was used to assess the behaviour of *S. punctillum* in the presence of odours from bean leaves and bean leaves infested with spider mites (Chapter 8).

Crop scale experiments often provide greater insight into observations found in laboratory experiments. Therefore the ability of *S. punctillum* to control spider mites in a semi-field trial of glasshouse cucumbers was investigated (Chapter 9).

All experimental results are discussed with a view to the inclusion of *S. punctillum* as a biological control agent against two-spotted spider mites in glasshouse cropping.

2 LITERATURE REVIEW

2.1 Biological Pest Control

2.1.1 Definition and scope

DeBach and Rosen (1991) defined pests as organisms that cause significant economic injury. Biological pest control may be defined as a reduction in population density of insect and mite pests using natural enemies (e.g. Speight *et al.*, 1999). The importance of predators as biocontrol agents was recognised as far back as 1752 by Linnaeus (Van Driesche and Bellows, 1996). Contemporary definitions of biological control may include human (e.g. DeBach, 1964) or genetic (e.g. Abercrombie *et al.*, 1992) manipulation.

Reports of successful biological pest control on outdoor crops are numerous, e.g. control of the cottony-cushion scale, *Icerya purchasi* Maskell (Homoptera: Margarodidae) on citrus in California by the vedalia ladybird, *Rodolia cardinalis* Mulsant (Coleoptera: Coccinellidae) (DeBach and Rosen, 1991). However, the more stable and predictable environment of a glasshouse lends itself well to biological pest control (*section 2.2*). Indeed, Van Lenteren *et al.* (1997) suggested that the extent of the glasshouse industry on continental Europe is the reason that more species of natural enemies are commercially available here compared with the United States of America.

There are five strategies of biocontrol, namely introduction, conservation, augmentation, inundation and inoculation (Dent, 1995). The first technique attempts to control introduced pests by introducing their natural enemy (e.g. Speight *et al.*, 1999). Habitats or alternative hosts may be utilised to encourage the survival and population increase of native or exotic natural enemies and this method is termed conservation (e.g. Speight *et al.*, 1999). Conservation techniques may be supplemented by additional releases of smaller quantities of natural enemy through augmentation (e.g. Speight *et al.*, 1999). Application of biocontrol agents can achieve short-term (inundative release methods) or long-term (inoculative release methods) control of pest populations where the former method requires frequent releases of large quantities of natural enemy to control minor pests in glasshouses for example (e.g. Van Lenteren and Woets, 1988). The use of large quantities of a biocontrol agent, for an immediate impact on the pest

population, is sometimes referred to as 'biotic-' or 'living-insecticide' application (Hodek and Honěk, 1996). The successful mass production of natural enemies is required to realise this technique and relies upon specific knowledge of life-history traits of predators and parasites, e.g. development rate and fecundity (McEwen, 1997).

General release licences are required for the importation of natural enemies and information such as lower temperature thresholds for development of natural enemies, lower lethal temperatures and the ability to survive on alternative native prey species is becoming necessary before the licences are issued (Ponsonby and Copland, 1997).

2.1.2 Importance of biological pest control

The use of chemicals to control arthropod pests has caused several problems, for example, the disruption of beneficial insects and mites (e.g. Hussey and Huffaker, 1976; Wilson *et al.*, 1998) and new or old pest resurgence, e.g. resurgence of the cottony cushion scale, *I. purchasi* following destruction of its natural enemy the vedalia ladybird, *R. cardinalis* by the pesticide, DDT (1,1,1-trichloro-2,2-bis-(p-chlorophenyl) ethane) and the insecticide, malathion (Hodek, 1973). In addition, pests have shown resistance to insecticides, e.g. resistance development in the two-spotted spider mite, *Tetranychus urticae* (e.g. Gould and Jessop, 1981; Edge and James, 1982; Jacobson and Croft, 1999). Evidence suggests that direct chemical damage to crops, especially young plants (Van Lenteren and Woets, 1988), has been caused by, for example, petroleum oils and acaricides (e.g. Hussey and Scopes, 1985; DeBach and Rosen, 1991) and damage to fruit has also been recorded (e.g. Van Lenteren and Woets, 1988; Waite, 1988), e.g. cyhexatin, dicofol and propargite were reported to cause blemishes on strawberry fruit in Australia (Waite, 1988).

Practical aspects of pesticide application that are not beneficial to crop management include disruption to harvesting schedules to allow for safety periods post spraying. Certain spray equipment, used to apply chemicals in crops, is not always effective in reaching the undersides of leaves (Waite, 1988), a problem which has increased in importance in cucumber crops where modern growing techniques favour dense foliage (Hussey and Scopes, 1985). This is of particular importance when the underside of the leaf is favoured by a pest, e.g. spider mites. Van Lenteren and Woets (1988) stated that

it is common for new pesticides to be used before determining their effect on natural enemies. Further problems include, increasing costs of chemicals, legislation that has resulted in limiting the choice of available pesticides and the extent of their application (Veanman, 1992). All these factors have contributed to the increase in demand for alternative control measures, e.g. biological control, and basic research on natural enemy biology is pivotal to the success of pest management programmes (e.g. DeBach and Rosen, 1991).

2.1.3 Characteristics of biological control agents

According to Huffaker *et al.* (1971), the main characteristics of a natural enemy that determine its effectiveness in pest control include: adaptability to the environmental conditions, searching capacity, capacity of increase relative to that of its prey, capacity for prey consumption and other inherent properties such as, synchronisation with the prey, prey specificity, degree of discrimination and the ability to survive prey-free periods.

2.2 Glasshouse Culture

Monocultures, e.g. in glasshouses, are not always conducive to the successful performance of natural enemies in pest control due to a lack of alternative foods, e.g. nectar and pollen, which may be utilised when pest levels are low (Hodek, 1973). For example, two species of *Hippodamia* (Coleoptera: Coccinellidae) were more common in maize-faba bean polycultures compared to corn monocultures and the reason for this was suggested to be the presence of extrafloral nectaries in faba beans (Trujillo-Arriega and Altieri, 1990).

Nevertheless, the glasshouse environment is isolated and thus relatively protected from outside influences such as extreme temperature and humidity changes, and immigration of pests on a large scale (Van Lenteren and Woets, 1988). Strict regulation of temperature makes predictions of arthropod development, in relation to temperature, relatively simple compared to outdoor situations. This is particularly important when evaluating the impact of natural enemies on pest populations in commercial glasshouse crops where the acceptable level of pest damage is limited (Van Lenteren *et al*, 1997).

Predictions of the rate of increase in plant damage in relation to temperature, e.g. damage caused by spider mites, are also possible (Hussey and Huffaker, 1976) and this in turn facilitates the establishment of successful pest control programmes.

Unlike hop gardens, for example, where the same plants and growing frames are used from year to year (Darby P., personal communication), glasshouses are cleaned in between growing seasons to create pest-free areas at the start of the season (Van Lenteren and Woets, 1988) and the subsequent introduction of pest-free plants from propagators is essential (Hussey and Scopes, 1985). Cleaning helps to eliminate the damaging effects caused by the emergence of overwintering pest populations, e.g. mites, when young plants are introduced, e.g. cucumbers, and the heating is turned on (e.g. Parr and Hussey, 1966; Hussey and Scopes, 1985).

2.3 Spider Mites

Spider mites are arachnids in the family Tetranychidae and order Acarina. One member is *T. urticae*, commonly known as the two-spotted spider mite or glasshouse red spider mite. The spider mite lifecycle consists of five developmental stages; egg, larva, protonymph, deutonymph and adult. Adult males are smaller than females and are easily distinguished from the latter by their distinctly pointed abdomens. Rate of development of spider mites depends on environmental changes such as temperature, e.g. the mean development time of *T. urticae* from egg to adult decreased from 32.9 to 6.7 days with an increase in temperature from 15 to 30 ^{0}C (Malais and Ravensberg, 1992).

Spider mites are one of the most important pests in glasshouses (e.g. Van Lenteren and Woets, 1988). Plants susceptible to spider mite damage are numerous and include glasshouse crops, e.g. tomato and cucumber (Helle and Sabelis, 1985ab) and fruit crops, e.g. strawberry (e.g. Oatman *et al.*, 1985; Waite, 1988; Raworth, 1990; García-Marí and González-Zamora, 1999).

Damage to plants is caused by the feeding action of both juvenile and adult spider mites. They pierce plant cells and suck out the contents, which leads to chlorosis of the plant tissue immediately around the feeding site. Spider mites are commonly found on

the abaxial leaf surface and the feeding damage appears on the adaxial surface as chlorotic patches of white and yellow. This damage may be assessed by using the leaf damage index. The index is composed of five categories of leaf damage severity and was formulated by the former Glasshouse Crops Research Institute, Littlehampton, UK (GCRI) (Hussey and Scopes, 1985).

Damaged leaves completely turn yellow as feeding levels increase and the leaves eventually die through a lack of photosynthetic area. Spider mites produce webbing which may also decrease the photosynthetic area of a leaf. Severe spider mite infestations may result in plant death.

2.3.1 Biological control of spider mites in glasshouses

Biological control in glasshouses started with the use of the predatory mite, *Phytoseiulus persimilis* to control spider mites in 1968 (Van Lenteren and Woets, 1988) and success in this field has been attributed to correct predator: prey ratios (Helle and Sabelis, 1985a). Successful biological control of spider mites has since been achieved on a number of horticultural crops (Helle and Sabelis, 1985a). For example, *P. persimilis* is widely used in combination with the acaricide, fenbutatin oxide, to control spider mites on protected tomatoes in the UK (Croft *et al.*, 1999).

Mass production of *P. persimilis* is widespread and other natural enemies of spider mites are found in a number of arthropod orders, e.g. Acarina, Araneida, Coleoptera (e.g. *Stethorus* sp.), Diptera [e.g. *Feltiella acarisuga* Vallot (Diptera: Cecidomyiidae)], Hemiptera, Neuroptera and Thysanoptera (Hussey and Huffaker, 1976).

2.4 Ladybirds

Ladybirds are in the family Coccinellidae, which belongs to the diverse order of Coleoptera or beetles. Approximately 350 000 species of beetle are known and over 5 200 of these are ladybirds (Majerus, 1994).

The lifecycles of nearly all Coccinellidae contain four larval stages. Juvenile development depends on, for example, temperature, food availability and prey species

(e.g. Putman, 1955; Hagen, 1962; Blackman, 1965; Miller and Paustian, 1992; Formusoh and Wilde, 1993; Ponsonby and Copland, 1997). Aphidophagous coccinellids are reported to take significantly less time to develop than coccophagous coccinellids (Dixon, 2000).

Some coccinellids are phytophagous (Drea and Gordon, 1990), but the majority predate on insects and mites (Hagen, 1962) and have been used in classical, augmentative and conservation programmes in biological control (e.g. Majerus, 1994). There is a vast amount of evidence to suggest that coccinellids are often a major cause of mortality in aphids, coccids and spider mites (Hodek, 1973). They also predate on whiteflies, mealybugs (DeBach and Rosen, 1991), psyllids and chrysomelid larvae (Drea and Gordon, 1990). Many species of coccinellid will feed on juvenile Lepidoptera in the absence of preferred food (Drea and Gordon, 1990). Species of the tribe Stethorini are exclusively mite predators (*section 2.4.1*). Reports suggest that ladybirds are cannibalistic, behaviour that would allow survival when prey are scarce (e.g. Majerus, 1994; Ponsonby, 1995; Ponsonby and Copland, 1997).

Ladybirds are costly to mass-produce because they require prey rather than synthetic foods to realise their full reproductive potential (Majerus, 1994). Hodek (1973) stated that a common problem when using coccinellids in biocontrol is providing them in sufficient numbers to control pests at the critical periods. The amount of plants needed to support adequate numbers of prey to rear large quantities of ladybirds is not cost effective in pest management compared to the cost of insecticides (Majerus, 1994). However, Lilley *et al.* (1999) stated that application costs of natural enemies may be reduced by exploiting the rate of effective dispersal of some predators and reducing the numbers that are released into a crop. Moreover, unlike the spider mite predator, *F. acarisuga*, for example, all mobile stages (i.e. larvae and adults) of coccinellids are able to attack pests and therefore have the ability to reduce pest populations (Hodek, 1973).

Insecticide use (e.g. Hoy and Smith, 1982) and the requirement for non-target insect foods (Hagen, 1987) are thought to be responsible for the lack of establishment of coccinellids in crops. Nevertheless, ladybirds have continued to play an important role in the development of biological control and many species of ladybirds have successfully been used in classical biological control programmes, e.g. the importation

of the vedalia ladybird to control the cottony-cushion scale in Californian citrus (e.g. DeBach and Rosen, 1991).

2.4.1 *Stethorus spp. including S. punctillum* Weise (Coleoptera: Coccinellidae)

All known members of the coccinellid tribe Stethorini are exclusive mite predators. Kapur (1948) and McMurtry *et al.* (1970) published reviews of the genus *Stethorus* (Coleoptera: Coccinellidae) including geographical distribution, prey host and host plant, and in particular described *S. punctillum* as an Old World species located in Europe and Asia.

In Britain, *S. punctillum* has been observed at a few localities in south east and central England (Majerus, 1994). It is said to have a local distribution in Surrey (Hawkins, 2000) and in relation to commercial fruit orchards in Essex, it was described as abundant in commercial orchards but rare in neglected ones (Collyer, 1953). The establishment of *S. punctillum* in North America, which was first reported in 1950 (Hodek, 1973; Majerus, 1994), is thought to have developed from an accidental introduction. Putman (1955) stated that *Stethorus punctum* (LeConte), the native species in Ontario was replaced by the Palaearctic species, *S. punctillum.*

Within the tribe Stethorini, several *Stethorus* species are considered to have worldwide importance wherever red spider mites occur (e.g. Collyer, 1953) although some plants that are susceptible to spider mite damage have been deemed unsuitable for *Stethorus* development. For example, the trichomes of wild raspberry leaves (Roy *et al.*, 1999) and French bean leaves, *Phaseolus vulgaris* L. (Putman, 1955) are thought to prevent the development of *S. punctillum* and in the latter case it was stated that the hooked trichomes tore the integuments of the beetles.

Stethorus spp. are regarded as important biological control agents of spider mites in agricultural crops and research in this area has mainly concentrated on fruit crops, for example, apple (e.g. Horsburg and Asquith, 1968; Injac and Dulič, 1992), strawberry (e.g. Waite, 1988; Raworth, 1990; García-Marí and González-Zamora, 1999), raspberry (e.g. Charles *et al.*, 1985; Raworth, 1989; Congdon *et al.*, 1993; Roy *et al.*, 1999) and peach (e.g. Putman and Hearne, 1966). The natural invasion of *Stethorus* sp. (e.g. Injac

and Dulič, 1992) and the impact of predator guilds, including *Stethorus* sp., on spider mites (e.g. García-Marí and González-Zamora, 1999) have been evaluated.

For example, Injac and Dulič (1992) reported that *S. punctillum* invaded apple orchards early in the growing season, from an unknown source, and together with other predators [e.g. *Orius minutus* L. (Heteroptera: Anthocoridae) and *Chrysopa carnea* Stephens (Neuroptera: Chrysopidae)] they were able to reduce the density of spider mites. Similar results were reported by García-Marí and González-Zamora (1999) in strawberry plantations in Spain where *S. punctillum* was one of the most common predators found. A guild of predators, including *S. punctillum*, was able to effectively control spider mite infestations and maintain them below damaging levels.

Information about *Stethorus* spp., which may be applied to the control of spider mites in glasshouses, is limited and includes; (i) evaluation of the relationship between temperature and development of *S. punctillum* (Raworth, 2001); (ii) its predatory behaviour in relation to temperature, humidity and host plant (Rott and Ponsonby 2000a); (iii) field trials reporting the establishment of the predator in glasshouse crops (Raworth, 2001); and (iv) evaluation of *S. punctillum* as part of a guild of spider mite predators (Rott and Ponsonby, 2000b).

For example, Raworth (2001) reported the development time and lower developmental temperature for *S. punctillum*. He presented his results as the rate of development of *S. punctillum* from egg hatch to adult versus temperature. However, the ages of some eggs at the start of the experiment were unknown and the duration of individual juvenile stages was not reported. In addition, larval voracity at three temperatures was recorded and no significant differences were found between the total numbers of spider mite eggs eaten by *S. punctillum* larvae and temperature. Raworth reported establishment of *S. punctillum* on pepper and cucumber but not tomato.

Rott and Ponsonby (2000ab) evaluated the predatory behaviour of *S. punctillum* larvae on *T. urticae* in relation to temperature (20, 25 and 30 ^{0}C), humidity (33, 65 and 90 % r.h.) and host plant (tomato, pepper, aubergine and cucumber). They found that the activity of *S. punctillum* significantly increased at higher temperatures, relative

humidity did not influence predatory behaviour and *S. punctillum* was most active on pepper and tomato and least active on aubergine.

Rott and Ponsonby (2000b) conducted semi-field trials on tomato and pepper. Various combinations of the spider mite predators, *P. persimilis*, *F. acarisuga*, *Amblyseius californicus* McGregor (Acari: Phytoseiidae) and *S. punctillum* were evaluated for their impact on spider mite pest populations. *S. punctillum* and *F. acarisuga* survived when prey was scarce and it was found that significantly more *P. persimilis* and *F. acarisuga* survived in the absence of *S. punctillum*. It was postulated that *S. punctillum* predated on the former two predators.

Stethorus spp. have often been regarded as high density predators (e.g. Bailey and Caon, 1986; Waite, 1988) with a population increase that does not match that of their prey (e.g. Putman, 1955; Bailey and Caon, 1986). In addition, Putman (1955) stated that adults and larvae of *S. punctillum* appeared to locate prey at random. However, reports that *Stethorus punctum picipes* Casey is able to locate rare prey patches early in the growing season of red raspberry when both the prey and predator populations are low (Congdon *et al.*, 1993) and that *S. punctum* was able to detect very low mite densities (Hull *et al.*, 1977) suggest that *Stethorus* sp. use a stimulus other than random search to locate their prey. Congdon *et al.* (1993) stated that the effectiveness of *Stethorus* spp. as biocontrol agents may depend primarily on behavioural mechanisms, such as area-restricted search or chemotaxis rather than a numerical response.

Stethorus spp. are attractive predators for use in the biocontrol of spider mites because they are obligate, voracious predators that are long-lived (Putman, 1955; Readshaw, 1971) and are highly mobile (e.g. Readshaw, 1971; Charles *et al.*, 1985). In particular, recent research on the ability of *S. punctillum*, as part of a predator guild, to control spider mites under glasshouse conditions has been encouraging (Rott and Ponsonby, 2000ab; Raworth, 2001).

3 GENERAL MATERIALS AND METHODS

3.1 Origin of *Tetranychus urticae* (spider mites) and *Stethorus punctillum* (beetles)

3.1.1 Spider mites

Initial spider mite cultures were inherited from stocks maintained at Canterbury Christ Church University College, Kent, UK. These were regularly supplemented with two-spotted spider mites that had been reared on bean plants (*Phaseolus vulgaris*[1]) and supplied by Biological Crop Protection (B.C.P.) Ltd., Wye, Kent, UK, on excised leaves.

3.1.2 Beetles

At the outset of this research a batch of approximately 50 *S. punctillum* adults was supplied by Applied Bio-Nomics Ltd, Sidney, B.C., Canada to supplement stocks already maintained at Canterbury Christ Church University College but that had originally been obtained from the same company.

3.2 Plant Cultures

Cucumber seeds (Thiram treated *Cucumis sativus* L. cv. Enigma RZ®) and two cultivars of bean seeds (Thiram treated *Vicia faba* L. cv. Aquadulce Claudia and *V. faba* cv. Maris Bead) were sown on a regular basis (refer to *Table 3.2.1* for seed suppliers).

Cucumber seeds were sown in peat pots (60 mm diameter by 60 mm height) impregnated with plant food. Peat pots containing plants were transferred into plastic pots (128 mm diameter by 111 mm depth) after the cotyledons had fully emerged and before the first true leaf had fully expanded.

Bean seeds were either sown in plastic plant pots as before (*V. faba* cv. Aquadulce Claudia) or sown in standard seed trays (230 mm width by 375 mm length by 55 mm depth) (*V. faba* cv. Maris Bead).

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[1] *P. vulgaris* is unsuitable for rearing *S. punctillum* because the hooked trichomes tear the integument of the beetle (Putman, 1955). Therefore mites had to be transferred from these leaves to more suitable plants for rearing purposes.

The seeds were sown in organic, peat-free multipurpose compost supplied by Sainsbury's Homebase or B&Q.

Sown seeds were reared to plant status in a ventilated greenhouse at 25-30 ^{0}C, 40-70 % r.h. and 16L: 8D.

Table 3.2.1 **Seed Suppliers**

Seed type	Supplier
Cucumber, *Cucumis sativus* cv. Enigma	**Rijk Zwaan Distribution B.V.** P.O. Box 40 NL-2678 ZG De Lier The Netherlands
Bean, *Vicia faba* cv. Aquadulce Claudia	**Tozer Seeds** Cobham Surrey. England KT11 3EH
Bean, *V. faba* cv. Maris Bead	**W. A. Church (Bures) Ltd** Bures Suffolk. England CO8 5JQ

3.3 Spider Mite Cultures

Clean (pest-free) plants were transferred from the plant-propagating greenhouse to a second greenhouse, under equivalent conditions, when they had reached a suitable growth stage (approximately three to five pairs of true leaves) to withstand healthy infestations of two-spotted spider mites.

3.4 Beetle Cultures

Initial maintenance of a stock culture of ladybirds involved placing excised bean leaves (*V. faba* cv. Aquadulce Claudia) infested with two-spotted spider mites into large Perspex boxes (*section 3.6.2*) lined with paper towels. The box lids were partly

replaced with polyester mesh (monofilament 52 cm $^{-1}$, 34 % open area) to overcome culturing problems associated with high humidity. The boxes were sealed with masking tape and maintained in a controlled temperature room (CT room) (*section 3.5.1*).

This method proved to be inefficient as the leaves were prone to drying out and the number of insects needed to complete experiments was not realised. However, this method was employed to culture ladybirds under specific temperatures in incubators (see below).

A larger stock culture was maintained on whole plants. Bean plants (*V. faba* cv. Maris Bead) infested with two-spotted spider mites were transferred to a four-tier grow house (1570 mm height by 700 mm width by 490 mm depth). Each shelf in the unit was supplied with overhead lighting from a daylight aquarium light (F20 W/154-RS) with a photoperiod regime of 16L: 8D. The whole unit was covered with a flexible PVC cover modified for ventilation with rectangles of polyester mesh (*section 3.4*) on all sides. The unit was held in the CT room and supplied with water twice a week by an automatic watering system.

For certain experiments it was necessary to rear *S. punctillum* at temperatures different to that provided by the CT room. In these cases, beetles were cultured in Perspex boxes with solid lids as described in *section 3.6.2*. The boxes were then transferred to incubators set at the required temperatures and photoperiods, with saturated salt solutions to maintain the relative humidity at the requisite levels (*Table 3.5.2.2*).

3.5 Experimental Conditions

3.5.1 Temperature control and measurement

A CT room was used for culturing beetles and in certain aspects of experimental research (refer to individual chapters for details). Air temperature in the CT room was maintained at 23 ± 2 ^{0}C and controlled with a Daikin® air conditioning unit. Polylux® XL or Sylvania® Studio 186 strip lights (F36W/860) were used for overhead lighting and provided a 16L: 8D photoperiod. Relative air humidity in the CT room was 50-60 %.

Sanyo® Gallenkamp cooled incubators were utilised in all laboratory-based experiments. L8W/20 Hellweiss® cool white tubes provided side lighting. Temperature was monitored using Gemini® miniature data loggers and for an immediate temperature check, alcohol thermometers were consulted.

3.5.2 Humidity control and measurement

Humidity within the experimental equipment was maintained at a constant level using saturated salt solutions as detailed in *Table 3.5.2.2.* Humidity was monitored using Gemini® miniature data loggers.

Table 3.5.2.2 **Constant percentage relative humidities (r.h.) maintained at various temperatures using saturated solutions of a range of salts (after Winston and Bates, 1960).**

Temperature (^{0}C)	Salt			
	Magnesium chloride	**Ammonium nitrate**	**Sodium chloride**	**Potassium chloride**
5	32.5	-	75	-
20	33	65.5	76	-
25	32.5	62.5	75.5	85
30	-	-	75.5	-
35	-	-	75.5	-

- Not used

3.6 Experimental Equipment

3.6.1 Plastic Petri dishes

Plastic Petri dishes (52 mm diameter by 20 mm depth) with lids partly made of polyester mesh (*section 3.4*) were used as development and oviposition arenas. A disc of plastic measuring 22 mm in diameter was removed from the lids and replaced with mesh. In certain experiments (refer to individual chapters for details) the dishes were lined with circles of filter paper (Whatman® grade 1; 42.5 mm diameter).

3.6.2 Perspex boxes

Perspex boxes (285 mm length by 162 mm width by 103 mm height) were used to contain cultures of *S. punctillum*, Petri dish arenas and glass vials. Lids were either completely made of Perspex or ventilated with mesh (*section 3.4*) and sealed using masking tape.

3.6.3 Controlled humidity units

Glass, controlled humidity units (CHUs) were used in experiments detailed in *Chapters 4* and *5*. The units were constructed from two glass jars (83 mm diameter by 75 mm depth), one inverted and placed on top of the other. Plastic lids that were screwed onto the mouths of the jars separated the two and these lids were held together with insulating tape. The lids had holes (3 mm diameter) in them and the holes in the top lid were covered with mesh (*section 3.4*) serving as a barrier between the arthropods and the saturated salt solution in the base jars. The saturated salt solution was used to maintain a constant relative humidity within the unit. The CHUs were sealed using sealing film (Nescofilm®) followed by masking tape.

3.6.4 Glass vials

Glass vials (33.5 mm depth by 22 mm diameter) were used to contain adult *S. punctillum*. The lids were either made from Nescofilm® or mesh (*section 3.4*) secured with elastic bands.

4 EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF *STETHORUS PUNCTILLUM.*

4.1 INTRODUCTION

The development time of an insect may be defined as the period from when an egg is laid to the time when the adult ecloses (Jervis and Copland, 1996). Insects are poikilotherms, thus their rates of development have been demonstrated to depend on environmental temperature changes (e.g. Miller and Paustian, 1992; Eraky and Nasser, 1993; Dreyer *et al.*, 1997).

Insects are either holometabolous (form pupae) or hemimetabolous (no pupal stage) in their development. This characteristic appears to influence the proportion of time they spend in each developmental stage of their lifecycle (Honěk and Kocourek, 1990). Ladybirds are holometabolous and Dixon (2000) stated that the proportion of time spent on development in each stage of a ladybird lifecycle does not vary with temperature and is referred to as rate isomorphy.

Physiological time is the amount of heat required over time for an insect to complete development or a particular stage of development and is generally adopted by entomologists to estimate parameters of insect development. The threshold temperature or developmental zero, *tl*, of a particular developmental stage of an insect and its rate of development in relation to temperature, are required to calculate physiological time. These parameters are obtained by reciprocating development times measured over a range of constant temperatures. The reciprocals are then plotted as development rate versus temperature (Dent, 1997). The relationship is generally considered to be non-linear following a sigmoidal curve with three distinct regions (*Figure 4.1.1*).

The lower region of the curve (A) levels out because at low temperatures insects can often survive long periods with little or no development. The *tl* of an insect is the temperature below which there is no (measurable) development (Jervis and Copland, 1996). This is determined by extrapolating the regression line back to the abscissa, where $tl = -a/b$ (a is the abscissa and b is the slope).

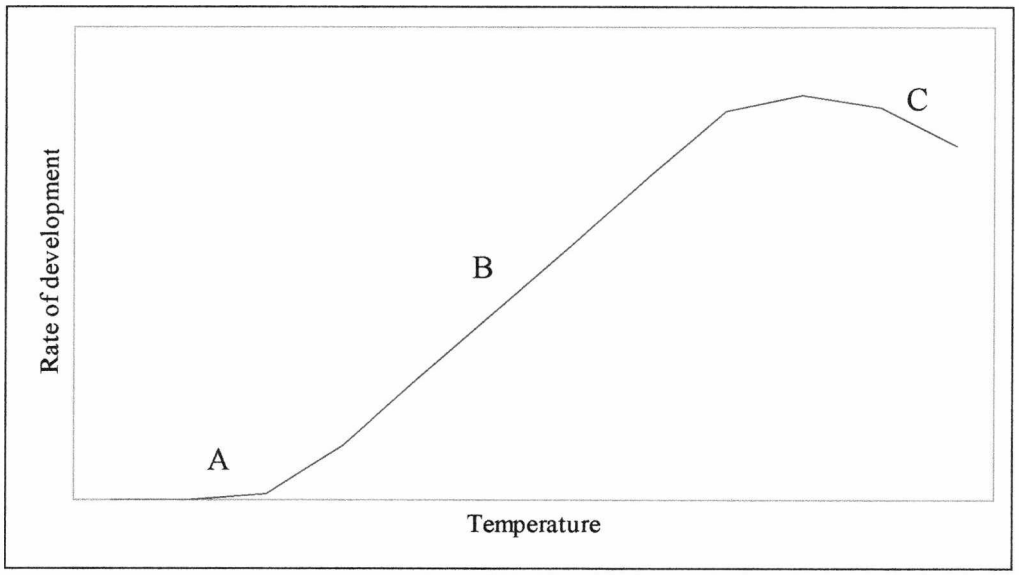


Figure 4.1.1 **Typical relationship between rate of development of a poikilothermic animal and temperature.**

Over the intermediate range of temperatures normally experienced by insects in the field, the rate of development increases linearly with temperature (Jervis and Copland, 1996) (B in *Figure 4.1.1*). These data may be expressed by the linear regression equation;

$$y = a + bT,$$

where y is the rate of development at temperature T, and a and b are constants (as before).

Development slows down after the intermediate range, up to an optimum temperature and above this, further increases in temperature result in only small increases in development rate. Development rates fall sharply with further increases in temperature and insect mortality rates are often high (C in *Figure 4.1.1*).

The thermal constant, K, is the amount of thermal energy required to complete development and is calculated from the reciprocal of the slope of the regression line; $1/b$. Summation procedures are used to determine the rate of development under any fluctuating temperature regime, once tl and K have been calculated from data for

constant temperatures. This is achieved by accumulating unit day- or hour-degrees above *tl* until the value of *K* is reached.

Several researchers have used regression analysis in this way for a number of different insect and mite species. For example, the pea aphid, *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae) (Siddiqui *et al.*, 1973); *Phytoseiulus persimilis* (Hamamura *et al.*, 1976); the mealybug parasitoids, *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae), *Leptomastix dactylopii* (Howard) (Hymenoptera: Encyrtidae) and *Leptomastidea abnormis* (Girault) (Hymenoptera: Encyrtidae) (Tingle and Copland, 1988) and the scale insect predator, *Chilocorus nigritus* (Fabricus) (Coleoptera: Coccinellidae) (Ponsonby and Copland, 1998).

Information about the effect that temperature has on development may be used when selecting biological agents for glasshouse introductions (Jervis and Copland, 1996) and this is especially important in the importation of insects (DeBach and Rosen, 1991). For example, the focus of Miller and Paustian's (1992) research was to investigate the development of the aphidophagous coccinellid, *Eriopis connexa* Mulsant (Coleoptera: Coccinellidae). In the early 1990s it was a recent import into the USA from South America as a possible control agent of the Russian wheat aphid (Reed and Pike, 1991). Mean development time from egg to adult at 14 and 34 ^{0}C was found to take 53.3 and 10.9 days, respectively. The authors compared these results with seven other species of aphidophagous coccinellids indigenous to North America and found that *E. connexa* developed at cooler temperatures compared to all the other species tested, except for *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae).

Previous research concerning the effect of temperature on the development of *Stethorus punctillum* has concentrated on a predator-prey complex typical in raspberry where the spider mite prey is *Tetranychus mcdanieli* McGregor (Acari: Tetranychidae) (Roy *et al.*, 2002; Roy *et al.*, 2003) and egg to adult development on strawberry (Raworth, 2001) where age of all the eggs was not established. The aim of the research presented here was to evaluate the development rate of *S. punctillum* at temperatures representative of those found in glasshouses, to determine the thermal constant (*K*) and the threshold temperature (*tl*) of the coccinellid and to establish to what extent development of this predator is synchronised with development of its host prey, *Tetranychus urticae*. In

order to do this, the rate of development of *S. punctillum* at a range of eight constant temperatures from 18 to 34 ^{0}C and cycling temperatures of 10/25 ^{0}C was determined (*experiment 1*). The resulting model was subsequently tested in simulated glasshouse conditions (*experiment 2*).

The aim of a further experiment was to provide detailed information about the development of *S. punctillum* at two temperatures in the linear range of development that could be used in further trials. In order to do this the development times of individual juvenile stages of *S. punctillum* at 21 and 26 ^{0}C were determined (*experiment 3*).

4.2 MATERIALS AND METHODS

4.2.1 Experiment 1: Effect of temperature on the development of *S. punctillum* from egg to adult at eight constant temperatures and one cycling temperature regime.

Adult *S. punctillum* of mixed ages were allowed to oviposit on excised bean leaves (*V. faba* cv. Aquadulce Claudia) infested with two-spotted spider mites (*T. urticae*) in Perspex boxes with ventilated lids (*section 3.6.2*). The boxes were sealed using masking tape and maintained in the CT room.

Leaves were checked for eggs on a daily basis. Eggs were counted and transferred to CHUs (*section 3.6.3*). These were maintained at a relative humidity in the range of 83 to 86 % (*section 3.5.2*). The units were then incubated at constant temperatures of 18, 20, 22, 24, 27, 30, 32 and 34 ^{0}C, and a cycling regime at 12 hours of 10/25 ^{0}C, with a 16L: 8D photoperiod. The CHUs were randomly moved between shelves in the incubators to overcome any temperature differences between different areas of the incubators.

Development times of eggs, larvae and pupae were established by checking individuals at 12-hourly intervals until adult eclosion. Developing larvae were provided with an excess of all spider mite stages to ensure satiation.

4.2.2 Experiment 2: Testing the model.

In order to the test the model used to interpret data collected from *experiment 1*, juvenile *S. punctillum* were reared from egg to adult under fluctuating temperatures (minimum 12.4 – maximum 29.2 ^{0}C) in an incubator. A data logger was placed in one of the rearing containers and the temperature was recorded every five minutes for the duration of development and used to estimate lifecycle duration using both the polynomial and accumulated heat methods.

Adult *S. punctillum* of mixed ages were allowed to oviposit on bean leaves infested with two-spotted spider mites in Perspex boxes. The sealed boxes were maintained in an incubator at a constant temperature of 24 ^{0}C, with a 16L: 8D photoperiod. The relative humidity within the boxes was maintained between 83 and 86 %. After 24 hours, all adult coccinellids were removed and the remaining leaves were checked for eggs. A total of 57 eggs were retrieved and divided as follows into four Perspex boxes with solid lids: 7, 8, 20 and 22 eggs[1].

The boxes were sealed and transferred to an incubator fluctuating between 12.4 and 29.2 ^{0}C with a 16L: 8D photoperiod. Relative humidity within the boxes was maintained between 83 and 86 %. A data logger as described in *section 3.5.1* was placed in one of the boxes to record the temperature every five minutes. The hourly mean was then calculated from these data for later use in calculating hour-degrees. The boxes were randomly transferred on a daily basis between two shelves in the incubator in an attempt to overcome possible temperature differences between different areas in the incubator.

The boxes were checked daily until all the eggs had hatched or it was clear that any remaining intact eggs were dead. Ten newly emerged larvae were transferred into each of five clean, Perspex boxes containing bean leaves infested with two-spotted spider mites (n = 50 coccinellid larvae). These boxes were checked daily until all adult coccinellids had reached eclosion. Developing *S. punctillum* larvae were provided with an excess of all spider mite stages to ensure satiation.

[1] The division of eggs was uneven to prevent excessive and unnecessary handling of freshly laid and thus delicate eggs.

4.2.3 Experiment 3: Effect of two constant temperatures on the development of the juvenile stages from egg to adult of *S. punctillum.*

Adult *S. punctillum* were held in CHUs in incubators at 21 and 26 ^{0}C, 75 - 76 % r.h. and with a 16L: 8D photoperiod. The beetles were allowed to oviposit on excised bean leaves infested with two-spotted spider mites for 24 hours. After 24 hours the adult coccinellids were removed and the remaining eggs were allowed to hatch. Eggs were checked every 12 hours and upon hatching, the first instar larvae were transferred singularly to vented Petri dishes (*section 3.6.1*). These were lined with filter paper and contained excised bean leaves infested with two-spotted spider mites. The Petri dishes were sealed using Nescofilm® and transferred to Perspex boxes with an interior humidity of 75 - 76 %. The boxes were sealed using masking tape and returned to the incubators at 21 ^{0}C (n = 11) and 26 ^{0}C (n = 12), as before.

Individuals were assessed every 12 hours to determine the duration of each juvenile stage and survivorship until adult eclosion. Individuals were supplied with an excess of all spider mite stages throughout development to ensure satiation.

4.3 STATISTICAL ANALYSES

4.3.1 Experiment 1

Data for development time of *S. punctillum* in relation to temperature were analysed using an analysis of variance (ANOVA, P<0.05). Differences between means were calculated using Fisher's test at the 5 % level. A QBASIC computer programme (Jervis and Copland, 1996) was used to convert the means to reciprocals and fit a polynomial regression line to the development data. The linear phase of the regression line was then used to extrapolate values for *tl* (the lower thermal threshold for development) and *K* (the thermal constant; the number of day-degrees needed for development).

The polynomial regression line was used to extrapolate the development time for *S. punctillum* at 25 ^{0}C. These data and the data for development at cycling temperatures of 10/25 ^{0}C were analysed in a QBASIC computer programme to determine the development of *S. punctillum* at a constant temperature of 10 ^{0}C.

Data for survivorship of *S. punctillum* at each temperature are presented as percentage survivorships. These were calculated by dividing the number of individuals that survived from egg to adult and dividing by the total number of individuals incubated and multiplying by 100.

Based on the mean development times for *S. punctillum* at constant temperatures of 18, 20, 22, 24, 27, 30, 32 and 34 ^{0}C, the proportions of time spent in each developmental stage were determined.

4.3.2 Experiment 2

Data for survivorship were calculated as described in *section 4.3.1*. The numbers of individuals to survive the egg period were calculated to provide percentage mortality data for the egg stage. The number of surviving individuals to complete development from egg to adult out of the original 50 eggs was determined to provide percentage mortality data for development from first instar larvae to adults.

The temperature data collected from the data logger, together with the results from *experiment 1*, were used in a QBASIC computer programme that predicted developmental period. The programme used both the polynomial method and the accumulated heat method and calculated the proportion of the total lifecycle completed in an hour at a given temperature. Hourly temperature data were examined and increments were summed until they reached unity (i.e. until development was complete).

4.3.3 Experiment 3

Transformation of the data did not improve their distribution, therefore, data for development of juvenile *S. punctillum* were analysed using non parametric analyses within treatments (Kruskal Wallis test, $P<0.05$) and across treatments (Mann Whitney *U*-test, $P<0.05$), adjusted for ties. However, for ease of interpretation data are presented as means followed by standard errors of the means.

Data for survivorship were calculated as described in *section 4.3.1* and are presented as cumulative percentage survivorships for each stage of development.

4.4 RESULTS

4.4.1 Experiment 1

Summary data for the effect of temperature on the development of *S. punctillum* are presented in *Table 4.4.1.1*. Analysis of variance indicated that temperature had a significant effect on the development of the predatory coccinellid. Mean development times for egg to first instar, egg to pupa and egg to adult, significantly decreased from 18 to 22 ^{0}C. There were no significant differences in development times between 22 and 24 ^{0}C. Further significant decreases in development times of egg to first instar and egg to adult were observed at 24 to 30 ^{0}C.

Polynomial regression analysis of the rate of development of *S. punctillum* from egg to adult eclosion showed that a third order polynomial curve, known to realistically fit the actual biological situation (Ponsonby, 1995), gave an r^2 value of 0.98 (*Figure 4.4.1.1*).

Temperatures in the linear range of the developmental curve were visually estimated as 18 to 32 ^{0}C, which gave an r^2 value of 0.98 (*Figure 4.4.1.2*). The regression line was used to calculate K which was evaluated as 279 day-degrees (≡ 6704.4 hour-degrees) and tl which was found to be 6.5 ^{0}C.

Percentage survivorships for each immature developmental stage and overall percentage mortalities at each temperature tested were calculated (*Table 4.4.1.2*). Percentage mortalities varied considerably across the constant temperatures used. The highest overall percentage mortality of 74 % was observed at 22 ^{0}C and the lowest overall percentage mortality of 0 % was observed at 32 ^{0}C.

For every constant temperature, except 32 ^{0}C and the cycling temperature regime of 10/25 ^{0}C, at least one individual died in the egg stage. Mortality occurred to a lesser extent in the larval stage. Survivorship of 100 % was observed in the pupal stage at all temperatures tested. These results indicated that in general the egg stage was the most vulnerable developmental stage and that the pupal stage was the least vulnerable.

Table 4.4.1.1 **Mean (SE) development time (days) of the immature stages of *Stethorus punctillum* when fed to satiation on two-spotted spider mites (*Tetranychus urticae*) infesting bean leaves (*Vicia faba* cv. Aquadulce Claudia) at eight constant temperatures and one cycling temperature regime at approximately 83 - 86 % r.h. with a 16L: 8D photoperiod.**

Stage of Development	Temperature (^{0}C)									*P*
	10[1]	18	20	22	24	27	30	32	34	10/25[2]	
Egg to first		6.57	5.93	4.45	4.45	3.09	3.58	2.82	2.88	6.58	**0.001**
		(0.13)a	(0.20)b	(0.09)c	(0.08)c	(0.16)d	(0.10)e	(0.08)d	(0.08)d	(0.26)	
n		7	7	10	11	17	13	11	8	12	
Egg to pupa		18.00	16.21	12.79	12.95	9.94	9.31	8.23	9.13	18.96	**0.001**
		(0.41)a	(0.33)b	(0.52)c	(0.20)c	(0.38)d	(0.24)d	(0.16)e	(0.13)de	(0.41)	
n		6	7	7	10	8	13	11	4	12	
Egg to adult	67.91	23.83	21.14	17.00	16.95	13.50	12.12	10.73	11.63	25.08	**0.001**
		(0.36)a	(0.43)b	(0.59)c	(0.23)c	(0.30)d	(0.20)e	(0.16)f	(0.13)ef	(0.36)	
n		6	7	7	10	8	13	11	4	12	

Means followed by the same letter in rows, for temperatures 18 to 34 ^{0}C, are not significantly different (ANOVA, $P<0.05$).

[1] Prediction of development using, extrapolated development data for 25 ^{0}C from the polynomial regression analysis and cycling data at 10/25 ^{0}C, in a QBASIC computer programme.

[2] Fluctuating temperature regime at 12 hours.

Table 4.4.1.2 **Cumulative percentage survivorships at each immature developmental stage and overall percentage mortality of *Stethorus punctillum* when fed to satiation on two-spotted spider mites (*Tetranychus urticae*) infesting bean leaves (*Vicia faba* cv. Aquadulce Claudia) at eight constant temperatures and one cycling temperature regime at 12 h, approximately 83 - 86 % r.h. and with a 16L: 8D photoperiod.**

Temperature	*n*	Cumulative Percentage Survivorship (number surviving)			% Mortality
		Egg	Larvae	Pupae	
18	9	78(7)	67(6)	67(6)	33
20	20	35(7)	35(7)	35(7)	65
22	27	37(10)	26(7)	26(7)	74
24	13	85(11)	77(10)	77(10)	23
27	29	59(17)	28(8)	28(8)	72
30	14	93(13)	93(13)	93(13)	7
32	11	100(11)	100(11)	100(11)	0
34	13	62(8)	31(4)	31(4)	69
10/25	16	75(12)	75(12)	75(12)	25

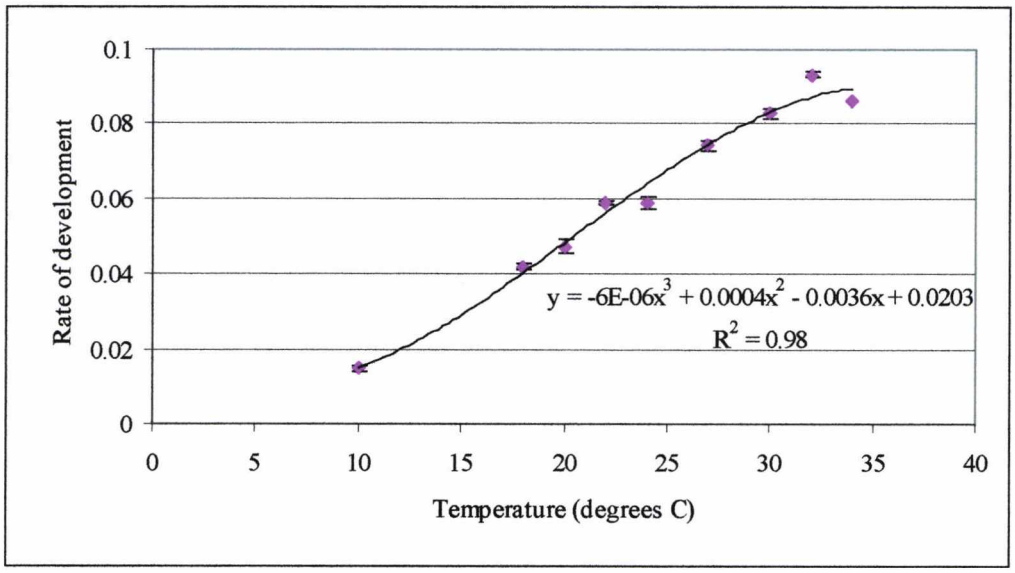


Figure 4.4.1.1 **Rate of development (1/mean development time) of *Stethorus punctillum* from egg to adult when fed to satiation on two-spotted spider mites (*Tetranychus urticae*) infesting bean leaves (*Vicia faba* cv. Aquadulce Claudia) at eight constant temperatures, 83-86 % r.h. with a 16L: 8D photoperiod.**

Rate of development at 10 ^{0}C extrapolated using data from cycling temperatures of 10/25 ^{0}C.

Vertical bars represent ± SE.

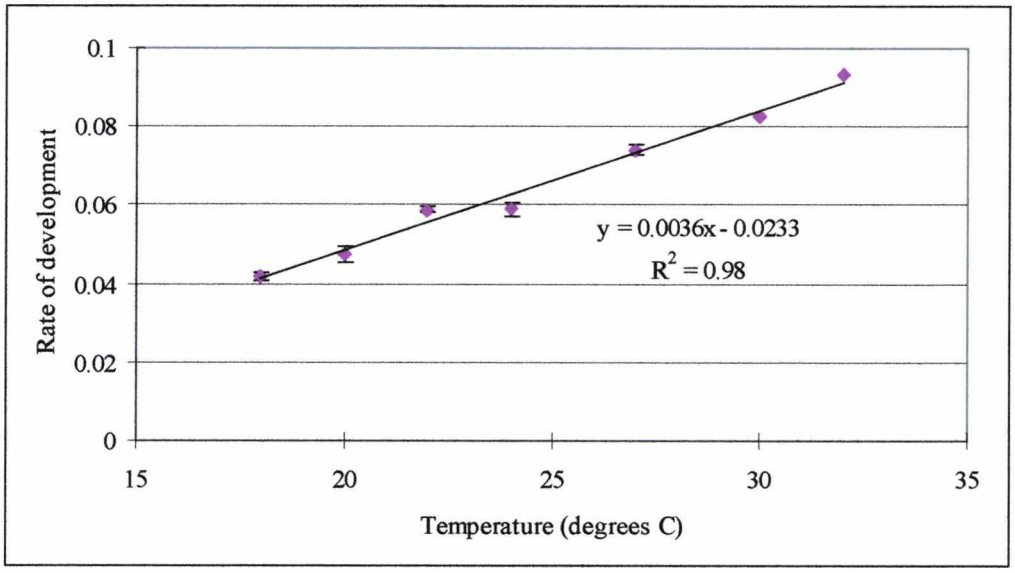


Figure 4.4.1.2 **Linear regression analysis for the rate of development (1/mean development time) of *Stethorus punctillum* from egg to adult when fed to satiation on two-spotted spider mites (*Tetranychus urticae*) infesting bean leaves (*Vicia faba* cv. Aquadulce Claudia).**

Vertical bars represent ± SE.

The proportions of time spent in each developmental stage of *S. punctillum* did not alter greatly with temperature and the data for development at 24 ^{0}C are displayed in *Figure 4.4.1.3*. The maximum amount of time spent in the egg stage, as a proportion of the whole lifecycle, was observed for predators reared at 27 ^{0}C (26 %) and the minimum was observed at 34 ^{0}C (21 %). The proportions of time spent in the larval stages ranged between 23 % (27 ^{0}C) and 30 % (30 ^{0}C). A similar range of proportions of time were observed in the pupal stage; 47 % (30 ^{0}C) to 54 % (34 ^{0}C).

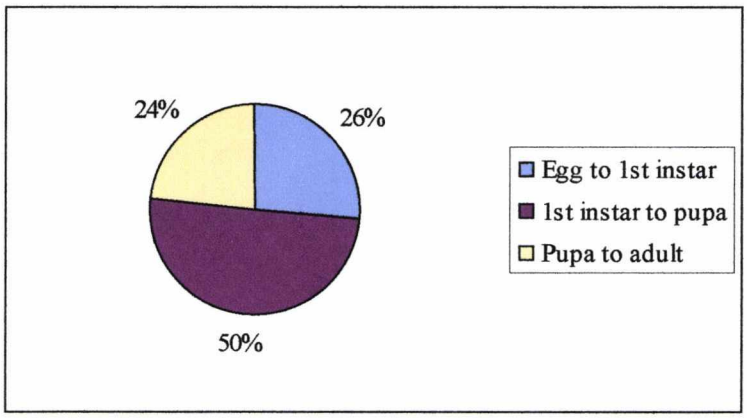


Figure 4.4.1.3 **Proportions of time spent in each stage of development of *Stethorus punctillum* based on mean development times of the predator when fed to satiation on two-spotted spider mites (*Tetranychus urticae*) infesting bean leaves (*Vicia faba* cv. Aquadulce Claudia) at 24 ^{0}C, 83 - 86 % r.h. and with a 16L: 8D photoperiod.**

4.4.2 Experiment 2

Mean development time (days) and percentage survivorships for *S. punctillum* reared at fluctuating temperatures between 12.4 and 29.2 ^{0}C, with a 16L: 8D photoperiod and fed to satiation on two-spotted spider mites infesting bean leaves are displayed in *Table 4.4.2.1*.

Percentages of individuals surviving the egg period and those surviving the period from first instar to adult eclosion, were 89 % and 86 %, respectively. These results are comparable with percentage survivorships recorded for individuals reared at 21 ^{0}C in *experiment 3*, below.

Mean (± SE) development time for *S. punctillum* under these conditions was 14.65 (± 2.23) days. The QBASIC programme described in *section 4.3.1* predicted the duration

of the lifecycle to be 15.61 days using the polynomial method and 15.70 days using the accumulated heat method (*Table 4.4.2.2*).

Table 4.4.2.2 **Mean development time (days) and percentage survivorships (*n* = number surviving) of *Stethorus punctillum* from egg to first instar (*n* = 57) and from first instar to adult (*n* = 50).**

Method 1:	beetles were incubated at fluctuating temperatures between 12.4 and 29.2 ^{0}C and a relative humidity of 83 – 86 %, with a 16L: 8D photoperiod.
Method 2:	prediction of the lifecycle duration using the Polynomial Method[1].
Method 3:	prediction of the lifecycle duration using the Accumulated Heat Method/Thermal Summation[1].

Method	Mean development time (days)	% Survivorship (*n*)	
		Egg to first instar	First instar to adult
1	14.65	89(51)	86(43)
2	15.61		
3	15.70		

[1] A QBASIC computer programme (Jervis and Copland, 1996) was used to calculate these predictions.
Standard error of the mean in method 1 = 2.23 days.
Beetles were fed to satiation on two-spotted spider mites (*Tetranychus urticae*) infesting bean leaves (*Vicia faba*).

4.4.3 Experiment 3

The results of this experiment are displayed in *Table 4.4.3.1*. Development time was significantly affected by temperature, apart from the length of the third instar. Incubation at 26 ^{0}C significantly reduced the development time of *S. punctillum* compared with individuals incubated at 21 ^{0}C.

The proportions of time spent in each stage of development of *S. punctillum* were comparable at 21 and 26 ^{0}C. Data for individuals reared at 21 ^{0}C are displayed in *Figure 4.4.3.1*.

Overall percentage survivorships at the two temperatures were high, with 82 % of the total number of individuals in the experiment surviving to adulthood at 21 ^{0}C and 67 % surviving to adulthood at 26 ^{0}C. In contrast to *experiment 1*, there was no mortality in the egg stage. At 21 ^{0}C, two individuals died as first instar larvae and at 26 ^{0}C, mortality was observed in the third (25 % mortality) and fourth instars (11 % mortality) only.

Table 4.4.3.1 **Mean (SE) development time (days) of each juvenile stage of *Stethorus punctillum* and cumulative percentage survivorships (CPS) at 21 and 26 ^{0}C, 75 - 76 % r.h. with a 16L: 8D photoperiod when fed to satiation on two-spotted spider mites (*Tetranychus urticae*) infesting bean leaves (*Vicia faba* cv. Aquadulce Claudia).**

Juvenile stage	**Mean (SE) development time (days)**				***P***
	21 ^{0}C		**26 ^{0}C**		
		CPS		**CPS**	
Egg	4.9 (0.1)a A	100	3.4 (0.1)b A	100	**0.018**
n	11		12		
First instar	2.4 (0.2)a B	82	1.8 (0.1)b B	100	**0.002**
n	9		12		
Second instar	1.7 (0.1)a C	82	1.1 (0.1)b C	100	**0.001**
n	9		12		
Third instar	1.6 (0.1)a C	82	1.6 (0.1)a D	75	**0.777**
n	9		9		
Fourth instar	3.5 (0.1)a D	82	2.6 (0.1)b B	67	**0.002**
n	9		8		
Pupa	4.5 (0.1)a E	82	3.2 (0.1)b A	67	**0.001**
n	9		8		
Egg to adult	18.4 (0.3)a	82	13.8 (0.2)b	67	**0.001**
n	9		8		
P	**0.001**		**0.001**		

Means followed in rows by the same lower-case letters and in columns by the same upper-case letters are not significantly different (Kruskal Wallis test or Mann Whitney *U*-test, $P<0.05$).

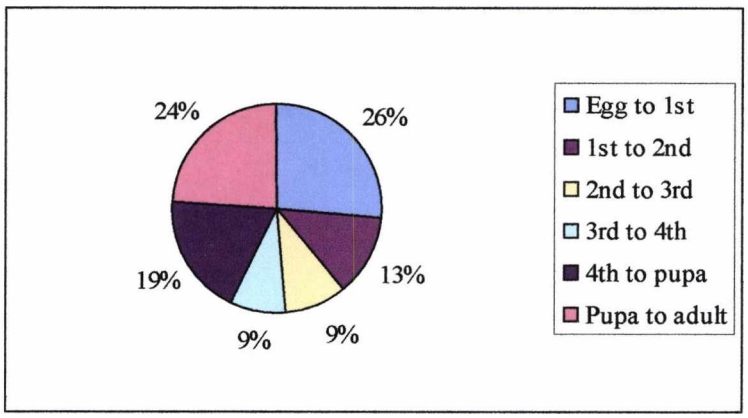


Figure 4.4.3.1 **Proportions of time spent in each stage of development of *Stethorus punctillum* based on mean development times of the predator when fed to satiation on two-spotted spider mites (*Tetranychus urticae*) infesting bean leaves (*Vicia faba* cv. Aquadulce Claudia) at 21 ^{0}C, 83 - 86 % r.h. and with a 16L: 8D photoperiod.**

4.5 DISCUSSION

4.5.1 Experiments 1 and 2

S. punctillum completed development at all eight constant temperatures and one cycling temperature regime tested (*experiment 1*). Putman (1955) stated that the mean development time from egg to adult of *S. punctillum* reared at 21.1 ^{0}C and approximately 79 % r.h., was 21.12 days. Although the experiments presented in *experiment 1* were not carried out at this temperature or humidity, those data for mean (± SE) development times at 20 ^{0}C (21.14 ± 0.43 days) and 22 ^{0}C (17.00 ± 0.59 days) are in close agreement with Putman's findings.

Mean development times of *S. punctillum* at each temperature had low standard errors and fitted closely to the polynomial regression curve. Polynomial regression analysis indicated that 98 % of the variation within the data for rate of development of *S. punctillum* may be explained by temperature. The rate of development for *S. punctillum* follows a shallow sigmoid velocity curve, as with other insects (e.g. Hamamura *et al.*, 1976; Ponsonby and Copland, 1996).

The linear range of the developmental curve for *S. punctillum* extended from 18 to 32 ^{0}C and is longer compared to linear ranges of other temperate species which are expected to be in the region of 20 to 24 ^{0}C (Copland M. J. W., personal

communication). The said range for *S. punctillum* is longer compared with that for the scale insect predator, *C. nigritus* (22 to 28 ^{0}C) (Ponsonby and Copland, 1996) and those for the insect parasitoids, *A. pseudococci*, *Leptomastix dactylopii* and *Leptomastidea abnormis* (20 to 26 ^{0}C) (Tingle and Copland, 1988).

The lower threshold for development is defined as the temperature at which no development occurs (Jervis and Copland, 1996). Development would be initiated with a rise above this temperature or halted with a fall below it. The *tl* for *S. punctillum* was estimated as 6.5 ^{0}C by extrapolation from the linear section of the temperature versus rate of development relationship. Raworth (2001) reported higher thresholds of 10.7 ± 0.95 ^{0}C for female *S. punctillum* and 12.7 ± 0.45 ^{0}C for males. The discrepancy between the two data sets may be explained by differences between geographical races of *S. punctillum*, the photoperiods used (16L: 8D used here and 15L: 9D used by Raworth), humidity (83 – 86 % r.h. used here and not indicated by Raworth) and/or the host plant used (bean used here and strawberry used by Raworth).

Siddiqui *et al.* (1973) stated that the lower threshold for development may be regarded as purely theoretical due to the curvature of the temperature velocity line at lower temperatures. As this parameter has no biological validity (Tingle and Copland, 1988), a regime of cycling temperatures was used in the present research to provide further information pertaining to the *tl*.

Polynomial (15.61 days) and thermal summation (15.70 days) techniques slightly over-predicted the development time of *S. punctillum* from egg to adult (14.65 days). However, the differences were within the standard error of two days. Over-prediction by these methods was probably due to a lack of data for development below 18 ^{0}C and above 34 ^{0}C in *experiment 1*. Over-prediction of development time by the polynomial method was also reported by Ponsonby and Copland (1996) for *C. nigritus* and by R. Priestly (personal communication) for *Feltiella acarisuga*.

The importance of day-degree modelling is highlighted by research such as that of Hanula *et al.* (2002). They used the model to decide timings of pesticide applications against a coneworm, *Dioryctria amatella* (Hulst) (Lepidoptera: Pyralidae) in loblolly pine seed orchards. Sprays were applied when the model predicted a 50 % hatch of the

spring generation of pest eggs. Predictions such as this are important in formulating pest management programmes with a reduced number of insecticide applications (Hanula *et al.*, 2002).

Tingle and Copland (1988) proposed that it is possible to determine the threshold for development from experimental data under cycling regimes, using thermal summation on an hourly basis. For *S. punctillum*, the developmental period for egg to adult under the cycling regime of 12 hours at 25 ^{0}C/12 hours at 10 ^{0}C was 25.08 days, which is shorter than twice that at a constant 25 ^{0}C (30.76 days), thus this indicates that the *tl* is below 10 ^{0}C. This is in agreement with the extrapolated *tl*.

In this study, the mean of the temperatures cycled is 17.5 ^{0}C and mean development at a constant 18 ^{0}C was 23.83 days, which is comparable with development data for the cycled temperatures. In order to establish the threshold more accurately, experiments using 12 hours at 25 ^{0}C and progressively lower temperatures would be required.

Despite the limitations imposed on extrapolated *tl* values, they are a useful tool for comparison amongst animals. For example, the *tl* for *P. persimilis*, a commercially available and widely used predator of *T. urticae* in glasshouses, is 11.6 ^{0}C (Hamamura *et al.*, 1976). The results presented here indicate that *S. punctillum* is more cool-tolerant than *P. persimilis*.

The *K* for *S. punctillum* was estimated as 279 day-degrees. *P. persimilis*, for example, has a thermal constant of 65.79 day-degrees, thus these results show that *S. punctillum* requires more thermal energy than the predatory mite to complete development. This requirement means that *S. punctillum* develops over a longer period than *P. persimilis*. The more rapidly an insect can complete development to a reproducing adult, the more rapidly it will increase in numbers in relation to its host and to its competitors. However, other factors must be considered. For example, it is possible that *S. punctillum* larvae may eat more prey in their lifetime than a juvenile predatory mite. Experiments to determine prey consumption by *S. punctillum* and *P. persimilis* are indicated.

In this study it was shown that *S. punctillum* spent approximately the same proportion of its total lifecycle in each developmental stage irrespective of temperature and this is known as rate isomorphy (Dixon, 2000). Similar results were reported by Ponsonby (1995) for the scale insect predator, *C. nigritus*.

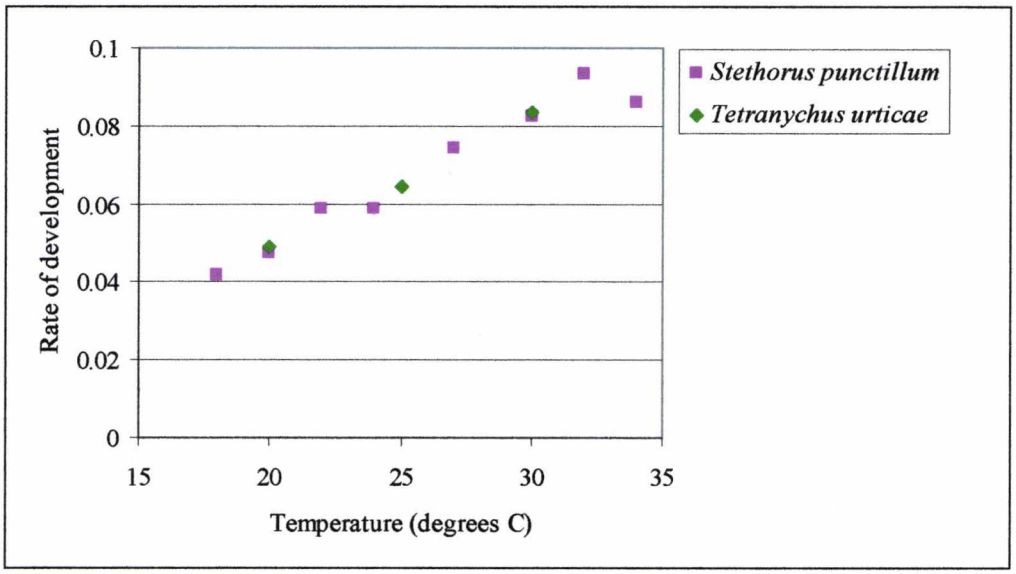


Figure 4.5.1.1 **Comparison between the rate of development (days) of *Stethorus punctillum* from egg to adult when fed to satiation on two–spotted spider mites (*Tetranychus urticae*) infesting bean leaves (*Vicia faba* cv. Aquadulce Claudia) and the rate of development of *T. urticae* from egg to adult maintained on kidney bean plants (after Rao *et al.*, 1996).**

According to Stinner (1977), one of four criteria for selecting entomophagous species for biological control is the general adaptive features of the natural enemy, including its synchronies and adaptations relative to the environment and its host or prey. Putman (1955) superimposed his own data regarding days to maturity relating to temperature for *S. punctillum* onto those of Cagle (1946) who studied the European red mite. He concluded that higher temperatures accelerate the development of the mite more than that of *S. punctillum* so that the efficiency of the predator decreases as the temperature increases. Indeed Roy *et al.* (1999) proposed that predator-prey asynchrony in relation to differing spring temperature thresholds was a possible explanation for the inability of *S. punctillum* to regulate mite populations in raspberry.

However, based on the results presented here and those of Rao *et al.* (1996), it appears that the rate of development of *S. punctillum* from 20 – 30 ^{0}C closely matches that of its prey, *T. urticae* (*Figure 4.5.1.1*).

ElLaithy (1996) studied integrated control of *T. urticae* on cucumbers grown under plastichouse conditions in Egypt. It was stated that humidities of no more than 30 % and temperatures of no less than 30 ^{0}C, favoured population build-up of *T. urticae*. *S. punctillum* may sufficiently control the spider mite pest at the beginning of the crop growing season, if introduced into the glasshouse early enough. However, it is likely that as temperatures increase, the rapidly reproducing spider mite may escape control by the predator. In addition, *S. punctillum* was shown to develop at a decreased rate at temperatures above 32 ^{0}C in my study.

However, it must be remembered that both predators and prey would not be subjected to a constant temperature of 32 ^{0}C under glasshouse conditions but as part of a heavily fluctuating temperature regime. It is also possible that if this problem occurred it may be overcome by additional releases of the adult predatory coccinellid.

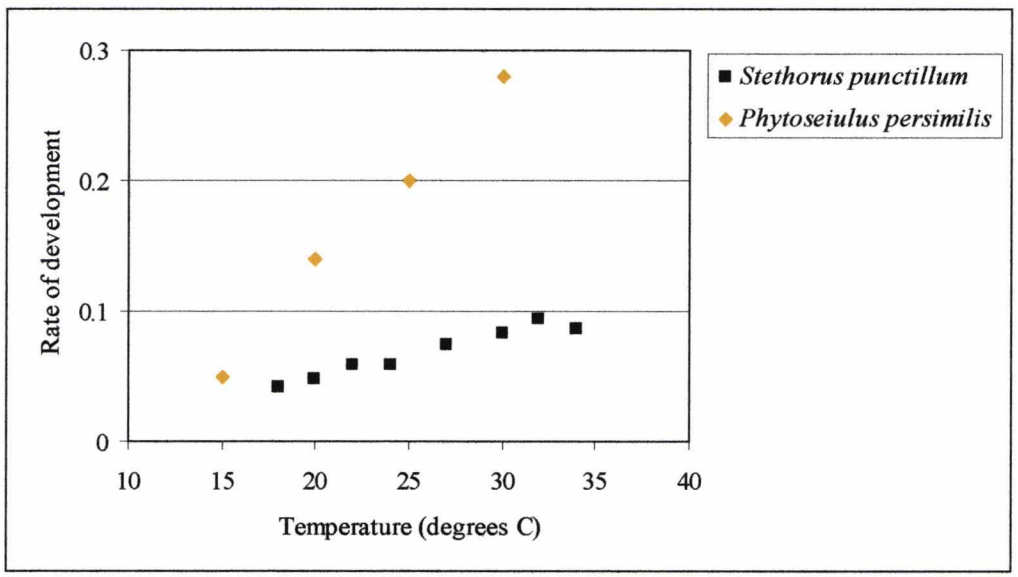


Figure 4.5.1.2 **Comparison between the rate of development (days) of *Stethorus punctillum* from egg to adult when fed to satiation on *Tetranychus urticae* infesting bean leaves (*Vicia faba* cv. Aquadulce Claudia) and that of *Phytoseiulus persimilis* from egg to adult maintained on *Tetranychus kanzawai* infesting bean leaves (after Hamamura *et al.*, 1976).**

In comparison to *P. persimilis*, the data presented here suggest that *S. punctillum* has a much slower rate of development (*Figure 4.5.1.2*). The data displayed for *P. persimilis* is based on results published by Hamamura *et al.* (1976) who used *Tetranychus kanzawai* Kishida (Acari: Tetranychidae) as prey. They reported that the shortest mean period of development for the predatory mite from egg to adult was 3.52 ± 0.28 days at

30 ^{0}C. At 32.5 ^{0}C development took on average 3.59 ± 0.18 days. Based on the results presented here, at 30 ^{0}C, *S. punctillum* requires an average (± SE) of 12.12 ± 0.20 days to complete development from egg to adult. In order to understand the full implications of these results, it is also necessary to take into account predation rates, as mentioned previously, and mortality rates for both spider mite predators.

Hamamura *et al.* (1976) reported that at 35 ^{0}C, 30 % of *P. persimilis* eggs hatched but all died at the larval stage. In comparison, although the percentage mortality at 34 ^{0}C was 69 % for *S. punctillum*, 62 % of all eggs hatched hence 50 % of the ensuing larvae *did* complete development. These results indicate that the predatory coccinellid is more tolerant of higher temperatures than *P. persimilis*. Rott and Ponsonby (2000a) reported increased activity of *S. punctillum* in the range 25 - 30 ^{0}C and combined with the tolerance of higher temperatures reported here, the coccinellid appears adept at withstanding the higher temperatures experienced in a glasshouse.

Nihoul (1992) investigated the effect of temperature (and r.h.) on the control of *T. urticae* by *P. persimilis* in glasshouse tomatoes. The results for the widely used predatory mite were surprisingly unsatisfactory. On one variety grown in soil, predator: prey ratios were high in the spring, but control of the pest could not be achieved in the summer and the ratio declined considerably making it necessary to use acaricides. Control was satisfactory on a second variety grown with and without soil and on the first variety grown without soil. Repetitively high temperatures (above 30 ^{0}C) (and low relative humidity) were suggested as a possible reason for a decrease in predator: prey ratios.

4.5.2 Experiment 3

In *experiment 3*, mean development times of each juvenile stage of *S. punctillum* at 21 ^{0}C closely relate to Putman's (1955) findings. It was found here that on average significantly more time was spent in the fourth instar stage compared to the other instars. Similarly, Putman reported that on average at least 1.06 more days were spent in the fourth instar compared with the other three instars. Considering all the immature stages, both Putman's findings and those presented here agree that the egg stage took on average the longest time to complete, with the pupa stage coming a close second.

Clausen (1940) reported that it is usual for coccinellids to spend more time in the first and the fourth instars compared to instars two and three.

The overall mean (SE) development time from egg to pupa at 21 ^{0}C and 75 - 76 % r.h. was 18.4 (0.3) days in the experiments presented here. This is slightly shorter in comparison to Putman's (1955) findings of 21.12 ± 0.12 days at 21 ^{0}C and 79 % r.h.. Slight discrepancies between data sets such as these are possibly explained by different biotypes of the coccinellid, differences in experimental conditions (e.g. microclimate) and/or equipment used.

In conclusion, the closely related rates of development of *S. punctillum* and its prey, *T. urticae* over the temperature range of 20 to 30 ^{0}C, render *S. punctillum* a promising predator of the pest. Whilst *P. persimilis* has an elevated rate of development compared to the predatory coccinellid, the latter appears more tolerant of temperature extremes. The data presented here indicate that *S. punctillum* is able to successfully develop over a wide range of constant temperatures when fed to satiation on two-spotted spider mites infesting bean leaves. It appears suited to glasshouse temperatures, which are expected to be in excess of 20 ^{0}C in a typical UK summer.

5 EFFECT OF TEMPERATURE ON THE FECUNDITY AND ADULT LONGEVITY OF STETHORUS PUNCTILLUM

5.1 INTRODUCTION

Information pertaining to the fecundity and adult longevity of insects is used to assess their performance as biocontrol agents. It is also pivotal to successful mass production techniques. Quality standards based on such traits have been set by the International Organisation for Biological Control (IOBC). Mass producers are required to conform to these guidelines to ensure that the natural enemies they provide are of a satisfactory quality (McEwen, 1997).

Information on age-specific fecundity and survival may be used to evaluate the intrinsic rate of increase of an insect population, which can subsequently be applied in ecological and agricultural entomology (Dent, 1997). The discovery of how ecological factors effect insect populations is important for formulating successful pest management programmes.

Fecundity refers to an insect's reproductive output in terms of the total number of eggs produced or laid during the lifetime of the female and is regarded as a measure of individual fitness in insects (Jervis and Copland, 1996).

Dent (1997) stated that temperature is the main abiotic factor influencing fecundity in insects and their rate of reproduction is dependent on temperature usually up to a critical maximum (Dent, 1995). For example, lower temperatures caused a decrease in the daily number of eggs laid per female cotton whitefly, *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) (Enkegaard, 1993) and in general, fecundity of the pea aphid, *Acyrthosiphon pisum* was said to decrease with an increase in constant temperature (Siddiqui *et al.*, 1973). In contrast, Ponsonby and Copland (1998) reported no significant effect of temperatures between 20 – 30 ^{0}C on either oviposition rate or total fecundity in *Chilocorus nigritus*.

Insects tend to display a triangular fecundity function, the peak of which is observed early in the adult life (Dixon, 2000) and this has been shown for *Coccinella*

transversalis Fabricius (Coleoptera: Coccinellidae) and *Menochilus sexmaculatus* Fabricius (Coleoptera: Coccinellidae) (Dixon and Agarwala, 2002) for example. The numbers of eggs produced per day by *C. transversalis* and *M. sexmaculatus* reached peaks early in the lives of the coccinellids. Temperature has been shown to affect the timing of the peak in the number of eggs laid per day for other insects, e.g. with an increase in temperature a peak in egg production by *B. tabaci* was observed earlier in its lifetime (Enkegaard, 1993) and with alternating temperatures this was also true for *A. pisum* (Siddiqui *et al.*, 1973).

Mating is another factor which effects fecundity and it is necessary for ladybirds to mate frequently to guarantee egg production (Copland M. J. W., personal communication). Drea and Gordon (1990) stated that a gravid female coccinellid may oviposit several hundred eggs if repeated matings are made. This is of particular consideration when rearing *Stethorus punctillum* as the females do not possess a spermatheca (Kovář, 1996).

The longevity of an insect is the period from when an egg hatches until adult death and this may be considered in two phases; (1) from egg hatch until adult eclosion and (2) the duration of the adult life (Jervis and Copland, 1996). Either phase may be extended via diapause, which is discussed in relation to *S. punctillum* in *Chapter 7*. It is the second phase, adult longevity, which will be considered in this chapter.

As with fecundity, longevity may also be used as an indicator of fitness in insects and adult longevity is considered such that; (a) the longer a male can live, the more females he can inseminate and therefore the more eggs he can fertilise and (b) the longer a female can live the more eggs she may lay (Jervis and Copland, 1996). In coccinellids, longevity is said to depend on climate, time of year and availability of hosts (Drea and Gordon, 1990). The life span of coccinellids is variable in nature and lasts for approximately a year (Honěk, 1996). However, a second hibernation has been recorded for some coccinellids, including *S. punctillum* (Putman, 1955).

As with insect development (*Chapter 4*) and fecundity, evidence suggests that temperature is a key abiotic factor affecting adult longevity and in general there is an

optimum range where longevity decreases with an increase in temperature (e.g. Tingle and Copland, 1989). For example, Enkegaard (1993) reported mean adult female longevities of 35.2 and 50.8 days for *B. tabaci* reared at 16 ^{0}C on tobacco and poinsettia, respectively, compared with 18 and 16 days when reared at 28 ^{0}C. In contrast, Ponsonby (1995) stated that the adult lifespan of *C. nigritus* was not significantly affected by temperature, although there was a trend for a decrease in longevity at constant temperatures approaching the upper and lower thresholds for development. Cycling temperatures further increased the adult lifespan.

The adult life of a female insect may be divided into three phases; (1) the pre-oviposition period: the period from adult eclosion until the day when egg production commences; (2) the oviposition period: the length of time over which eggs are produced; and (3) the post-oviposition period: the period of time from the cessation of egg production until death or hibernation. Honěk (1996) stated that the teneral pre-oviposition period decreases with temperature and this has been recorded for a number of coccinellids, e.g. *Hyperaspis notata* Mulsant (Coleoptera: Coccinellidae) (Dreyer *et al.*, 1997) and *C. nigritus* (Ponsonby and Copland, 1998).

Research on the total fecundity, adult longevity (Putman, 1955) and life history of *S. punctillum* under natural conditions (Collyer, 1953; Putman, 1955) have been reported. Age-specific fecundity of *S. punctillum* when fed on *Tetranychus mcdanieli* infesting raspberry leaves has been determined (Roy *et al.*, 2003). However, information relating to the oviposition rate of *S. punctillum* that may be used to assess the effectiveness of *S. punctillum* as a biological control agent of spider mites in glasshouses is limited. Therefore, the aim of this research was to examine the fecundity and longevity of *S. punctillum* when fed to satiation on *T. urticae* infesting bean leaves at temperatures typical to UK glasshouses.

5.2 MATERIALS AND METHODS

Juvenile *S. punctillum* were reared on whole bean plants (*Vicia faba* cv. Maris Bead) infested with two-spotted spider mites (*T. urticae*) in a CT room (*section 3.5.1*). Those leaves holding pupae were excised and transferred to a Perspex box (*section 3.6.2*) with a ventilated lid and returned to the CT room under equivalent conditions. Pupae were checked on a daily basis and newly eclosed adults were sexed and placed in male and female pairs in CHUs (*section 3.6.3*). Saturated salt solutions were placed in the base containers to maintain a relative humidity of 85 % (*section 3.5.2*). Excised bean leaves (*V. faba* cv. Aquadulce Claudia) infested with *T. urticae* were placed in the top containers, providing the adult coccinellids with an excess of all spider mite developmental stages. Five pairs of adults were transferred to each of two incubators at 20 and 26 ^{0}C with a 16L: 8D photoperiod.

Leaves were checked on a daily basis for the number of beetle eggs laid. The eggs were counted and removed and the spider mites were replenished as necessary to ensure that the beetles were fed to satiation. Daily egg lay was observed for 85 days.

5.3 STATISTICAL ANALYSES

Pre-oviposition data were analysed using a Mann Whitney *U*-test ($P<0.05$), adjusted for ties. However, for ease of interpretation means followed by standard errors of the means are presented in the table of results (*Table 5.4.1*).

Where it was not possible to count eggs every day, the numbers of eggs collected over 2-3 days were converted to means for those days prior to statistical analysis of oviposition rate (eggs day $^{-1}$). The mean daily oviposition rate for each day was calculated by dividing the total number of eggs laid each day by the total number of replicates. Data were complicated by the fact that some individuals died before the end of the experiment while others were still alive and ovipositing on day 85. Therefore data were standardized and oviposition rate was analysed from the end of the pre-oviposition period to day 43 for beetles surviving the whole of this period. In this way sufficient replication was achieved for statistical analysis.

Data were not normally distributed and transformation of the data did not improve their distribution, therefore a Friedman test, was used to analyse oviposition rate over time (each time was treated as a block). However, for ease of interpretation the mean oviposition rate and standard error of the mean for each temperature treatment are presented. Raw data is also included in *Table 5.4.1* to facilitate interpretation of the results.

5.4 RESULTS

Effect of temperature on the longevity and fecundity of *S. punctillum* is displayed in *Table 5.4.1*. The frequency with which *S. punctillum* ceased to lay eggs is displayed in *Table 5.4.2*.

5.4.1 Longevity

At 20 ^{0}C, four female *S. punctillum* out of a total of five were still alive at the end of the experiment and thus had been alive for 85 days. The fifth female died on day 62. Two males died on days 66 and 68 and were replaced with adult males of a similar age from a stock culture.

At 26 ^{0}C, three out of a total of five females died before the end of the experiment on days 19, 30 and 60. The death on day 30 was the result of drowning in salt solution and a further pair of adults was set up to overcome the reduction in replicates (replicate 6 in *Table 5.4.1*). At the end of the experiment, three females had been alive for 46 (one beetle) and 85 (two beetles) days. No male beetles died during the experimental period at 26 ^{0}C.

The experiment continued for a further 14 days in which time no further deaths occurred. However, the data for oviposition rate were not analysed beyond day 85 because adults were not fed to satiation due to a lack of spider mites.

5.4.2 Fecundity

Analysis indicated no significant effect of temperature on pre-oviposition period of *S. punctillum* incubated at 20 ^{0}C and 26 ^{0}C (Mann Whitney *U*-test, P=0.175). Two-way analysis indicated that oviposition rate was significantly effected by temperature (Friedman test, P<0.05). Beetles incubated at 26 ^{0}C tended to lay at an increased mean rate of oviposition (11.95 ± 0.67 eggs day $^{-1}$) compared with beetles that were incubated at 20 ^{0}C (8.99 ± 0.46 eggs day $^{-1}$).

Oviposition rate of *S. punctillum* was variable within temperature treatments especially at 26 ^{0}C indicated by the large standard error of the mean. At 26 ^{0}C one female beetle laid 394 eggs over an oviposition period of 58 days compared with a total of 502 eggs laid over an oviposition period of 43 days for another female. The maximum total number of eggs laid by any one female was 1214 over an oviposition period of 84 days at 26 ^{0}C.

At 20 ^{0}C, one female beetle oviposited 288 eggs over an oviposition period of 83 days, compared to a female beetle who laid 462 more eggs over an oviposition period of 81 days and compared to a female that died on day 62 having laid a total of 338 eggs over an oviposition period of 61 days.

Beetles ceased egg lay for one to four days on a number of occasions. This cessation was more frequent for beetles incubated at 20 ^{0}C compared with beetles incubated at 26 ^{0}C (*Table 5.4.2*).

Table 5.4.1 **Effect of temperature on the fecundity and longevity (until the end of experimentation on day 85) of *Stethorus punctillum* when fed to satiation on two-spotted spider mites (*Tetranychus urticae*) infesting bean leaves (*Vicia faba* cv. Aquadulce Claudia) with 85 % r.h. and a 16L: 8D photoperiod.**

Temperature (^{0}C)	Replicate pair	Longevity (days)		Pre-oviposition period (days)			Total eggs	Oviposition rate[3] (eggs day $^{-1}$)		
		Female	Male	Mean	Range	SE		Mean	Range	SE
20	1	85+	85+				288			
	2	62[1]	85+				338			
	3	85+	68[1]	2.40a	1-4	0.51	640	8.99a	0-35	0.46
	4	85+	85+				750			
	5	85+	66[1]				850			
26	1	60[1]	85+				394			
	2	19[1]	85+				140			
	3	85+	85+				1214			
	4	85+	85+	1.50a	1-3	0.34	929	11.95b	0-33	0.67
	5	30[2]	85+				417			
	6	46+	85+				502			
P				**0.175**				**0.001**		

Means followed by the same letter in columns are not significantly different [Mann Whitney *U*-test (pre-oviposition period) or Friedman test (oviposition rate), $P<0.05$]. + Individual still alive at the end of experimentation; [1] mortality of individual (males were replaced); [2] drowned in salt solution; [3] measured from the end of the pre-oviposition period to day 43 – $n = 5$ and 4 for 20 and 26 ^{0}C, respectively.

Table 5.4.2 **Average frequencies in cessation of egg lay for female *Stethorus punctillum* incubated at 20 and 26 ^{0}C, 85 % r.h. and under a 16L: 8D photoperiod when fed to satiation on two-spotted spider mites (*Tetranychus urticae*) infesting excised bean leaves (*Vicia faba* cv. Aquadulce Claudia) for 43 days (excluding pre-oviposition period).**

Temperature (^{0}C)	*n*	Number of days in period of cessation			
		1	2	3	4
20	5	0.40	0	0.20	0
26	4	0.25	0	0	0.25

n = number of mating pairs of *S. punctillum*.

5.5 DISCUSSION

Eggs were laid at both temperatures indicating that neither 20 ^{0}C nor 26 ^{0}C had a detrimental effect on the oviposition of *S. punctillum per se*. Temperature did not significantly effect the pre-oviposition period of *S. punctillum*. However, there was a trend for a decrease in mean pre-oviposition period with an increase in temperature from 20 ^{0}C to 26 ^{0}C. This is in broad agreement with Roy *et al.* (2003) who reported a decrease in pre-oviposition period from 51.4 to 2.5 days in *S. punctillum* when fed on *T. mcdanieli* infesting raspberry leaves and incubated at 16 to 30 ^{0}C, respectively.

High variability in fecundity by female *S. punctillum* within treatments was observed despite stable climatic conditions and a regular food supply. Variation in fecundity was also reported for *S. punctillum* by Putman (1955) and appears to be a trait shared by all coccinellids (Hodek, 1973) suggesting that an increase in replication may not have overcome variability. Despite this variability oviposition rate was shown to be significantly effected by temperature.

The maximum number of eggs laid by any one individual female *S. punctillum* was 1214 over 84 days at 26 ^{0}C. However, this female had possibly not realised her lifetime fecundity as she was still alive at the end of the experiment. Putman (1955) reported a maximum of 1290 eggs laid by a female *S. punctillum* maintained in an insectary

(temperatures not specified although reportedly similar to temperatures in orchards). However, the eggs were laid over two seasons in 1951 and 1952 in oviposition periods of 75 and 123 days, respectively.

Putman (1955) reported that 28 eggs was the greatest number laid by any one female *S. punctillum* in 24 hours in the insectary. This is slightly lower than the maximum number of 35 eggs laid by a female incubated at 20 ^{0}C in this study. However, mean oviposition rate was much lower for females incubated at 20 ^{0}C in this study (9.0 eggs day $^{-1}$) and similar to that reported by Putman (1955) for females held in the insectary (8.5 eggs day $^{-1}$). Putman (1955) also reported a mean of 11.1 eggs day $^{-1}$ for female *S. punctillum* held at 21.1 ^{0}C. Slight discrepancies between these data sets may be explained by differences in biotypes of *S. punctillum*, the environmental (e.g. microclimate) and/or experimental equipment.

In this study, eggs were laid on bean leaf surfaces or on the surfaces of the CHUs. There was a tendency for eggs on leaves to be laid near leaf veins and this has also been reported for *S. punctillum* in apple orchards (Injac and Dulič, 1992). In general, *S. punctillum* laid eggs singly. However, observations included a cluster of seven eggs out of a total of 19 eggs laid in 24 hours by one female at 20 ^{0}C. The reproductive tactic of laying eggs in clusters has been reported for aphidophagous (e.g. Dixon, 2000) and coccidophagus (e.g. Quezada and DeBach, 1973; Ponsonby and Copland, 1997) coccinellids, but in the case of *S. punctillum* previous reports have suggested that eggs are laid singly (Putman, 1955).

The general shape of an *S. punctillum* egg is an oval measuring approximately 0.5 mm in length. In the course of this experiment shorter eggs, measuring approximately a half to two-thirds the length of 'normal' ones, were intermittently observed. Honěk (1996) stated that when food supply is scarce, female insects may adjust either the number or the size of their eggs accordingly. Although some insects reduce the size of the eggs and maintain the number of eggs laid, coccinellids tend to produce fewer eggs but maintain the size of the eggs laid (Honěk, 1996). Considering Honěk's statement and the fact that the food supply in this study was maintained at a constant level to ensure coccinellids were fed to satiation, it is suggested that egg size was not a consequence of

food deficiency in this case. The reason(s) for the variation in egg size and the effect, if any, on egg viability remain unclear.

The frequency of cessation in egg laying was greater for *S. punctillum* incubated at 20 ^{0}C. Cessation of egg lay in *S. punctillum* has previously been recorded (Putman 1955; Roy *et al.*, 2003). Putman (1955) observed that occasionally a female *S. punctillum* would stop ovipositing for a day or sometimes longer and that when egg lay resumed it was at an increased rate. The reason(s) for periods without egg lay in this study is uncertain but it is unlikely to be a function of amount of prey supplied as beetles were fed to satiation. Although Injac and Dulič (1992) stated that *S. punctillum* was sensitive to changes in food under laboratory conditions, they did not present results to support their observation. Ladybirds are cannibalistic (e.g. Majerus, 1994; Ponsonby and Copland, 1998) and in particular Putman (1955) observed that *S. punctillum* females occasionally ate their own eggs. It was not within the scope of the experiment presented here to determine the occurrence and extent of cannibalism in *S. punctillum* and further research is indicated to evaluate this.

The results presented here are an indication of the potential egg laying ability of *S. punctillum* under stable conditions of temperature and food supply. This information may be applied to *S. punctillum* maintained in an insectary under constant conditions. However, in glasshouses beetles would be exposed to 20 and 26 ^{0}C as part of a fluctuating temperature regime and Dixon (2000) stated that ladybirds provided with excess prey lay twice as many eggs compared to when the same beetles are fed at a third of this rate. Further research would be required to evaluate the effect of fluctuating temperatures and prey consumption on daily and total egg lay in *S. punctillum*. Further work is also indicated to determine the adult longevity, total lifetime fecundity and oviposition rate of *S. punctillum* in a wider range of temperatures.

The results presented here indicate that *S. punctillum* is able to oviposit at temperatures typical to UK glasshouses and information such as this is important in the development of successful mass production and pest management strategies when other factors that affect population dynamics (e.g. development and prey consumption) are incorporated.

6 EFFECT OF COLD STORING STETHORUS PUNCTILLUM ADULTS ON TIME TAKEN TO RESUME OVIPOSITION, FECUNDITY AND EGG VIABILITY POST STORAGE

6.1 INTRODUCTION

Successful mass production of insects and mites for use in biological control programmes and flexibility within this process probably rest on the ability of the biocontrol agents to withstand cold storage (Osman and Selman, 1993). Production costs may be reduced by cold storing insects and mites when rearing in greenhouses is slow and expensive, i.e. in winter (e.g. Gilkeson, 1990; McEwen, 1997). Cold storage is crucial to pest management programmes (e.g. Van Lenteren and Woets, 1988).

In glasshouse cultivation, there are times when the pressure for supplies of natural enemies is low. However, cultures of predators and parasites must be maintained in order to meet future demands and cold storage allows mass producers to overcome discrepancies between supply and demand.

Research of cold storage techniques associated with biological control has either concentrated on a particular life stage of natural enemy that is perceived to be a suitable stage for introduction into the glasshouse (e.g. Hämäläinen and Markkula, 1977) or exploitation of a particular developmental stage that enters diapause (e.g. Hämäläinen, 1977; Tauber *et al.*, 1993).

Majerus (1994) stated that mass production costs of coccinellids are often high and suggested that if they are reared in large quantities, successful storage of them is essential until they are required. Coccinellids attempt survival of cold periods in the adult stage (Majerus, 1994). However, evaluation of their ability to withstand cold storage has included eggs, larvae and pupae (e.g. Quezada and DeBach, 1973; Hämäläinen and Markkula, 1977; Abdel-Salam and Abdel-Baky, 2000) as well as adults (Quezada and DeBach, 1973; Abdel-Salam and Abdel-Baky, 2000).

Under natural conditions, *Stethorus punctillum* may hibernate in deep litter in woodlands, under very sparse cover in orchards (Putman, 1955), under or in the

crevices of bark, or on deciduous trees (Majerus, 1994). *S. punctillum* hibernate as adults indicating that this is the most tolerant development stage to withstand cold temperatures and therefore was the focus for evaluation of cold storage here.

Putman (1955) investigated the survival of *S. punctillum* from various hibernation materials and reported 20 %, 27.5 %, 27.5 % and 0 % beetle emergence from tin cans containing dead leaves, earth, sod and loose-barked tree limb, respectively. He concluded that beetles might hibernate successfully in various types of ground cover or on nearly bare soil but not above ground level. It was also reported that beetles were frequently found in autumn beneath bark scales on peach and apple trunks and in corrugated paper bands around trunks but no living ones were found there in April. Putman surmised that low temperatures may destroy them on the trees but low humidity may also be important.

To the author's knowledge, no previous studies have been carried out on the effect of cold storage that may be applied in the mass production of *S. punctillum*. Therefore, the aim of this research was to determine the ability of *S. punctillum* to withstand cold storage and thus establish its suitability for this technique.

Tauber *et al.* (1993) stated that factors such as high survival in storage and continued high fecundity and fertility are required for cold storage to be of any importance to mass production. Therefore, the present study evaluated the survival of *S. punctillum* adults in cold storage at 6 ^{0}C, 75 % r.h. and in the dark and compared it with the survival of adults that were not cold stored. Oviposition rate (eggs day $^{-1}$) of adults post storage was assessed and compared with the oviposition rate of *S. punctillum* adults that were not cold stored, to indicate any abnormalities caused by storage. The ages of *S. punctillum* adults were unknown as they were obtained directly from Applied Bio-Nomics, Canada and subjected to cold storage 24 hours after their arrival to the laboratory at Canterbury Christ Church University College (*experiment 1*).

In a further experiment (*experiment 2*), 15-day old *S. punctillum* adults were cold stored at 6 ^{0}C, 75 % r.h. and in the dark for five, 10 and 15 days. Survival of *S. punctillum* adults in cold storage and oviposition rate of adults post storage were compared with

survival and oviposition of *S. punctillum* adults that were not cold stored. The aim here was to repeat the first experiment using *S. punctillum* adults of a known age and include an additional cold storage period of 15 days.

It is possible that any adverse effects of cold storage on *S. punctillum* adults may not be apparent in the actual number of eggs laid post storage, but rather in the viability of those eggs. Therefore, the viability of eggs laid by *S. punctillum* adults that had been previously cold stored for five, 10 and 15 days in *experiment 2* was determined (*experiment 3*).

6.2 MATERIALS AND METHODS

6.2.1 Experiment 1: Effect of cold storage for five and 10 days on *S. punctillum* adults of unknown age.

Adult *S. punctillum* were obtained directly from Applied Bio-Nomics, Canada and transferred to a Perspex box (*section 3.6.2*), lined with tissue paper and containing excised bean leaves (*V. faba* cv. Aquadulce Claudia) infested with two-spotted spider mites (*T. urticae*) after their arrival to the laboratory at Canterbury Christ Church University College, UK. The level of spider mite infestation on the bean leaves was sufficient to feed *S. punctillum* to satiation. The box was sealed using masking tape and maintained in the CT room (*section 3.5.1*).

After 24 hours, 34 males and 34 females were transferred singly to glass vials (*section 3.6.4*) with Nescofilm® lids. In this way 10 replicate pairs were provided for each of two cold storage treatments (*treatment 1*: cold storage for five days; *treatment 2*: cold storage for 10 days) with a surplus of 14 males and 14 females to overcome possible poor survival in storage.

The glass vials were held in a sealed Perspex box (as before) containing a saturated salt solution to maintain a relative humidity of 75 % (*section 3.5.2*). This box was transferred to a refrigerator at 6 ^{0}C and in the dark.

After the storage periods, individual pairs of males and females were transferred to Petri dish arenas (*section 3.6.1*). The arenas contained excised bean leaves infested with two-spotted spider mites and were lined with filter paper to absorb excess moisture. The lids were made of inverted Petri dish bases that had mesh (*section 3.4*) vents in them and were held in place using Nescofilm®. The arenas were transferred to a Perspex box containing a saturated salt solution to maintain 75 % r.h. (*section 3.5.2*). The box was sealed and incubated at 24 ^{0}C with a 16L: 8D photoperiod.

A further 10 pairs of males and females were not cold stored (control) and transferred from the CT room to Petri dish arenas incubated under identical conditions as described above.

Adequate numbers of all spider mite stages were provided throughout experimentation to ensure satiation of the beetles. The number of days taken to resume oviposition after storage and the oviposition rate (eggs day $^{-1}$) were determined for each beetle pair for four days.

6.2.2 Experiment 2: Effect of cold storage for five, 10 and 15 days on 15-day old *S. punctillum* adults.

S. punctillum were reared as detailed in *section 3.4*. Adult beetles were 15 days old at the start of the experiment. Adults were transferred individually to glass vials (*section 3.6.4*), which were then sealed using discs of mesh secured with elastic bands. The glass vials were transferred to Perspex boxes (*section 3.6.2*) containing saturated salt solutions to maintain the humidity within the boxes at approximately 75 % r.h. (*section 3.5.2*). The boxes were then sealed using masking tape and transferred to a refrigerator at 6 ^{0}C and in the dark. Adults were maintained under these cold storage conditions for five, 10 or 15 days.

After these time periods, percentage survivorship was determined for each of the three cold storage treatment groups. Ten male and female pairs from the remaining live adults were transferred to Petri dishes (*section 3.6.1*), which had mesh vents in the lids. The dishes contained excised bean leaves (*V. faba* cv. Maris Bead) bearing a high infestation of two-spotted spider mites to ensure that *S. punctillum* were fed to satiation

throughout experimentation. The arenas were transferred to Perspex boxes containing saturated salt solutions to maintain a relative humidity of 75 % (*section 3.5.2*). The boxes were sealed using masking tape and incubated at 25 ^{0}C with a 16L: 8D photoperiod (conditions for oviposition).

A further group of 10 male and female pairs of *S. punctillum* were not subjected to cold storage but transferred directly to Petri dish arenas and maintained at the conditions for oviposition as described above to provide a control.

The number of days taken to resume oviposition after storage (days from the end of storage to the first egg) and oviposition rate (egg day $^{-1}$) for 22 days were determined for each pair of beetles.

6.2.3 Experiment 3: Viability of eggs oviposited by *S. punctillum* adults that were previously cold stored.

Egg viability was tested for 10, 10, six and seven replicate pairs of adults from each of the four treatment groups in *experiment 2* post 0 (control), five, 10 or 15 days in cold storage, respectively. Within five days of setting up the Petri dish arenas to observe fecundity post cold storage as described in *section 6.2.2*, five eggs from each ovipositing pair were transferred to similar Petri dish arenas and incubated under equivalent conditions to the adults. These eggs were checked daily for hatching.

6.3 STATISTICAL ANALYSES

6.3.1 Experiment 1

Percentage survivorship was calculated for beetles that had been cold stored and a control group of beetles that had not been cold stored.

Data for the number of days taken to resume oviposition after storage were analysed using a Kruskal Wallis test ($P<0.05$), adjusted for ties. However, for ease of interpretation all data are presented as means followed by the standard errors of means and ranges in *Table 6.4.1.1*.

The mean oviposition rate (eggs day $^{-1}$) for each day was calculated by dividing the total number of eggs laid each day by the total number of replicates. Oviposition rate was analysed for four days from the start of the oviposition period (*Table 6.4.1.1*). Data were not normally distributed and transformation of the data did not improve their distribution therefore a Friedman test was used to analyse oviposition rate over time (each time was treated as a block). However, for ease of interpretation the mean followed by the standard error of the mean for each cold storage treatment are presented.

6.3.2 Experiment 2

Percentage survivorship was calculated for beetles that had been cold stored and a control group of beetles that had not been cold stored. The number of days taken to resume oviposition after storage of adult *S. punctillum* was analysed using a Kruskal Wallis test ($P<0.05$), adjusted for ties. However, for ease of interpretation data are presented as means followed by the standard errors (*Table 6.4.2.1*).

Where it was not possible to count eggs every day, the numbers of eggs collected over two to three days were converted to means for those days prior to statistical analysis of oviposition rate (eggs day $^{-1}$). The mean oviposition rate for each day was calculated by dividing the total number of eggs laid each day by the total number of replicates. Oviposition rate was analysed from the beginning of the oviposition period to day 22 (*Table 6.4.2.1*).

Data were not normally distributed and transformation of the data did not improve their distribution, therefore, a Friedman test was used to analyse oviposition rate over time (each time was treated as a block) ($P<0.05$). However, for ease of interpretation means followed by standard errors for each cold storage treatment are presented.

6.3.3 Experiment 3

Data for egg viability were calculated for each pair of *S. punctillum* adults by dividing the number of eggs that hatched into first instar larvae by the total number of eggs observed ($n = 5$) and multiplying by 100. Replicates, including eggs laid on leaves that deteriorated prior to all or any of the eggs hatching, were discounted from the statistical

analyses. Percentage egg viabilities for each treatment were analysed using a Kruskal Wallis test ($P<0.05$), adjusted for ties. However, for ease of interpretation data are presented as mean percentage egg viabilities for each treatment.

6.4 RESULTS

6.4.1 Experiment 1

There was a trend for decreased survivorship of *S. punctillum* that were cold stored (47 %) compared to a group of beetles (control) that were not stored (89 %). Mean (SE) numbers of days to resume oviposition post storage and oviposition rates for beetles in each treatment group are displayed in *Table 6.4.1.1.*

Overall, the longest period to resume oviposition post storage of seven days was observed after five days in cold storage. However, the mean (± SE) period to resume oviposition post storage for beetles in this group was a much lower 2.87 (± 0.77) days. A Kruskal Wallis test indicated that there was no significant effect of cold storage on the number of days taken to resume oviposition ($P>0.100$). However, there was a tendency for beetles to display an increase in mean (± SE) number of days taken to resume oviposition from 2.00 (± 0.44) to 3.75 (± 1.03) days, with an increase in storage period from 0 to 10 days, respectively.

Two-way analysis indicated that oviposition rate was not significantly affected by cold storage ($P>0.100$). However, there was a tendency for beetles that had not been cold stored to oviposit at a higher mean (± SE) rate (7.25 ± 0.81 eggs day $^{-1}$) compared with beetles that had been cold stored for five (6.42 ± 1.11 eggs day $^{-1}$) and 10 days (6.37 ± 1.80 eggs day $^{-1}$).

Table 6.4.1.1 **Effect of cold storage on the number of days to resume oviposition (end of storage to first egg) and oviposition rate for *Stethorus punctillum* adults of unknown age: cold storage conditions were 6 ^{0}C, 75 % r.h. and 24 D.**

Days at 6 ^{0}C	*n*	Days to resume oviposition (days)			*n*	Oviposition rate[1] (eggs day $^{-1}$)		
		Mean	Range	SE		Mean	Range	SE
0	7	2.00a	0-4	0.44	7	7.25a	0-16	0.81
5	8	2.87a	1-7	0.77	6	6.42a	1-13	1.11
10	4	3.75a	2-6	1.03	4	6.37a	1-18	1.80
P		**0.410**				**0.779**		

Means followed by the same letter in columns are not significantly different [Kruskal Wallis test (days to resume oviposition) or Friedman test (oviposition rate), $P<0.05$].

Conditions for oviposition when fed to satiation on two-spotted spider mites (*Tetranychus urticae*) infesting excised bean leaves (*Vicia faba* cv. Maris Bead) were 24 ^{0}C, 75 % r.h. and 16L: 8D.

n = Number of male and female pairs of *S. punctillum* adults.

[1] Measured from the start of the oviposition period to day four.

6.4.2 Experiment 2

Data indicated that there was no obvious effect of cold storage on the survival of *S. punctillum* where 95, 96, 96 and 100 % of *S. punctillum* beetles survived in the control group and in cold storage for five, 10 and 15 days, respectively. Mean numbers of days taken to resume oviposition post storage and oviposition rates (eggs day $^{-1}$) for *S. punctillum* adults are displayed in *Table 6.4.2.1.*

A Kruskal Wallis test indicated that cold storage had a significant affect on the number of days taken to resume oviposition post storage (P=0.001). Beetles that were cold stored for 10 and 15 days had mean (± SE) numbers of days taken to resume oviposition of 0.56 (± 0.18) days and 1.29 (± 0.36) days, respectively, compared with no days to resume oviposition recorded for any beetles that were not cold stored and beetles that were stored for five days.

Two-way analysis indicated that oviposition rate was not significantly affected by cold storage (P=0.159). However, there was a tendency for beetles that were not cold stored to oviposit at an increased mean (± SE) rate (12.19 ± 0.67 eggs day $^{-1}$) compared with beetles that were cold stored for five (10.13 ± 0.59 eggs day $^{-1}$), 10 (9.04 ± 0.45 eggs day $^{-1}$) or 15 (9.61 ± 0.52 eggs day $^{-1}$) days.

Table 6.4.2.1 **Effect of cold storage on the number of days to resume oviposition (end of storage to first egg) and oviposition rate for 15-day-old *Stethorus punctillum* adults: cold storage conditions were 6 ^{0}C, 75 % r.h. and 24 D.**

Days at 6 ^{0}C	*n*	Days to resume oviposition (days)			*n*	Oviposition rate[1] (eggs day $^{-1}$)		
		Mean	Range	SE		Mean	Range	SE
0	10	0.00a			6	12.19a	2-28	0.67
5	9	0.00a			9	10.13a	0-29	0.59
10	9	0.56b	0-1	0.18	8	9.04a	1-17	0.45
15	7	1.29b	0-3	0.36	7	9.61a	1-19	0.52
P		**0.001**				**0.159**		

Means followed by the same letter in columns are not significantly different [Kruskal Wallis test (days to resume oviposition) or Friedman test (oviposition rate), $P<0.05$].

Conditions for oviposition when fed to satiation on two-spotted spider mites (*Tetranychus urticae*) infesting excised bean leaves (*Vicia faba* cv. Maris Bead) were 25 ^{0}C, 75 % r.h. and 16L: 8D.

n = Number of male and female pairs of *S. punctillum* adults.

[1] Measured from the start of the oviposition period to day 22.

6.4.3 Experiment 3

Data for effect of cold storage on egg viability are presented in *Table 6.4.3.1*. For adults that were cold stored for 10 and 15 days, the mean percentages of viable eggs were 63 and 71 %, respectively, compared with 94 and 98 % viable eggs oviposited by beetles that were not cold stored and beetles that were cold stored for five days, respectively. A Kruskal Wallis test indicated a significant effect of cold storage for 10 and 15 days on egg viability (P=0.002).

Table 6.4.3.1 **Mean percentages of viable *Stethorus punctillum* eggs (i.e. surviving to first instar larvae) post cold storage (6 ^{0}C, 75 % r.h. and 24D) of their parents[1] for five, 10 and 15 days compared to eggs oviposited by parents that were not cold stored[2].**

Days at 6 ^{0}C	*n*			Mean % viable eggs
	Parental pairs[2]	Eggs	Hatched	
0	10	50	47	94a
5	10	50	49	98a
10	6	30	19	63b
15	7	35	25	71b
P				**0.002**

Mean percentages followed by the same letters in columns are not significantly different (Kruskal Wallis test, P<0.05).

[1] Adult beetles were 15 days old at the onset of cold storage.

[2] Conditions for oviposition by adults were 25 ^{0}C, 75 % r.h. and 16L: 8D.

6.5 DISCUSSION

Previous research has investigated the effect of low temperatures on biological and physiological aspects of insects used as biological control agents (e.g. Hämäläinen, 1977; Hämäläinen and Markkula, 1977; Hofsvang and Hågvar, 1977; Tauber *et al.*, 1993; Abdel-Salam and Abdel-Baky, 2000). Successful cold storage techniques facilitate flexibility in the mass production of natural enemies (Osman and Selman, 1993). Coccinellids are commonly used in augmentative biological control (Obrycki and Kring, 1998) and Abdel-Salam and Abdel-Baky (2000) stated that successful biological control may be assisted by storage of coccinellids.

When large numbers of coccinellids are required for biological control, rearing costs may be high and this has been documented for *Stethorus picipes* when compared to rearing costs of phytoseiids (McMurtry, 1995). However, production costs may be reduced by the implementation of cold storage techniques (e.g. Gilkeson, 1990; McEwen, 1997). This study demonstrates, for the first time, that cold storage of adult *S. punctillum* is possible. However, egg viability was significantly affected after cold storage for 10 and 15 days. Cold storage for five, 10 and 15 days at 6 ^{0}C did not significantly affect oviposition rate (eggs day $^{-1}$) post storage.

Previous research concerning the cold storage of coccinellids, has investigated the effect it has after months, rather than days. Due to time constraints it was not possible to subject *S. punctillum* to cold storage conditions for more than a few days. Nevertheless, previous research can facilitate the interpretation of the results discussed here. For example, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) hibernates in sheltered environments covered by snow; in litter, under tussocks, shrubs or stones (Hämäläinen, 1977), which are comparable to the hibernation sites of *S. punctillum* (Putman, 1955; Majerus, 1994). Hämäläinen (1977) reported a high mortality rate in *C. septempunctata* after one to two months storage at 6 ± 1 ^{0}C and this was attributed to an unsuitable microclimatic condition. It was stated that the hibernacula used for storage may have been too dry (70 - 90 % r.h.) and too warm for the beetles.

In *experiment 1* there was a tendency for lower survival of adults in cold storage compared with adults that were not cold stored. This may have been explained by unsuitable storage conditions. However, similar conditions were implemented in *experiment 2* where survival was comparable between all treatments groups.

Coccinellids store fat in preparation for overwintering and diapausing coccinellids typically have an increased fat content (Hodek, 1973). Low levels of energy reserves are not conducive to survival of winter conditions (Majerus, 1994). As the low temperatures of cold storage in the experiments presented here simulated at least part of what is endured by overwintering adults in nature, it is possible that the lower survival of cold stored beetles in *experiment 1* may be attributed to a lack of preparation for cold storage. In this experiment, *S. punctillum* adults were allowed to feed for 24 hours after their arrival from Applied Bio-Nomics, Canada, before being subjected to cold storage conditions. However, this may not have been a sufficient length of time for the beetles to replenish food reserves, possibly diminished during transportation. In contrast, in *experiment 2*, *S. punctillum* adults were fed to satiation for 15 days post-eclosion before being placed in cold storage.

Evidence suggests that feeding, prior to cold storage of coccinellids affects survival in storage (e.g. Deng 1982; Abdel-Salam and Abdel-Baky, 2000). Abdel-Salam and Abdel-Baky (2000) reported that feeding prior to cold storage of adult *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae) significantly increased the survival of adults in cold storage compared to a control group that were stored directly after eclosion. In contrast, Hämäläinen (1977) reported a more rapid rate of mortality in adults of *C. septempunctata* that had food reserves compared with those with limited reserves.

A further possible explanation for the lower survivorship observed among beetles in *experiment 1* is that these beetles were received directly from Applied Bio-Nomics, Canada, and may have already been stored in conditions of low temperature prior to their arrival in the UK. As the fitness of insects is reduced by cold storage (Van Lenteren and Woets, 1988), it is possible that the risk to fitness is exacerbated by repeated cold storage of the same beetles.

Commercial application of cold storage techniques requires synchrony in the initiation of reproduction post-storage (Tauber *et al.*, 1993) and the results presented here suggest that this is achievable for *S. punctillum* after cold storage for five (*experiments 1* and *2*) and 10 days (*experiment 1*). However, conflicting results were reported in *experiment 2* where beetles that had been cold stored for 10 days took a significantly longer period of time to commence egg lay compared to beetles that had not been stored and those that had been stored for five days. In general, the mean numbers of days taken to resume oviposition post storage observed in *experiment 1* were considerably longer compared with the mean numbers of days observed in *experiment 2*. Possible differences in the physiological state of beetles used in the two experiments may account for the discrepancies observed. Results presented by Abdel-Salam and Abdel-Baky (2000) indicated that prior feeding by *C. undecimpunctata* had an effect (not tested for significance) on pre-oviposition period post cold storage. They found that the mean pre-oviposition periods of adults stored for seven and 15 days were longer for adults that were not fed prior to storage (6.3 and 7 days, respectively) compared with adults that had been fed for five days (5.8 and 6.2 days, respectively) and adults that had been fed for 10 days (5.9 and 6.8 days, respectively) prior to storage.

In addition, the high variability observed within treatments (indicated by large standard errors of the means), particularly for *treatment 3* in *experiment 1*, may account for the lack of significance observed for the numbers of days taken to resume oviposition post storage between treatments in *experiment 1*. Standard errors in *experiment 2* were lower and means were based on a higher number of replicates. Therefore, an increase in replication is indicated for future experiments.

For practical application it is important to determine whether cold storage has a detrimental effect on the production of off-spring (Hofsvang and Hågvar, 1977) and cold storage has been shown to significantly effect the total number of eggs produced per female *C. undecimpunctata* (Abdel-Salam and Abdel-Baky, 2000) for example. The results presented here indicated no significant effect of cold storage for five, 10 or 15 days on oviposition rate of *S. punctillum*. However, previous evidence indicated that cold storage had a significant effect on oviposition period and the total number of eggs laid per female in some insects (e.g. Tauber *et al.*, 1993; Abdel-Salam and Abdel-Baky,

2000). Oviposition rate was not established for the longevity of the female beetles used in this study and future experiments are indicated to ascertain the oviposition period and total number of eggs produced per female *S. punctillum* post cold storage.

Previous research on the effect of cold storage on the egg stage of insects has mainly concentrated on eggs that have themselves been cold stored (e.g. Hämäläinen and Markkula, 1977; Osman and Selman, 1993; Abdel-Salam and Abdel-Baky, 2000). However, in my study the effect of cold storage on eggs laid by adults previously cold stored was investigated and shown to have a significantly negative effect on egg viability when beetles were stored for 10 and 15 days, compared with eggs that were laid by females stored for 0 and five days. Similar results were reported by Tauber *et al.* (1993) for *Chrysoperla carnea* where females that were not cold stored produced, on average, 480 fertile eggs during the first 30 days of oviposition compared with approximately 250 to 350 fertile eggs produced by females that had been cold stored for six to 31 weeks at 5 ^{0}C. However, the reduction in fertile eggs caused by cold storage was only significant when adults had been stored for 31 weeks.

Securing a suitable method for cold storing *S. punctillum* is of great importance to mass producers and distributors of the predator as a biocontrol agent. Obviously there are peaks of spider mite infestation in the growing season under glass and demands for the control agent may be high at this time. Cold storage techniques provide a valuable method of manipulating cultures to meet high demands. In addition, rearing insects is prone to culture losses from time to time, resulting from lack of prey, invasion of unwanted predators or parasites, disease or reduced vigour. Cold storage provides a way of overcoming these problems.

In conclusion, cold storage of *S. punctillum* is possible with no significant effect on oviposition rate. However, results suggest that the physiological state of adults is a possible factor affecting survival in cold storage. Future experiments are indicated to investigate this further. Induction of diapause prior to cold storage may also aid survival (e.g. Tauber *et al.*, 1993) and the factors that induce diapause provide an insight into the processes involved in cold storage techniques. Temperature has been reported as the dominant factor in diapause induction for some insects [e.g. Anderson

and Hales, 1986; *Coccinella repanda* Thunberg (Coleoptera: Coccinellidae)] but not for others where changes in photoperiod are important (e.g. Hodek, 1986; *Adalia bipunctata*). Photoperiod changes are discussed further in relation to *S. punctillum* and diapause in *Chapter 7*.

Future experiments are also indicated to determine the maximum period of low temperature storage tolerated before unacceptable traits are observed, e.g. reduction in adult longevity and fecundity, and to investigate the efficacy of adult *S. punctillum* released into the glasshouse post cold storage.

7 SHORT-DAY PHOTOPERIODIC RESPONSE IN STETHORUS PUNCTILLUM

7.1 INTRODUCTION

Insect lifecycles have evolved to include an inactive phase to overcome periods unfavourable to growth and morphogenesis. For example, in ladybirds, reproduction and dormancy are alternated to synchronize the lifecycle with environmental change and thus aid survival (e.g. Hodek *et al.*, 1984; Anderson, 1986). In this phase, food reserves and a reduced metabolic rate are relied upon for survival (Hodek, 1996a).

Hodek (1996a) described diapause as any period of arrested development that includes changes in behaviour, structure and/or the biochemistry of insects. Until the summer diapause in short-day insects and periods of aestivation in tropical insects were discovered, this state of quiescence was originally considered to have evolved only in order to overcome periods of intense cold (Hodek, 1996a).

Hodek (1965) provided a review of adult diapause in insects. Use of the term diapause causes confusion not only because there are different types of diapause but also because the same individuals are able to arrest or resume development in different ways (Hodek, 1996a). For example, based on their results for *Coccinella septempunctata brucki* Mulsant (Coleoptera: Coccinellidae), Sakurai and Takeda (1986) concluded that aestivation is a true diapause whereas hibernation is only facultative dormancy caused by low temperatures. Hodek (1996a) described dormancy as *any* arrest in development and in contrast, adaptive functions (e.g. enlarged fat body, reduced metabolism) are used in diapause to synchronize development with favourable conditions. Usually in temperate climates completion of the overwintering diapause occurs in early/mid winter (Hodek, 2002).

Understanding of the underlying processes in insect diapause is important to mass production (Tauber *et al.*, 1993) and dormant coccinellids are frequently collected for use in biological control either for immediate or eventual use (Hodek and Růžička, 1979). However, if they are to be used for immediate success, insects collected in the dormant phase must first be subjected to the correct environmental cues. For example, DeBach and Rosen (1991) stated that insects in diapause imported for immediate use in

biological control programmes would not complete development under normal insectary conditions. Such environmental cues may also be exploited to increase survival in cold storage (e.g. Tauber *et al.*, 1993) as previously discussed in *Chapter 6*.

Physiological induction of diapause has been linked to activity of the *corpora allata* and its production of juvenile hormone and ecdysone (Hodek, 1996a). However, I examined the environmental effects on diapause.

Environmental factors implicated in diapause induction in insects include changes in photoperiod, food supply and temperature (e.g. Anderson and Hales, 1986; Hodek, 1996a) but the level of their importance appears to depend on insect species. For example, Anderson and Hales (1986) reported that the dominant factors that effected diapause in *Coccinella repanda* were food and temperature. In contrast, a decrease in temperature did not induce diapause in *Adalia bipunctata* and *C. septempunctata* but changes in photoperiod either maintained (short-day) or terminated (long-day) diapause (Hodek, 1986). Hodek *et al.* (1984) reported opposing responses to photoperiod in two sub-species of *C. septempunctata* namely *C. septempunctata septempunctata* (long-day response) and *C. septempunctata brucki* (short-day response).

Inducing environmental factors are not necessarily mutually exclusive in their influence on diapause and there may be a temperature threshold above which the photoperiodic response is inhibited (Hodek *et al.*, 1984; Hodek and Okuda, 1993). For example, photoperiod may govern diapause in *Dolycoris baccarum* (L.) (Heteroptera: Pentatomidae) and long days may prevent it. However, under short day-lengths diapause may be prevented in this species by exposing it to high temperatures (Hodek and Hodkova, 1993).

Diapause induction has been evaluated for a number of coccinellids and in particular *C. septempunctata* (Hodek *et al.*, 1977; Hodek and Růžička, 1979; Hodek *et al.*, 1984; Hodek, 1986; Hodek and Okuda, 1997) and diapause in multivoltine populations are typically induced by photoperiod (Hodek, 1986). Brief discussions on the stage at which *S. punctillum* hibernates and survival in various hibernation materials were included in *Chapter 6* and for further information the reader is directed to publications by Collyer (1953) and Putman (1955).

Indirect evidence suggests that pre-imaginal stages in insects are sensitive to diapause inducing stimuli, for example Tauber *et al.* (1993) stated that larvae of *Chrysoperla carnea* were reared under a short day-length of 10L: 14D at 24 ^{0}C to induce diapause. The aim of *experiment 1* was to investigate the feasibility of rearing *S. punctillum* larvae under short-day conditions in terms of development period and survival.

The effect of decreasing day-length on egg lay in *Stethorus nigripes* Kapur has been reported (Hoy and Smith, 1982) but to my knowledge no previous studies have investigated the effect of photoperiod on egg lay in *S. punctillum.* The aim of *experiment 2* was to establish the effect on oviposition rate (eggs day $^{-1}$) of rearing *S. punctillum* under short-day conditions. *S. punctillum* that had been reared under short-day or long-day conditions were then transferred to long-day or short-day conditions, respectively, to evaluate the effect of changes in photoperiod on oviposition rate.

7.2 MATERIALS AND METHODS

7.2.1 Experiment 1: The effect of short day-length on juvenile development in *S. punctillum.*

Mixed-age adult *S. punctillum* were allowed to oviposit for 24 hours on excised bean leaves (*Vicia faba* cv. Maris Bead) that were infested with two-spotted spider mites (*Tetranychus urticae*) in a Perspex box with a solid lid (*section 3.6.2*). The box was sealed using masking tape and maintained in an incubator at 25 ^{0}C with a 16L: 8D photoperiod and contained a saturated salt solution to maintain 75 % r.h. (*section 3.5.2*). After 24 hours, *S. punctillum* eggs were counted and transferred to two Perspex boxes. One box was incubated under equivalent conditions as before (n = 17) and the second box was incubated at 25 ^{0}C with a 12L: 12D photoperiod (short day-length) (n = 13).

Eggs were checked every 12 hours for development. When the eggs hatched the ensuing first instar larvae were transferred to Petri dishes (one larva per dish) (*section 3.6.1*) that contained excised bean leaves infested with two-spotted spider mites. The lids of the Petri dishes were vented with mesh (*section 3.4*) and sealed using Nescofilm®. The dishes were maintained in a Perspex box under equivalent conditions of temperature and photoperiods as described above for incubation of the eggs.

Developing *S. punctillum* were fed to satiation and checked every 12 hours until adult eclosion to determine the developmental period and survivorship.

7.2.2 Experiment 2: The effect of short day-length on survivorship and fecundity of *S. punctillum.*

S. punctillum were reared as described in *section 3.4.* Pupae were collected from the insectary and transferred to a Perspex box with a vented lid (*section 3.6.2*) and maintained in the CT room (*section 3.5.1*) until adult eclosion. Newly eclosed adult *S. punctillum* were transferred in male and female pairs to Petri dish arenas (*section 3.6.1*) that contained bean leaves (*V. faba* cv. Maris Bead) infested with two-spotted spider mites (*T. urticae*). The lids of the dishes were vented with mesh and sealed using Nescofilm®. The Petri dishes were transferred to Perspex boxes containing saturated salt solutions to maintain a 75 % r.h. (*section 3.5.2*). The boxes were incubated at 25 ^{0}C under photoperiods of 16L: 8D, 12L: 12D or 8L: 16D (*Table 7.2.2.1*).

Pre-oviposition periods and oviposition rates (egg day $^{-1}$) were observed for each mating pair of *S. punctillum* adults. *S. punctillum* eggs were removed daily and two-spotted spider mites were replenished to ensure that *S. punctillum* were fed to satiation.

Table 7.2.2.1 **Duration (days) of photoperiod regime for each treatment group (*n* = number of mating pairs) of *Stethorus punctillum* (excluding pre-oviposition period) in experiment 2.**

Treatment	*n*	Hours of light (L) and dark (D) per day				
		16L: 8D		12L: 12D		8L: 16D
1	**11**	66				
2	**8**	20	←	30		
3	**10**	15	→	20	→	20

Direction of arrows (← or →) indicates direction of insect transfer.

7.3 STATISTICAL ANALYSES

7.3.1 Experiment 1

Data for development of *S. punctillum* from egg to adult eclosion under long day- (16L: 8D) and short day- (12L: 12D) lengths were tested for an approximation to the normal distribution before carrying out a *t*-test ($P<0.05$). For data that were not normally distributed a non parametric analysis was used; the Mann Whitney *U*-test ($P<0.05$). However, for ease of interpretation all data are presented as means followed by standard errors of the means.

Data for survivorship of *S. punctillum* from egg to adult eclosion are presented as percentages and were calculated by dividing the number of individuals that survived from egg to adult by the total number of individuals tested and multiplying by 100.

7.3.2 Experiment 2

Effect of short day-length (12L: 12D) on pre-oviposition period was analysed using a Mann Whitney *U*-test ($P<0.05$), adjusted for ties. However, for ease of interpretation all data are presented as means followed by standard errors of the means.

Where it was not possible to count eggs every day, the numbers of eggs collected over two to three day intervals were represented as means for those days prior to statistical analysis of oviposition rate (eggs day $^{-1}$). Data for oviposition rate of beetles in each treatment were tested for an approximation to the normal distribution before carrying out an analysis of variance (ANOVA, $P<0.05$) within and across treatments. Replicates that did not survive to the end of experimentation were excluded from the statistical analyses.

Data for survivorship of *S. punctillum* in each treatment are presented as percentages and were calculated as described in *section 7.3.1*.

7.4 RESULTS

7.4.1 Experiment 1

The effect of short day-length (12L: 12D) on mean development period and survivorship in *S. punctillum* is displayed in *Table 7.4.1.1.* Short day-length did not significantly effect the duration of juvenile development in *S. punctillum* when compared with the duration of development under long day-lengths (16L: 8D) ($P>0.05$).

Cumulative percentage survivorship from egg to adult of *S. punctillum* reared under short day-lengths (46 %) was higher compared with beetles reared under long day-lengths (29 %). The egg stage was shown to be the most vulnerable stage under short day-lengths and the egg and first larval stage were shown to be the most vulnerable stages under long day-lengths.

7.4.2 Experiment 2

When *S. punctillum* were reared under long day-lengths (16L: 8D) in *treatment 1* 100 % and 82 % survivorship was recorded for male and female beetles, respectively. In *treatment 2*, 75 % survivorship was recorded for both males and females when reared under short day-lengths (12L: 12D). When these beetles were transferred to long day-lengths no further mortality was observed. In *treatment 3*, 100 % and 90 % survivorship was observed for male and female beetles under long day-lengths, respectively. When beetles were transferred to short day-lengths of 12L: 12D no further deaths were recorded. No male deaths were recorded when beetles were then transferred to shorter day-lengths of 8L: 16D. However, 67 % survivorship was recorded for the female beetles.

Effect of photoperiod on pre-oviposition period and oviposition rate (eggs day $^{-1}$) of *S. punctillum* is displayed in *Table 7.4.2.1.* Statistical analysis indicated that photoperiod had a significant effect on pre-oviposition period ($P<0.05$). Mean (± SE) pre-oviposition period for beetles incubated under short day-lengths (12L: 12D) was 6.87 ± 1.12 days compared with 2.54 ± 0.16 days for beetles incubated under long day-lengths (16L: 8D).

Table 7.4.1.1 **The effect of long day- (16L: 8D) (*n* = 17) and short day- (12L: 12D) (*n* = 13) length on the mean (SE) development time (days) and cumulative percentage survivorship (CPS) of *Stethorus punctillum* at 25 ^{0}C and 75 % r.h. when fed to satiation on two-spotted spider mites (*Tetranychus urticae*) infesting bean leaves (*Vicia faba* cv. Maris Bead).**

Development period	Photoperiod						*P*
	16L: 8D			12L: 12D			
	Mean (SE)	*n*	CPS	Mean (SE)	*n*	CPS	
Egg to first instar	3.9(0.2)a	5	29	3.6(0.1)a	10	77	**0.197**
First instar to second instar	1.1(0.2)a	5	29	1.2(0.2)a	8	61	**1.000**
Second instar to third instar	0.6(0.1)a	5	29	0.8(0.1)a	6	46	**0.383**
Third instar to fourth instar	0.8(0.1)a	5	29	0.8(0.1)a	6	46	**0.913**
Fourth instar to pupa	1.9(0.1)a	5	29	2.2(0.1)a	6	46	**0.130**
Pupa to adult	2.8(0.1)a	5	29	3.2(0.1)a	6	46	**0.071**
Egg to adult	11.1(0.5)a	5	29	11.4(0.2)a	6	46	**0.598**

Means followed by the same letter in rows are not significantly different (*t*-test or Mann Whitney *U*-test, $P<0.05$).

Table 7.4.2.1 **Effect of photoperiod on pre-oviposition period and oviposition rate of *Stethorus punctillum* when fed to satiation on two-spotted spider mites (*Tetranychus urticae*) infesting bean leaves (*Vicia faba* cv. Maris Bead) at 25 ^{0}C and 75 % r.h..**

Treatment	Photoperiod regime	*n*	Pre-oviposition period (days)			*n*	Oviposition rate (egg day $^{-1}$)		
			Mean	Range	SE		Mean	Range	SD
1	16L: 8D	11	2.54a	2-3	0.16	10	12.24a	0-31	
2	12L: 12D 16L: 8D	8	6.87b	3-13	1.12	6	7.33b	0-22	2.36
3	16L: 8D 12L: 12D 8L: 16D					6	10.63a	0-29	
P			**0.001**				**0.003**		

Means followed by the same letters in columns are not significantly different [Mann Whitney *U*-test (pre-oviposition period) or ANOVA (oviposition rate), $P<0.05$).

n = Number of male and female pairs.

A significantly lower mean (± SD) oviposition rate (eggs day $^{-1}$) was achieved by females in *treatment 2* (7.33 ± 2.36 eggs day $^{-1}$) compared with *treatments 1* (12.24 ± 2.36 eggs day $^{-1}$) and *3* (10.63 ± 2.36 eggs day $^{-1}$) (ANOVA, P=0.003) (*Table 7.4.2.1*). There were no significant differences in oviposition rates between *treatments 1* and *3*, although the mean rate of oviposition in *treatment 1* was higher compared with *treatment 3*.

Further analysis of oviposition rate (eggs day $^{-1}$) within treatments (*Table 7.4.2.2*) showed that when beetles were reared under short day-lengths (12L: 12D) and then transferred to long day-lengths (16L: 8D) in *treatment 2* there was a significant increase in oviposition rate (ANOVA, P<0.05). When beetles were reared under long day-lengths (16L: 8D) and then transferred to short day-lengths (12L: 12D) in *treatment 3* there was no significant effect on oviposition rate. However, when beetles were then transferred to shorter day-lengths of 8L: 16D, oviposition rate significantly decreased from 14.11 to 4.38 eggs day $^{-1}$ (ANOVA, P<0.05). The incidence of reproductive arrest also increased from 0 to 50 % (0 to 3 beetles) (*Table 7.4.2.2*).

In *treatment 2*, two females ceased egg lay at 12L: 12D for 15 and 20 days after pre-oviposition periods of eight days followed by oviposition periods of 15 and 10 days, respectively. When the females were transferred to 16L: 8D, oviposition resumed one to two days later. The oviposition rates of these females were 1.23 and 0.67 eggs day $^{-1}$ at 12L: 12D, and 9.75 and 8.85 eggs day $^{-1}$ at 16L: 8D, respectively. Overall the range of oviposition rates under long-days was 8.85 to 13.60 eggs day $^{-1}$, therefore the rates of egg lay for these two females were comparable with the other beetles in this treatment. In contrast, females that were transferred to 12L: 12D for 20 days after being reared from eclosion at 16L: 8D continued to lay eggs throughout this period.

Table 7.4.2.2 **Effect of transferring *Stethorus punctillum* between photoperiod regimes of long day- (16L: 8D), short day- (12L: 12D) and shorter day- (8D: 16L) lengths on oviposition rate[1] when beetles were fed to satiation on two-spotted spider mites (*Tetranychus urticae*) infesting bean leaves (*Vicia faba* cv. Maris Bead) at 25 ^{0}C and 75 % r.h..**

Treatment	Photoperiod (duration in days)	*n*	Incidence (%) of reproductive arrest[2]	Oviposition rate (eggs day $^{-1}$)		
				Mean	Range	SE
1	16L: 8D	10	0	12.24[3]		
2	12L: 12D	6	33	5.12a	0-21	0.39
	16L: 8D		0	10.66b	0-22	0.47
P				**0.014**		
3	16L: 8D	6	0	14.31a	4-28	0.74
	12L: 12D		0	14.11a	0-29	0.64
	8L: 16D		50	4.38b	0-17	0.55
P				**0.001**		

Means followed by the same letter in columns are not significantly different (ANOVA, $P<0.05$).

[1] Not including pre-oviposition period.

[2] Percentage of females displaying a reproductive arrest of two to 20 days.

[3] Included for comparison only, not included in the within-treatment analyses.

7.5 DISCUSSION

7.5.1 Experiment 1

Short day-length (12L: 12D) did not significantly effect development period in *S. punctillum* from egg to adult and this has also been shown for *C. septempunctata* larvae (Hodek, 1958) and *Coccinella novemnotata* Herbst (Coleoptera: Coccinellidae) larvae (McMullen, 1967). However, previous evidence shows that insect larvae are sensitive to diapause inducing stimuli (e.g. Tadmor and Applebaum, 1971; Storch and Vaundell, 1972; Tauber *et al.*, 1993). For example, Tadmor and Applebaum (1971) reported that pre-imaginal stages of *Chilocorus bipustulatus* L. (Coleoptera: Coccinellidae) are sensitive to stimuli controlling the onset of diapause. Iperti and Prudent (1986) reported a gradual photoperiodic activation of *A. bipunctata* reared before adult emergence at 12L: 12D and reared as adults at 13L: 11D. Similarly, Tauber *et al.* (1993) reared larvae of *C. carnea* under short day-lengths of 10L: 14D to, as they stated, induce diapause. Retarded ovogenesis has been reported in females who were subjected to diapause inducing conditions as juveniles (Hodek, 1996a).

7.5.2 Experiment 2

Differences in the length of pre-oviposition periods under opposing photoperiod regimes are usually used as a measure of the photoperiodic response (Hodek and Růžička, 1979). Data for *S. punctillum* indicated that pre-oviposition period was significantly longer in beetles reared from adult eclosion under short day-lengths (12L: 12D) compared with beetles that had been reared under long day-lengths (16L: 8D). Similar results have been reported for other species of coccinellids [e.g. Hodek and Růžička, 1979: *C. septempunctata*; Hodek and Iperti, 1983: *Semiadalia undecimnotata* (Schneider)]. Hodek and Růžička (1979) reported that the majority of *C. septempunctata* females collected in October began laying eggs within 14 days of exposure to long-day conditions and in contrast the majority of females exposed to short-day conditions of 12L: 12D oviposited after 56 to 72 days.

Frequently it is necessary to isolate results for individuals of test populations to gain a clearer picture of events. For example, Hodek and Růžička (1979) concluded from their observations on diapause in *C. septempunctata* that estimations based on sample populations may be misleading because these populations are rarely homogenous.

Although short day-lengths of 12L: 12D were considered to be diapause-inducing in *Hippodamia tredecimpunctata* (L.) (Coleoptera: Coccinellidae), one third of females tested continued to lay eggs (Storch and Vaundell, 1972).

In the present study, half the test population of *S. punctillum* (three female beetles) in *treatment 3* continued to lay eggs after transfer to conditions of 8L: 16D but at a reduced rate and egg lay ceased eight to 10 days after transfer from 12L: 12D to 8L: 16D for the remainder of the test population. The apparent discrepancies observed in the results where a photoperiod of 12L: 12D appeared to induce a reduction in oviposition rate in *treatment 2* but not in *treatment 3* may be explained by comparing oviposition rates for beetles in *treatments 1* and *3* held under 16L: 8D. It appears that inadvertent selection was made of females that tended to display an elevated oviposition rate and these were included in *treatment 3*. Indeed 100 % of the females tested in *treatment 3* had oviposition rates in excess of 10 eggs day $^{-1}$ when held under photoperiod conditions of 16L: 8D, compared with 70 % of females in *treatment 2* when held under equivalent conditions.

Polymorphism amongst coccinellids in relation to fecundity is common (Hodek, 1973) and indeed the plasticity of *C. septempunctata* is said to be largely due to polymorphism in the photoperiodic response (Hodek and Růžička, 1979), which enables it to make use of temporary niches (Hodek, 1986). In the present study, differences in the fecundity of *S. punctillum* subjected to the same conditions of photoperiod may explain the overall similarity in oviposition rates between *treatments 1* and *3* (*Table 7.4.2.1*) despite the decrease in photoperiod, which was shown to have an effect on oviposition rate in *treatment 2*.

Alternatively, the age of beetles tested and their sensitivity to photoperiod may explain the differences observed. When *S. punctillum* were reared from eclosion under short-days (12L: 12D) two female beetles ceased egg lay which was resumed when they were transferred to long-day conditions. In contrast, beetles that were reared from eclosion under long-day conditions and then transferred to short-day conditions continued to lay eggs. This may suggest that once *S. punctillum* had started to oviposit under long day-lengths transferral to short day-lengths of 12L: 12D for 20 days was not sufficient to prevent egg lay. However, a reproductive arrest could be induced in 50 % of the test

population if beetles were reared from eclosion under short-days at 12L: 12D. Newly eclosed adults of *S. punctillum* (*treatment 2*) appeared to be more sensitive to short day-lengths (12L: 12D) compared with 16- to 18-day old adults (*treatment 3*). My research confirms McMullen's (1967) who stated that the stage sensitive to diapause induction in *C. novemnotata* is the young adult aged one to seven days. However, this was challenged by Hodek (1996a) who postulated that the results were based on dissections possibly carried out too early to be of any relevance.

In the present study, despite the fact that adults were aged between 36 and 38 days old, a further decrease in day-length from 12L: 12D to 8L: 16D prevented oviposition in 50 % of the test population of *S. punctillum*, suggesting a sensitivity to reduced photoperiod even in older adults.

Previous evidence has suggested that temperature, food supply, population density (e.g. Anderson and Hales, 1986; Hodek, 1986; Hodek, 1996a) and age (e.g. McMullen, 1967) modify the photoperiodic response. Here the results suggest that newly eclosed adults are more sensitive to short day-lengths of 12L: 12D compared with 16- to 18-day old adults, that is, the photoperiodic response of *S. punctillum* is possibly modified by age. This confirms earlier research by McMullen (1967).

8 OLFACTORY RESPONSES OF STETHORUS PUNCTILLUM

8.1 INTRODUCTION

Prior to 1980, researchers tended to conclude that host location by arthropods was random and relied upon physical contact (e.g. Fleschner, 1950; Banks, 1957). Fleschner (1950) studied the searching capacity of larval predators of the citrus red mite, *Paratetranychus citri* (McGregor) (Acari: Tetranychidae), i.e. *Stethorus picipes* Casey (Coleoptera: Coccinellidae), the green lacewing, *Chrysopa californica* Coquilet (Neuroptera: Chrysopidae) and the dusty wing, *Conwentzia hageni* Banks (Neuroptera: Coniopterygidae) and concluded that searching was random with perception of prey occurring upon physical contact only.

Behaviour that is thought to render predator-prey encounters more probable includes tactic responses (Hodek, 1996b), e.g. positive phototaxis and negative geotaxis of a predator corresponding to that of its prey (e.g. Fleschner, 1950; Dixon, 1959; Ng, 1986) and movement related to plant structure, e.g. direction of predator movement was thought to be determined by leaf edges, prominent leaf veins and the arrangement of plant leaves (e.g. Banks, 1957; Dixon, 1959). Modification of search patterns in the presence of host odours, e.g. decrease in speed and increased turning in shorter distances (e.g. Banks, 1957, Dixon, 1959; Nakamuta, 1985b), is suggested to increase the efficiency of searching for aggregated prey (Dixon, 2000).

Banks (1957) reported that coccinellid larvae wasted time and energy by repeatedly searching plant parts already visited. A subsequent study by Marks (1977) indicated that larvae of the aphidophagous coccinellid, *Coccinella septempunctata* marked plants chemically while searching; presumably to indicate previously searched patches. It was stated that aphids may be disturbed, facilitating prey capture by coccinellids crossing their original path. However, attempts to repeat this study were unsuccessful (Dixon, 2000).

Further research provided evidence to support the involvement of olfaction in host location by coccinellids (e.g. Heideri and Copland, 1992; Ponsonby and Copland, 1995;

Hattingh and Samways, 1995; Hamilton *et al.*, 1999; Raymond *et al.*, 2000) and ignorance of this perception in earlier experiments was thought to be because the distances involved are very short to the human observer (Stubbs, 1980). In addition, the apparent neglect of aphids shown by coccinellids in the laboratory (McAllister and Roitberg, 1987) may be due to the coccinellids being in a phase of the behavioural cycle where eating an aphid is not a priority (Hodek, 1996b).

Sensillae in the prolegs [e.g. Storch, 1976; *Coccinella transversoguttata* (Falderman)] and in the tips of the antennae (e.g. Hamilton *et al.*, 1999; *Hippodamia convergens* Guérin-Méneville) have been implicated in distant prey location by predatory coccinellidae.

Research to determine the mechanisms underlying the ability of *Stethorus* spp. to locate their prey remains inconclusive. For example, Putman (1955) reported that adults of *Stethorus punctillum* displayed no attraction towards two-spotted spider mites on broad bean leaves and that larvae only detected prey by physical contact. Subsequent research on adults of *Stethorus punctum* reported an attraction of the coccinellid to the European red mite, *Panonychus ulmi* (Koch) (Acari: Tetranychidae) and *P. ulmi*-infested apple leaves (Colburn and Asquith, 1970). However, the apparatus used allowed both sight of, and direct contact with, the mites. The ability of *Stethorus* spp. to locate rare prey patches (Hull *et al.*, 1977; Congdon *et al.*, 1993) suggests that a stimulus other than random search is used to locate their prey and this is thought to involve chemotaxis (Congdon *et al.*, 1993).

Four-arm olfactometers have been used to evaluate orientation to odours by parasitoids (e.g. Vet *et al.*, 1983) and coccinellids (e.g. Ponsonby and Copland, 1995; Raymond *et al.*, 2000). The relative attractiveness, for example, of volatiles emitted by host prey and host plants may be evaluated by measuring parameters of insect movement, e.g. distance travelled, turning rate and speed (e.g. Vet *et al.*, 1983; Ponsonby and Copland, 1995). The aim of my study was to evaluate the ability of *S. punctillum* to detect host odours from a distance of more than a few centimetres using a four-arm olfactometer.

8.2 MATERIALS AND METHODS

8.2.1 Olfactometer assembly

A four-arm olfactometer (glass chamber measurements: 230 mm x 230 mm), as described by Vet *et al.* (1983), was used to investigate the olfactory ability of *S. punctillum* adults to orientate towards bean leaves (*Vicia faba* cv. Maris Bead) only and bean leaves infested with two-spotted spider mites (*Tetranychus urticae*). The olfactometer was modified in the following ways:

- Air was drawn through PVC tubing (6 mm in diameter) from outside the laboratory and passed through an activated carbon filter before entering the glass jars;
- The exposure chamber was edged with 'O' ring rubber held in place with sealant, and the lid was secured with plastic clamps in order to prevent leakage;
- Each arm of the olfactometer was connected to a set of three glass jars (capacity of 250 ml). The first jars, nearest to the olfactometer chamber, served to trap any beetles that ventured out of the arena and along the connecting tubing. The second jar contained the odour source and the third contained 200 ml of water to maintain a constant humidity within the chamber;
- Hard, plastic tubing (9 mm by 50 mm) with one end sealed with mesh (*section 3.4*) was used to introduce the beetles into the arena;
- A glass bell jar (capacity of 10 L) was connected between the vacuum pump and olfactometer to smooth the airflow;
- The arena itself was placed inside a wooden box that was white inside and only allowed the entry of light from a circular, fluorescent tube through a Perspex ceiling;
- Activity was viewed on a monitor attached to a black and white video mini-camera with a 3.5 mm lens.

The four odour fields were visualised by placing ammonium hydroxide (NH_4OH) in the jars used for containing the odour source and hydrochloric acid (HCl) in the trap jars. Results were similar to those of Vet *et al.* (1983), with the most definite edges to the

odour field occurring at 0.30 l min $^{-1}$. Results also agreed with Ponsonby and Copland (1995), where a small, turbulent area was found between odour fields. All subsequent trials were carried out at 27 to 30 ^{0}C, with an airflow rate of 0.30 l min $^{-1}$.

Beetles used in the trials were reared as described in *section 3.4* and were two to 40 days post eclosion when used in the experiments. To account for the possible affect of circadian rhythms, beetles were tested between 9 am and 4 pm. Each beetle was starved for 18 to 24 hours, in an attempt to overcome heterogeneity in hunger levels, before being introduced into the arena and each beetle was tested once only.

Preliminary tests showed that beetles were more likely to enter the test arena if allowed to alight from the central plastic ring (*Figure 8.2.1.1*) before the air pump was connected and this procedure was incorporated into the trials. The video recorder was set to record beetle movement in the arena after connection of the arena to the air pump. Beetles were then recorded for the next 10 minutes. Beetles that failed to enter the arena within 10 minutes of introduction into the holding tube were discounted from any statistical analyses. After 15 beetles were introduced into the apparatus, the arena was thoroughly washed with Decon 90® (Decon Laboratories Ltd, East Sussex, UK), rinsed with distilled water and swabbed with 70 % ethanol. Between odour trials the apparatus was soaked overnight in Decon 90® pre rinsing, as before.

8.2.2 Odour trials

Odourless trials (*treatment 1*) were used to provide a control and to test for any directional bias. In these trials clean, humidified air was released into the arena through all four arms of the olfactometer.

Two further treatments followed where the host odour source was placed in one arm of the olfactometer. The second treatment tested the behaviour of *S. punctillum* adults in response to possible odours emitted from clean bean leaves of *V. faba* cv. Maris Bead, not previously exposed to two-spotted spider mites, *T. urticae* (*treatment 2*). The third treatment tested the behaviour of *S. punctillum* adults in response to possible odours

emitted from bean leaves infested with all developmental stages of two-spotted spider mites (*treatment 3*).

When a beetle crossed line '*a*' (*Figure 8.2.1.1*) in the direction of the odour source, this was accepted as a choice for that odour field. A computer/video measuring package (*Micromeasure*; Varley *et al.*, 1994) was used to analyse five variables:

Distance walked	(**mm**);
Time spent walking in each field	(**s**);
Turning of a beetle's path related to distance (turning rate)	(^{0} **mm** $^{-1}$);
Turning of a beetle's path related to time (angular velocity)	(^{0} **s**$^{-1}$);
Net speed (average speed of a beetle whilst it was moving)	(**mm s**$^{-1}$).

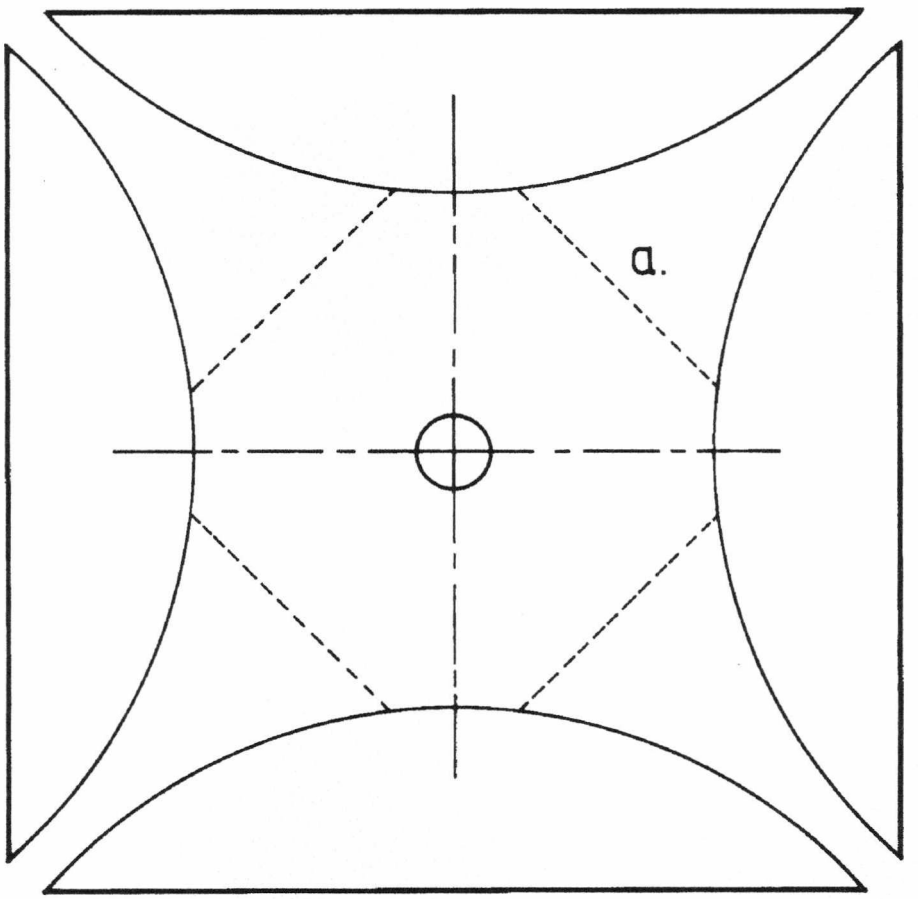


Figure 8.2.1.1 Diagrammatic representation of the glass chamber of a four-arm olfactometer indicating the first choice line (a). (*Courtesy of K. F. Beverley*)

8.3 STATISTICAL ANALYSES

The Chi-squared goodness-of-fit test was used to test for directional preferences in terms of first choices made by *S. punctillum* in all three treatments (*Table 8.4.1*). Odourless trials assumed that each arm had an equal chance of selection. Trials where odour was supplied through one arm assumed that a beetle would have a one in four chance of selecting the odour-carrying field.

Treatment by treatment total means were tested for approximation to a normal distribution before carrying out a non parametric version of a one-way analysis of variance; Kruskal Wallis test ($P<0.05$), adjusted for ties (*Table 8.4.2*). Field by field means were tested for approximation to a normal distribution before carrying out a two-way analysis of variance ($P<0.05$). For data that was not normally distributed, a non parametric version of a two-way analysis of variance; the Friedman test ($P<0.05$) was used (*Table 8.4.3*). However, for ease of interpretation all data are presented as means followed by the standard errors of the means.

Comparisons were made between the field containing odour from bean leaves or bean leaves plus two-spotted spider mites and the fields containing clean air. Data were tested for an approximation to a normal distribution before carrying out a paired *t*-test ($P<0.05$). Data that were not normally distributed were analysed using a non parametric version of the paired *t*-test; Wilcoxon signed rank test ($P<0.05$). Analyses of data for distance walked and time in odour field assumed a null hypothesis that 25 % of time or distance walked was in the odour field containing the host odours. Beetles that spent all of their time in the odour field containing host odours or all of their time in the other three fields were excluded from the statistical analyses as their relative behaviour in the four fields could not be compared. For ease of interpretation all data are presented as means followed by the standard errors of the means (*Table 8.4.4*).

8.4 RESULTS

Odourless controls indicated no significant difference in directional response by *S. punctillum* demonstrating that there was no bias in the equipment. Trials involving odours from the host plant, *V. faba* and those involving a mixture of odours from *V. faba* plus the host prey, *T. urticae* also revealed no directional bias (*Table 8.4.1*).

Table 8.4.1 **Directional response of *Stethorus punctillum* to odourless air, odour of host bean plant, *Vicia faba* cv. Maris Bead and odour of host bean plant infested with two-spotted spider mites, *Tetranychus urticae* in a four-arm olfactometer: first choice of odour field.**

Treatment	*n*	No. of first choices/odour field		X^2	*P*
		1	2 3 4		
		odour source	clean air		
(1) Control	28[1]	8	6 6 8	0.57	0.95
(2) Host plant only	35	13	22	2.46	0.50
(3) Host plant plus prey mite	37	12	25	3.29	0.50

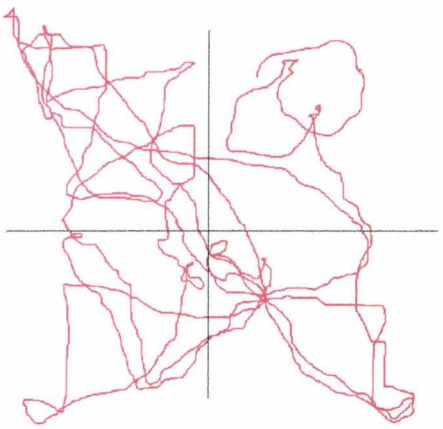
[1] 29 beetles were tested in total. However, one beetle failed to cross a choice line.

S. punctillum adults were observed to orientate towards host odours in the four-arm olfactometer and typical traces of one beetle from each treatment are displayed in *Figure 8.4.1*. The presence of bean leaves with and without spider mites modified the behaviour of *S. punctillum*; the distance walked and the time spent walking were significantly increased in the presence of host odours (*Table 8.4.2*). In general, turning rate, angular velocity and net speed were not significantly altered in the presence of host odours.

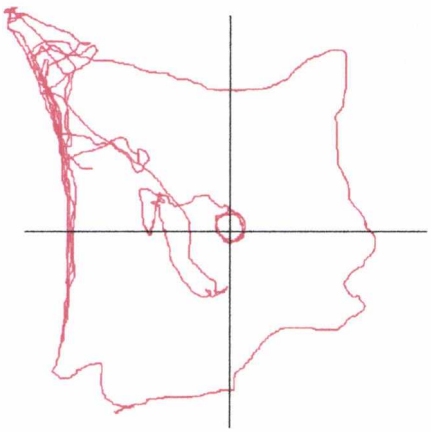
No directional bias in terms of first choice was detected in the presence of host plant odours in *treatment 2* (*Table 8.4.1*) and this was also the case in the field by field analysis for this treatment (*Table 8.4.3*). When comparing the behaviour of *S. punctillum* in the field containing host plant odours to the behaviour in the fields

containing clean air (*Table 8.4.4*), in general beetles walked further, spent more time walking, displayed an increased turning rate, angular velocity and net speed although the response was only significant for turning rate (P=0.015).

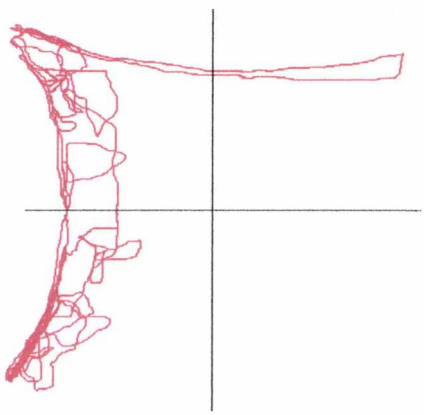
Similar results were observed in response to host plant plus host prey odours and the responses were significant for distance walked (P=0.037), turning rate (P=0.026) and angular velocity (P=0.019) (*Table 8.4.4*). Although the results for time spent walking and time spent stationary in the field containing the host plant plus host prey odours were not significantly different from the fields containing clean air, the probability statistic for the former behavioural parameter was 0.060, indicating a weak tendency to significantly increased time spent walking in the field containing host odours compared with the other three fields. Mean time spent stationary in the odour field containing host plant odours plus host prey odours was 113.50 s compared with 61.67 s for the other three fields but the difference was not significant (P=0.261).



(a) Typical trace of a beetle in response to clean air from all four arms of the olfactometer.



(b) Typical trace of a beetle in response to *V. faba* odour only, in the top left-hand field.



(c) Typical trace of a beetle in response to *V. faba* plus *T. urticae* odour in the top left-hand field.

Figure 8.4.1 **Typical traces of *Stethorus punctillum* beetles responding to either clean air in treatment 1 (*fig. a*), odour from *Vicia faba* cv. Maris Bead in treatment 2 (*fig. b*), or odours from *V. faba* plus *Tetranychus urticae* in treatment 3 (*fig. c*), in 600s of exposure in a four-arm olfactometer.**

Table 8.4.2 **Responses of *Stethorus punctillum* to clean air (treatment 1), odour from bean leaves, *Vicia faba* cv. Maris Bead (treatment 2) and odour from *V. faba* cv. Maris Bead infested with two-spotted spider mites, *Tetranychus urticae* (treatment 3) when exposed for 600s in a four-arm olfactometer: mean ± SE.**

Treatment	*n*	Distance walked (mm)	Time spent walking (s)	Turning rate (^{0} mm $^{-1}$)	Angular velocity (^{0} s $^{-1}$)	Net speed (mm s^{-1})
(1) Control	29	346.64 ± 30.77a	48.28 ± 4.46a	18.38 ± 0.92a	130.03 ± 6.19a	6.80 ± 0.33a
(2) Host plant only	35	533.86 ± 34.71b	77.25 ± 4.89b	18.05 ± 0.49a	129.69 ± 5.66a	8.60 ± 1.49a
(3) Host plant plus prey mite	37	499.81 ± 31.85b	74.32 ± 5.04b	18.23 ± 0.36a	130.03 ± 6.19a	6.66 ± 0.20a
P		**0.001**	**0.001**	**0.882**	**0.157**	**0.450**
H		14.40	18.44	0.25	3.70	1.60

Comparisons made between treatments using the Kruskal Wallis test ($P<0.05$).
Odour trials were carried out at 27 – 30 ^{0}C. Activity was viewed on a monitor attached to a black and white video mini-camera with a 3.5 mm lens. A computer/video measuring package (*Micromeasure*; Varley *et al.*, 1994) was used to analyse the variables.

Table 8.4.3 **Responses of *Stethorus punctillum* to the odour from bean leaves, *Vicia faba* cv. Maris Bead (treatment 2) when exposed for 600s in a four-arm olfactometer ($n = 35$): mean ± SE.**

Odour field[1]	Distance walked (mm)	Time spent walking (s)	Time spent stationary (s)
Leaf	553.93 ± 65.48	81.35 ± 10.13	67.66 ± 15.68
Clean air (a)	562.48 ± 80.56	86.99 ± 10.63	87.94 ± 21.94
Clean air (o)	497.49 ± 66.26	72.54 ± 9.54	72.07 ± 18.02
Clean air (a)	514.41 ± 66.15	67.27 ± 8.78	61.24 ± 19.61
P	**0.797**	**0.477**	**0.830**

Odour field[1]	Turning rate (^{0} mm $^{-1}$)	Angular velocity (^{0} s $^{-1}$)	Net speed (mm s^{-1})
Leaf	17.99 ± 1.44	123.25 ± 8.56	11.30 ± 5.32
Clean air (a)	17.49 ± 1.22	123.58 ± 7.76	8.03 ± 1.38
Clean air (o)	17.66 ± 1.32	138.71 ± 21.60	7.71 ± 1.68
Clean air (a)	19.07 ± 1.48	133.22 ± 8.47	7.37 ± 0.75
P	**0.934**	**0.960**	**0.960**

Comparisons made between behaviour in odour fields using a two-way analysis of variance or Friedman test ($P<0.05$).
[1] a = fields adjacent to odour source, o = fields opposite odour source. Odour trials were carried out at 27 – 30 ^{0}C. Activity was viewed on a monitor attached to a black and white video mini-camera with a 3.5 mm lens. A computer/video measuring package (*Micromeasure*; Varley *et al.*, 1994) was used to analyse the variables.

Table 8.4.4 **Responses of *Stethorus punctillum* to the odour from bean leaves, *Vicia faba* cv. Maris Bead (treatment 2) and odours from *V. faba* cv. Maris Bead plus two-spotted spider mites, *Tetranychus urticae* (TSSM) (treatment 3) when exposed for 600s in a four-arm olfactometer: mean ± SE.**

Treatment	*n*	Distance walked (mm)	Time spent walking (s)	Time spent stationary (s)	Turning rate ($^0mm^{-1}$)	Angular velocity ($^0s^{-1}$)	Net speed ($mm\ s^{-1}$)
(2)Leaves	30	616.49 ± 69.98	91.43 ± 10.14	76.99 ± 17.22	20.42 ± 1.08	139.07 ± 4.62	12.91 ± 6.17
Clean air		551.80 ± 57.12	79.74 ± 6.71	78.93 ± 15.17	17.75 ± 0.93	134.51 ± 11.32	8.16 ± 1.27
P		**0.742**	**0.337**	**0.837**	**0.015**	**0.195**	**0.886**
(3)Leaves+TSSM	36	630.73 ± 69.73	92.91 ± 10.54	113.50 ± 23.08	20.66 ± 0.69	143.54 ± 6.02	7.09 ± 0.32
Clean air		467.87 ± 43.84	70.60 ± 6.49	61.67 ± 8.24	17.68 ± 0.86	127.31 ± 7.45	6.59 ± 0.41
P		**0.037**	**0.060**	**0.261**	**0.026**	**0.019**	**0.261**

Comparisons made between behaviour in odour and clean air using paired *t*-tests or Wilcoxon signed rank tests ($P<0.05$).
Odour trials were carried out at 27 – 30 ^{0}C. Activity was viewed on a monitor attached to a black and white video mini-camera with a 3.5 mm lens. A computer/video measuring package (*Micromeasure*; Varley *et al.*, 1994) was used to analyse the variables.

8.5 DISCUSSION

Typical traces of beetle responses (*Figure 8.4.1*) to odours from the host plant and a mixture of odours from the host plant plus host prey were similar to those published by Ponsonby and Copland (1995) for *Chilocorus nigritus* in response to odours from the scale insect, *Abgrallaspis cyanophylli* (Signoret) (Homoptera: Diaspididae) and host plant, *Solanum tuberosum* L. In general, *S. punctillum* showed directed movement towards host odours from *V. faba* and *V. faba* plus *T. urticae*. Trace *c* in *Figure 8.4.1* indicates that beetles were not confined to the field carrying the host plant plus host mite odours and this was also reported by Ponsonby and Copland (1995) for *C. nigritus*. The authors observed *C. nigritus* showing directed movement towards odour fields containing volatiles from *S. tuberosum* plus *A. cyanophylli*, followed by downwind movement towards the odour field boundary. At the boundary the authors proposed that the cue was lost. Indeed, Nakamuta (1985ab) reported that intensive searching by *C. septempunctata* was varied and depended on the quality of the signal.

Treatment by treatment analyses indicated that the behaviour of *S. punctillum* was altered by the presence of *V. faba* odours either with or without two-spotted spider mite infestation compared with the control treatment (*Table 8.4.2*). However, field by field analyses (*Table 8.4.3*) and comparisons between the odour field containing the host plant odour and the fields containing clean air (*Table 8.4.4*) failed to show significant changes in response by *S. punctillum* apart from an increased turning rate. Responses for time spent walking narrowly missed significance. Raymond *et al.* (2000) tested the olfactory responses of *Adalia bipunctata* to *Aphis fabae* Scopoli (Homoptera: Aphididae) and the two plant species, *V. faba* cv. 'The Sutton' and *Tropaeolum majus* L. cv. 'Alaska'. The results suggested that the presence of aphids or their products was required to elicit an olfactory response in unfed coccinellids. In contrast, *H. convergens* has been shown to exhibit an olfactory response to aphid-free and aphid-infested radish plants (Hamilton *et al.*, 1999).

The behaviour of *S. punctillum* was only modified significantly in the presence of odours from the host plant plus host prey and although this did not include changes in net speed, there was a tendency for beetles to decrease their speed in the presence of

host plant plus host prey odours (*Table 8.4.2*). In contrast, Ponsonby and Copland (1995) showed that the net speed of *C. nigritus* tended to increase in the presence of odours from *S. tuberosum* or *S. tuberosum* plus *A. cyanophylli*. *S. punctillum* showed a tendency to arrest in response to odours from the host plant plus host prey (although this response was not statistically significant) and this has also been shown for *Exochomus* sp. (Coleoptera: Coccinellidae) and *Diomus* sp. (Coleoptera: Coccinellidae) in response to wax and honeydew from the mealybugs, *Phenococcus manihoti* Matile-Ferrero (Homoptera: Pseudococcidae) and *Planococcus citri* (Risso) (Homoptera: Pseudococcidae) (Van den Meiracker *et al.*, 1990).

The increased activity of *S. punctillum* in the presence of mite-infested bean plants may facilitate host finding by the coccinellid (see below). In biological control this is of particular importance when predators are often released into a glasshouse remote from the specific sites of pest infestation. The inclusion of a biological control agent that is as highly mobile as *S. punctillum* (Readshaw, 1971) and able to orientate towards host odours, as indicated here, would be of great advantage to two-spotted spider mite pest management in glasshouses.

Previous research evaluating olfaction in *Stethorus* spp. has been inconclusive. For example, in experiments with *P. ulmi* and apple leaves, *S. punctum* was shown to be significantly attracted to *P. ulmi* and apple leaves versus apple leaves alone (Colburn and Asquith, 1970). However, the predators' attraction to these odours compared with clean air was not evaluated. Furthermore, the structure of the odour fields was not determined.

The results presented in this study contradict those of Putman (1955), who reported no attraction of *S. punctillum* to spider mites on broad bean leaves and my results suggest that the behaviour of *S. punctillum* is modified in the odour plumes from *V. faba* and *V. faba* plus *T. urticae* when compared with a clean air treatment. Physical contact was not necessary to elicit a response in this research.

The ability of *S. punctillum* to locate rare prey patches (Hull *et al.*, 1977; Congdon *et al.*, 1993) is perhaps a consequence of behavioural changes in response to host odours, as exhibited here, which would increase the probability of host location. Congdon *et al.* (1993) postulated that factors other than the numerical response of *S. punctillum* are critical to its positive impact on pest populations. Area restricted search, where movement is slower and the frequency of turns is increased (e.g. Hattingh and Samways, 1995; Ponsonby and Copland, 1995) allows a predator to stay in a particular location for longer, facilitating prey capture if the prey are aggregated (Dixon, 2000). Spider mite infestations are often patchy on groups of plants intermittently spread throughout a glasshouse (e.g. Hussey and Scopes, 1985). Thus the indication is that this kind of searching behaviour would enhance spider mite capture by *S. punctillum.*

In conclusion, the results presented here show, for the first time, that *S. punctillum* is able to orientate towards *V. faba* and *V. faba* plus *T. urticae* using olfaction. The behaviour of *S. punctillum* was significantly modified in odour fields containing odours from *V. faba* plus *T. urticae.* Hierarchical host finding mechanisms in coccinellids that include vision, as well as olfaction, have previously been postulated (Hattingh and Samways, 1995; Ponsonby and Copland, 1995) and future work is also indicated to evaluate other possible processes involved (e.g. visual cues) in prey location by *S. punctillum.*

9 EVALUATION OF STETHORUS PUNCTILLUM AS A PREDATOR OF TETRANYCHUS URTICAE IN SEMI-FIELD TRIALS

9.1 INTRODUCTION

Ladybirds are widely used in augmentative biological control against aphids and coccids (Dixon, 2000). However, in outdoor (e.g. Hagen, 1962) and indoor (Hodek and Honěk, 1996) plant cultivation, their success has been hampered by unwanted dispersal away from the target crop. Other set-backs include high rearing costs of coccinellids. For example, rearing costs of *Stethorus picipes* were higher in comparison to the costs associated with phytoseiids (McMurtry, 1995). A thorough understanding of the organisms involved in biological control is a prerequisite for success in this field (DeBach and Rosen, 1991).

Stethorus spp. have been evaluated as predators of spider mites in a number of outdoor crops, e.g. seed lucerne (Bailey and Caon, 1986), raspberry (e.g. Charles *et al.*, 1985; Roy *et al.*, 1999) and strawberry (e.g. García-Marí and González-Zamora, 1999; Oatman *et al.*, 1985). A decrease in the density of phytophagous mites (e.g. Charles *et al.*, 1985; Bailey and Caon, 1986; Injac and Dulič, 1992) has been attributed to the action of a guild of predators including *Stethorus* spp. but also including, e.g. predatory thrips, mites and lacewings (e.g. Bailey and Caon, 1986; Injac and Dulič, 1992).

In contrast, trials that have investigated the efficiency of *Stethorus punctillum* as a predator of spider mites on edible glasshouse crops have received little attention. Such trials have included tomato, pepper, aubergine and cucumber plants (Raworth, 2001; Rott and Ponsonby, 2000ab). Failure of *S. punctillum* to establish on tomato was attributed to plant trichomes (Raworth, 2001). In contrast, Rott and Ponsonby (2000a) reported that *S. punctillum* was most active on tomato and pepper and least active on aubergine when distance travelled, time spent walking, walking speed, angular velocity and turning rate were measured on leaf discs under laboratory conditions. In semi-field trials on tomato and pepper, Rott and Ponsonby (2000b) found that *S. punctillum* showed considerable potential for controlling spider mites when used in combination with other predatory species.

Results from laboratory experiments often differ considerably from field situations (Force, 1974; Ehler and Hall, 1983). Information from field trials assists in the development of successful pest management strategies. The timing of release of natural enemies is essential to achieving the correct predator: prey ratios for sustained pest control (DeBach and Rosen, 1991). Therefore the following trials were generated to consider the efficacy of *S. punctillum* as a predator of *Tetranychus urticae* on cucumber plants in a greenhouse. The aim was to evaluate the establishment and response of *S. punctillum* on potted cucumber plants infested with *T. urticae* (*experiment 1*) and in a semi-field trial of cucumbers in a greenhouse (*experiment 2*).

9.2 MATERIALS AND METHODS

9.2.1 Experiment 1: Effect of releasing *S. punctillum* 0, 5, 10 and 15 days post establishment of *T. urticae* on cucumber plants.

Cucumber plants (*Cucumis sativus* cv. Enigma RZ®) were grown in peat pots as described in *section 3.2*. At the two-leaf stage they were transferred to large cylindrical insect cages (30 cm diameter; 42 cm height) that had circular vents of mesh (*section 3.6.1*) in the sides and in the lids for ventilation (one plant per cage). Petroleum jelly (Vaseline®) was smeared around the vents to aid prevention of arthropod migration into or out of the cylinders. The insect cages were arranged in a five by five randomised complete block design, where each block contained five treatments in total (i.e. 25 plants), in the CT room (*section 3.5.1*) and sealed using Nescofilm® followed by masking tape. *S. punctillum* were introduced as 4- to 19-day old adults (one male and one female per plant). Two-spotted spider mites (*T. urticae*) were introduced directly onto one leaf per plant as fecund adult females (10 per plant). Treatments were checked after five days for numbers of live two-spotted spider mites (eggs, nymphs and adults) and *S. punctillum* (eggs, larvae and adults). The experiment was repeated on two separate occasions and the treatments were as follows:-

Treatment 1: control (plant only);
Treatment 2: spider mites (*T. urticae*) and *S. punctillum* introduced together;
Treatment 3: *S. punctillum* introduced five days after spider mites;
Treatment 4: *S. punctillum* introduced 10 days after spider mites;
Treatment 5: *S. punctillum* introduced 15 days after spider mites.

9.2.2 Experiment 2: Semi-field trials on cucumber to evaluate the efficacy of *S. punctillum* as a predator of *T. urticae.*

Cucumber plants (*Cucumis sativus* cv. Enigma RZ®) were grown in peat pots as described in *section 3.2.* When the first true leaf had emerged 20 plants were transferred in peat pots to peat-free bags (2 May 2002). The peat-free bags were arranged in two rows of five bags and each bag contained two plants. The trial was carried out in a ventilated greenhouse and miniature data loggers were used to monitor temperature and relative humidity.

Data loggers (*section 3.5*) were positioned away from direct sunlight and in the upper and lower canopies of the crop as close to the leaf surfaces as possible to measure temperature and humidity throughout the experimental period. Plants were trained to the wire and all side shoots were removed until the introduction of *S. punctillum*, after which no foliage was removed to prevent removal of the predator from the crop. Plants were watered by an automatic watering system and fed with liquid tomato plant food (containing 5 % nitrogen, 5 % phosphate and 10 % potassium). Plants were fed at every watering.

The original intention was to introduce two-spotted spider mites when the first plant(s) reached the wire. However, natural levels of two-spotted spider mite infestation were so great at this point that no introductions were required. Eight male and female *S. punctillum* (16 adults in total) were evenly introduced into the crop on 2 August 2002. Two-spotted spider mite densities were measured on every leaf at the beginning of the trial using the damage index (Hussey and Scopes, 1985), and thereafter monitored on each assessment date, although overall damage indices per plant were high (4.0).

Each leaf of each plant was categorised as either upper or lower depending on its position relative to the middle of the plant. Six weekly destructive samples following the introduction of *S. punctillum* were conducted. Leaves from the upper and lower parts of alternate plants were removed on each sampling date. The numbers of live two-spotted spider mites (nymphs, adults and eggs) and *S. punctillum* (eggs, larvae, pupae and adults) per leaf were counted. Natural invasion of *Feltiella acarisuga* occurred in the experimental period and thus counts of this predator were included in the assessments.

9.3 STATISTICAL ANALYSES

9.3.1 Experiment 1

Forty eight percent of the plants died due to infestation of sciarids in the first run of this experiment and therefore, only the data from the second run were analysed. Data for spider mite and *S. punctillum* densities were not normally distributed and transformation of the data did not improve their distribution. However, data were analysed using a two-way ANOVA (general linear model; GLM) ($P<0.05$), as ANOVA offers a valid test even when data are not normally distributed (Zar, 1996). Data are presented as means (SE). Analysis of the means was carried out using a Tukey test with a 95 % confidence interval.

9.3.2 Experiment 2

Mean (SE) data for densities of *T. urticae*, *F. acarisuga* and *S. punctillum* on upper and lower leaves at each sampling date were calculated and displayed in *Figures 9.4.2.1* to *9.4.2.3*. Leaf samples were not taken from the lower crop canopy on the last assessment date (6 September 2002) due to substantial leaf death. Therefore data for the final assessment date were omitted from the statistical analyses. Data for *T. urticae* and *S. punctillum* were not normally distributed and transformation of the data did not improve their distribution. However, data were analysed using a two-way ANOVA (GLM) ($P<0.05$), as ANOVA offers a valid test even when data are not normally distributed (Zar, 1996). Data for *F. acarisuga* were not normally distributed and therefore data were square root transformed before carrying out a two-way ANOVA (GLM) ($P<0.05$) on the transformed data. Analysis of the means was carried out using a Tukey test with a 95 % confidence interval.

9.4 RESULTS

9.4.1 Experiment 1

Mean numbers of *T. urticae* and *S. punctillum* per plant are displayed in *Table 9.4.1.1*. *S. punctillum* were able to establish on cucumber plants and generally showed a significant increase with a significant increase in two-spotted spider mite density despite the relatively short length (five days) of the treatments post introduction of the predator (Two-way ANOVA, $P<0.05$).

Table 9.4.1.1 **Mean (SE) numbers of *Tetranychus urticae* and *Stethorus punctillum* per cucumber plant (*Cucumis sativus* cv. Enigma) five days after *S. punctillum* introduction, 22 ± 2 ^{0}C and 72-88 % r.h. within the insect cages and 23 ± 2 ^{0}C and 50-60 % r.h. outside the insect cages.**

Treatment[a]	*n*[b]	Mean (SE)	
		T. urticae	*S. punctillum*[c]
1	5	42.00(6.42)a[d]	0
2	5	123.00(9.35)a	1.00(0.77)a
3	5	18.80(5.10)a	0.60(0.60)a
4	5	102.60(10.45)a	22.20(6.51)b
5	4	926.75(236.16)b	32.50(7.96)b
P		**0.001**	**0.001**

Means followed by the same letters in columns are not significantly different (Two-way ANOVA, $P<0.05$).

[a] Treatment 1: control (plant only);

Treatment 2: spider mites (*T. urticae*) and *S. punctillum* introduced together;

Treatment 3: *S. punctillum* introduced five days after spider mites;

Treatment 4: *S. punctillum* introduced 10 days after spider mites;

Treatment 5: *S. punctillum* introduced 15 days after spider mites.

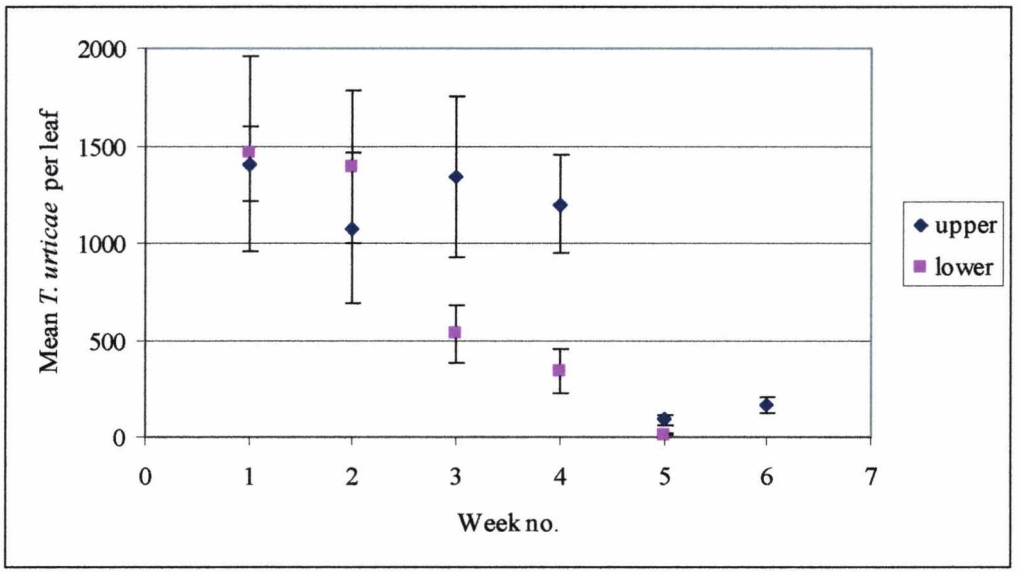
[b] *n* = Number of plants.

[c] Not including adults originally introduced.

[d] Spider mites migrated into the cages containing clean plants, despite the procedures followed to assist in preventing this.

9.4.2 Experiment 2

Data for mean (SE) numbers of *T. urticae*, *S. punctillum* and *F. acarisuga* per leaf in the upper and lower crop canopies for each assessment date are presented in *Figures 9.4.2.1*, *9.4.2.2* and *9.4.2.3*, respectively.



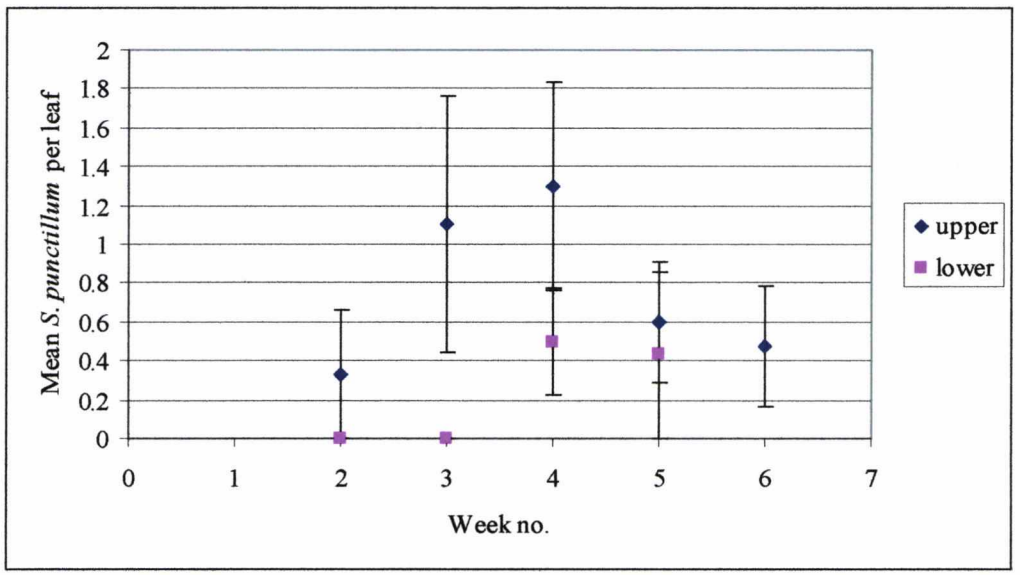
Factor	d.f.	*F* ratio	*P* value
Plant level	1	3.08	0.083
Assessment date	4	6.44	0.001
Error	84		

***Figure 9.4.2.1* Semi-field trial in a ventilated glasshouse showing mean density (vertical bars represent ± SE) of *Tetrancyhus urticae* per cucumber leaf (*Cucumis sativus* cv. Enigma) in the upper and lower canopies [average temperature 25 ^{0}C (upper) and 24 ^{0}C (lower) and average relative humidity 54 % (upper) and 53 % (lower)].**

Number of leaves sampled per week: 9, 9, 10, 10, 10 and 17 in the upper canopy and 7, 8, 10, 10 and 7 in the lower canopy.

There was no significant difference in two-spotted spider mite densities between crop canopy levels ($F_{1,84}$=3.08, P>0.05). However, there was an indication that two-spotted spider mite density varied significantly between assessment dates ($F_{4,84}$=6.44, P=0.001). Analysis of the means revealed that the mean (± SE) two-spotted spider mite density per leaf in week five was significantly lower than mean densities recorded in weeks one to

three. There was a narrowly missed significant difference between the mean densities recorded in weeks four and five (P=0.0781).

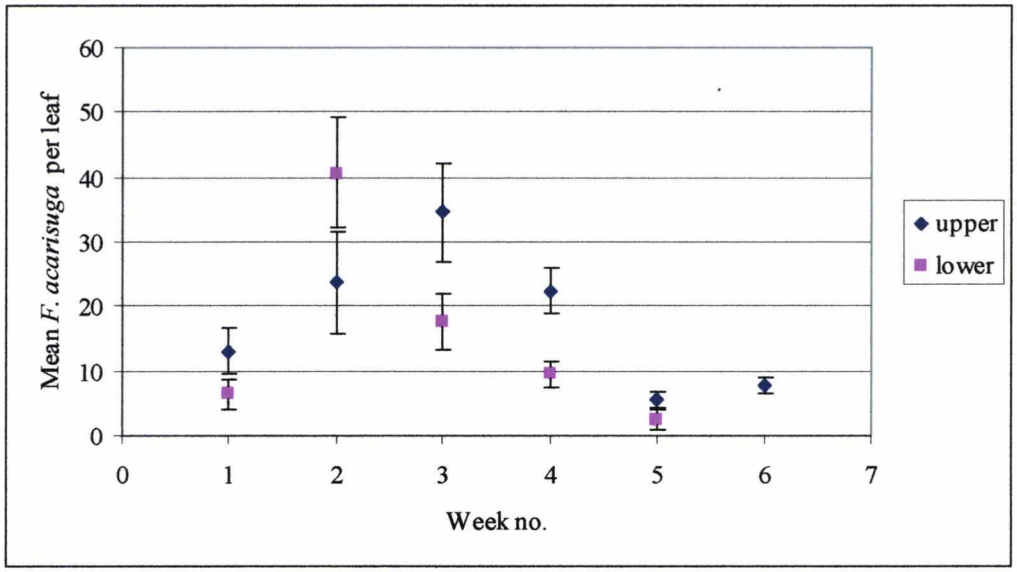


Factor	d.f.	*F* ratio	*P* value
Plant level	1	5.17	0.026
Assessment date	3	1.21	0.314
Error	69		

Figure 9.4.2.2 **Semi-field trial in a ventilated glasshouse showing mean density (vertical bars represent ± SE) of *Stethorus punctillum* per cucumber leaf (*Cucumis sativus* cv. Enigma) in the upper and lower canopies [average temperature 25 ^{0}C (upper) and 24 ^{0}C (lower) and average relative humidity 54 % (upper) and 53 % (lower)].**

Number of leaves sampled per week: 9, 9, 10, 10, 10 and 17 in the upper canopy and 7, 8, 10, 10 and 7 in the lower canopy.

Statistical analysis indicated no significant difference in densities of *S. punctillum* between assessment dates ($F_{3,69}$=1.21, P=0.314). However, there was an indication that densities of *S. punctillum* varied between plant levels ($F_{1,69}$=5.17, P=0.026) where higher densities of *S. punctillum* per leaf were recorded in the upper crop canopy compared with the lower canopy. In fact, *Figure 9.4.2.2* reveals that *S. punctillum* were recorded on leaves from the upper canopy two weeks prior to being recorded on leaves in the lower canopy.



Factor	d.f.	*F* ratio	*P* value
Plant level	1	3.87	0.053
Assessment date	4	10.88	0.001
Error	84		

Figure 9.4.2.3 **Semi-field trial in a ventilated glasshouse showing mean density (vertical bars represent ± SE) of *Feltiella acarisuga* per cucumber leaf (*Cucumis sativus* cv. Enigma) in the upper and lower canopies [average temperature 25 ^{0}C (upper) and 24 ^{0}C (lower) and average relative humidity 54 % (upper) and 53 % (lower)].**

Number of leaves sampled per week: 9, 9, 10, 10, 10 and 17 in the upper canopy and 7, 8, 10, 10 and 7 in the lower canopy.

There was an indication that densities of *F. acarisuga* varied between assessment dates ($F_{4,84}$=10.88, P=0.001). Analysis of the means revealed that mean (SE) numbers of *F. acarisuga* per leaf significantly increased between weeks one and two, and weeks one and three. From weeks three to five there was a significant decrease in mean numbers of *F. acarisuga* per leaf. Statistical analysis indicated a weakly significant variation in densities of *F. acarisuga* between plant levels ($F_{1,84}$=3.87, P=0.053).

9.5 DISCUSSION

Results from this study showed that *S. punctillum* was able to establish on cucumber plants (*C. sativus*) in both laboratory (*experiment 1*) and glasshouse conditions (*experiment 2*). This is in agreement with previous research by Elliot (Applied Bio-Nomics, Ltd., personal communication) and Raworth (2001).

Provisions were made to prevent spider mites entering the cages in *experiment 1*, other than those that were manually introduced. However, the inclusion of a treatment containing clean plants only indicated that this was unavoidable. The inclusion of a control treatment containing spider mites only, would have indicated whether the presence of *S. punctillum* was a factor that caused a spider mite population decrease. The inclusion of a control treatment such as this is indicated for future experiments of this nature.

In *experiment 1*, numbers of *S. punctillum* showed a significant increase when introduced onto cucumber plants 10 and 15 days post two-spotted spider mite introductions. This information is of particular importance to the development of successful pest management techniques. Success of the so-called 'pest-in-first' method was first reported by Huffaker and Kennet (1956) and involves the deliberate introduction of pests into a crop. This overcomes the patchy distribution of pests often encountered at the beginning of the growing season and encourages the establishment of a natural balance between prey and predators. DeBach and Rosen (1991) reported successful control of *T. urticae* by *Phytoseiulus persimilis* on glasshouse cucumbers by using this technique. Based on the results presented here, there is some evidence to suggest that the 'pest-in-first' method may be suitable for pest management of *T. urticae* by the introduction of *S. punctillum*.

There are a number of factors that influence the distribution of arthropods in habitats. In particular, determinants of coccinellid distribution include geographical latitude, population processes (i.e. immigration, emigration, natality and mortality), microclimate, host plant, prey abundance (Honěk and Hodek, 1996) and food preference (e.g. Ewert and Chiang, 1966). There was a high possibility that accidental establishment of *F. acarisuga* in *experiment 2* would have lead to tritrophic interactions between the two predators and two-spotted spider mites. However, the trial itself was

not designed to take these into account. Nevertheless, data on the presence of *F. acarisuga* was collected in an attempt to overcome this and it was found that while levels of *F. acarisuga* significantly decreased from weeks three to five, levels of *S. punctillum* remained similar and mean two-spotted spider mite densities per leaf decreased significantly. *F. acarisuga* was present in much higher numbers than *S. punctillum*. Predation rates for *F. acarisuga* and *S. punctillum* were not assessed in this study and this factor would help to explain the consequence of the differences in densities between the two predators.

S. punctillum are able to endure long periods of prey scarcity (Rott and Ponsonby, 2000b) probably attributed to their cannibalistic tendencies (Putman, 1955) and possible predation on other predators (Readshaw, 1971; Rott and Ponsonby, 2000b). Rott and Ponsonby (2000b) reported that significantly more *P. persimilis* and *F. acarisuga* survived in semi-field trials on tomato and pepper in the absence of *S. punctillum* and suggested that the latter natural enemy predated on *P. persimilis* when prey was scarce. In this study it is possible that *S. punctillum* predated on *F. acarisuga* causing a reduction in numbers of the cecidomyiid. However, further work is indicated to clarify possible interactions between the two predators.

A further possible explanation for differences in density changes displayed by *F. acarisuga* and *S. punctillum* with progression of the trial may include threshold densities of prey. For example, *C. septempunctata* are attracted by low levels of aphids and both the coccinellid and aphid prey simultaneously migrate into crops (Honěk and Hodek, 1996). However, Honěk (1980) stated that oviposition commences when a threshold density of one aphid per 300 cm^2 of leaf area is attained. Previous records of threshold prey densities for *Stethorus* spp. remain inconclusive. For example, Fleschner (1950) reported that a final instar larva of *S. picipes* required one adult female citrus red mite per 43.2 $inch^2$ of uniform surface for maturity. However, as stated by Putman (1955), Fleschner's results are not easily applied to field situations where plant leaves are rarely uniformly structured. Putman (1955) reported difficulties in attaining threshold densities of the European red mite sufficient to sustain *S. punctillum*. However, it was reported that at least one mite per leaf was the average minimum density. Despite this statement, Putman also comments that when mite densities are considerably greater than this, only a few predatory larvae reach maturity.

In this study, it is possible that the relatively low levels of two-spotted spider mites recorded towards the end of the trial (compared with the onset of the trial) were sufficient to support development of *S. punctillum*. In contrast, prey levels may have decreased past the threshold density for further development of *F. acarisuga* and thus a subsequent decrease in levels of this predator was observed between weeks three and five. Further evaluation of threshold densities of two-spotted spider mites required to support a population increase in *S. punctillum* is indicated.

Mean numbers of *S. punctillum* were lower compared to mean numbers of *F. acarisuga*. However, this is not necessarily a reflection of the individual impact that the predators had on the mite population. As with other insects, the impact of *S. punctillum* is not only determined by growth potential ratios in relation to prey but also by voracity and an aggregative response (Roy *et al.*, 2003). In addition, Congdon *et al.* (1993) suggested that the dispersal and searching ability of *Stethorus punctum picipes* rather than the numerical response of the predator are key to its predation on *T. urticae* in red raspberry. Further evaluation of behavioural parameters (e.g. voracity, dispersal and searching ability) is indicated to determine the individual impacts of *S. punctillum* and *F. acarisuga* on population densities of *T. urticae*.

S. punctillum were found on leaves in the upper canopy before they were found on leaves in the lower canopy. There was a significant effect of plant level on the density of *S. punctillum*. In this study there were no obvious differences between recorded temperatures or humidities in the upper and lower crop canopies. Although the data loggers used to measure these environmental parameters were positioned as close to the abaxial leaf surfaces as possible, measurement of microclimate experienced by the arthropods may not have been achieved. Previous research has indicated the importance of microclimate for distribution of coccinellids (Honěk and Hodek, 1996). For example, vertical gradient of humidity, and possibly trophic specificity, were shown to affect the vertical distribution of *Hippodamia convergens*, *Hippodamia tredecimpunctata* and *Coleomegila maculata* (DeGeer) (Coleoptera: Coccinellidae) in maize and barley (Ewert and Chiang, 1966). However, a lack of behavioural response by *S. punctillum* in differing humidities of 33, 65 and 90 % has been reported by Rott and Ponsonby (2000a), suggesting that humidity is possibly not the principal factor affecting *S. punctillum*.

Positive correlations between coccinellid abundance and the size of aphid colonies have previously been recorded (Honěk and Hodek, 1996). In this study, although there were no significant differences in two-spotted spider mite densities between crop levels, in weeks three to five, mean densities per leaf were higher in the upper crop level compared with the lower level. This may have influenced the position of *S. punctillum* relative to the two crop levels.

Microhabitat preference has been shown to be related to food preference in *C. maculata* (Ewert and Chiang, 1966). Evidence showed that this species of coccinellid displayed a microhabitat preference for the lower level of corn fields. This was explained by the requirement for pollen in the diet of *C. maculata*. In the present study, levels of two-spotted spider mite infestation were high at the start of the trial and in particular, leaves at the base of the plants were heavily infested. New plant growth was rapidly colonised by two-spotted spider mites. Houck (1991) stated that female *Stethorus punctum* displayed a definite preference for mite eggs over all other stages of development of *T. urticae*. Young colonies of spider mites are characterised by an abundance of eggs. If *S. punctillum* displays a preference for a particular developmental stage of *T. urticae* and this stage is found mainly on new plant growth at the apex of the plant then this may be a possible factor accounting for the position of *S. punctillum* relative to crop canopy levels. However, further research is indicated to determine possible prey preference displayed by *S. punctillum*.

On sampling dates, harvesting of leaf samples commenced in the morning and the daily movements of *S. punctillum* may have been reflected in the data collected. For example, Honěk and Hodek (1996) stated that several species of coccinellid bask in the early morning sun to increase their body temperature. Indeed, Benton and Crump (1981) observed *C. maculata* to climb up a vertical rod in the morning while the majority of beetles descended in the afternoon. It is possible that similar cyclical movements are made by *S. punctillum* in a crop. However, further observations are indicated to determine this.

Previous research has highlighted the importance of tactic responses that increase the probability of predator-prey encounters and include positive phototactic and negative geotactic responses of a predator corresponding to that of its prey (e.g. Fleschner, 1950;

Dixon, 1959; Ng, 1986). Photopositive and geonegative responses have been reported for *Stethorus* sp. (Fleschner, 1950; Putman, 1955). Putman (1955) reported that larvae of *S. punctillum* show a gradual upward movement on twigs in peach orchards.

The significant differences in densities of *S. punctillum* between crop canopies in this study may be explained by tactic responses to light and gravity. For example, the direction of movement shown by the predator would eventually place it in the upper crop canopy and it is possible that by comparing leaf samples from the upper and lower crop canopies this tactic behaviour has been exposed. Differences in tactic responses to light, humidity and gravity shown by *H. convergens*, *H. tredecimpunctata* and *C. maculata* were said to explain their differential distribution in crop fields (Ewert and Chiang, 1966).

This study indicates the importance of ensuring suitable levels of two-spotted spider mite infestation before the introduction of *S. punctillum*, to encourage a significant numerical response by the latter. There is some evidence to suggest that the 'pest-in-first' method may be suitable for two-spotted spider mite control by *S. punctillum*. Encouraging results have shown that *S. punctillum* is able to establish on cucumber plants under glasshouse conditions. This was met with a decrease in mean numbers of two-spotted spider mite, although the establishment of *F. acarisuga* made it impossible to determine the individual impact of *S. punctillum* on the pest population. Exclusion experiments would assist in preventing the introduction of unwanted predators into an experimental crop. For example, individual whole plants could be enclosed in cages. However, this would present an unrealistic view of the environmental factors (e.g. temperature and humidity) experienced in a glasshouse.

10 GENERAL DISCUSSION

Stethorus punctillum is a predator of tetranychids, e.g. *Tetranychus urticae*, which have the ability to cause considerable yield losses in commercial agricultural and horticultural crops. Previous research has suggested that *Stethorus* spp. are important candidates for inclusion in biological control programmes (e.g. Horsburg and Asquith, 1968; Raworth, 1990; Roy *et al.*, 1999). They are mobile and voracious (e.g. Readshaw, 1971) and attempts to establish them under glasshouse conditions have been promising (Rott and Ponsonby, 2000ab; Raworth, 2001).

Previously the focus of research involving *S. punctillum* as a control agent has concentrated on strawberry plantations and fruit orchards (e.g. Collyer, 1953; Putman and Hearne, 1966; Injac and Dulič, 1992; García-Marí and González-Zamora, 1999). However, recent publications by Rott and Ponsonby (2000ab) and Raworth (2001) for example, have assisted in increasing the profile of *S. punctillum* as an important predator for inclusion in biological control programmes under glass. Rott and Ponsonby (2000ab) showed that the activity of *S. punctillum* significantly increased at higher temperatures and the performance of the predator was strongly influenced by host plant species.

The focus of my study was to further explore areas of *S. punctillum* biology and behaviour, with a view to providing practical information that could be applied within the glasshouse environment.

10.1 Using *Stethorus punctillum* in the glasshouse

For effective use of *S. punctillum* in the glasshouse, the predator would be required to develop and reproduce on spider mites under temperature conditions typical to this environment. In this study, *S. punctillum* was able to develop over a range of temperatures from 18 to 34 ^{0}C when fed to satiation on *T. urticae* infesting *Vicia faba* leaves. A higher mean oviposition rate (eggs day $^{-1}$) was recorded for beetles incubated at a constant 26 ^{0}C compared with those incubated at a constant 20 ^{0}C.

The results presented here suggested that although *S. punctillum* requires more thermal energy for completion of development than *P. persimilis*, *S. punctillum* appears to be more tolerant of higher temperatures than *P. persimilis* (Hamamura *et al.*, 1976). This

research supports previous reports suggesting a tolerance of *S. punctillum* to higher temperatures, where an increase in activity was observed in the range 25-30 ^{0}C (Rott and Ponsonby, 2000a). Considering that temperatures in glasshouses in the UK are likely to exceed 20 ^{0}C in the summer, *S. punctillum* appears to be an attractive predator for inclusion in biological control programmes under glass.

Spider mites rapidly develop at high temperatures and it was shown by Putman (1955) that higher temperatures accelerate the development of the European red mite, *Panonychus ulmi* more than that of *S. punctillum*. In this way the efficiency of the predator was said to decrease as the temperature increased. However, based on the results presented in my study and those of Rao *et al.* (1996), the rate of development of *S. punctillum* from 20–30 ^{0}C closely matches that of its prey *T. urticae*.

The results of this study and previous studies provide evidence to support a confidence in the efficient use of *S. punctillum* in glasshouses, as it is able to develop and reproduce over a wide range of temperatures. This is in contrast to the less tolerant but widely used predatory mite, *P. persimilis*.

10.2 Practical application of cold storage techniques

The use of ladybirds in biological control has been met with surprising reticence, despite the vast amount of evidence to suggest that coccinellids are often a major cause of mortality in aphids, coccids and spider mites (Hodek, 1973). One of the reasons for this is the cost of rearing. Therefore techniques such as cold storage, which help to reduce rearing costs (e.g. Gilkeson, 1990; McEwen, 1997) are invaluable. A coccinellid that could be cold stored has obvious merits.

This investigation demonstrated for the first time the ability of *S. punctillum* to withstand cold storage. Cold storage for five, 10 and 15 days at 6 ^{0}C did not significantly affect oviposition rate (eggs day $^{-1}$) post storage. However, it was suggested that the physiological state of the beetles prior to storage was an important factor in determining their survival in storage and the synchrony in initiation of reproduction post-storage.

Despite the ability of adults to withstand cold storage it was demonstrated that this technique had a significantly negative effect on egg viability when beetles were stored for 10 and 15 days compared with eggs that were laid by females stored for 0 and five days. There was some evidence to suggest that under the correct conditions, a suitable method for the cold storage of *S. punctillum* is possible. However, the detrimental affect that cold storage had on the ensuing eggs must be overcome before this technique is of any value.

Although, Putman (1955) provided information on the survival of hibernating *S. punctillum* in various materials, the effect of cold storage *per se* on the coccinellid had not been investigated prior to my research. Therefore my study has significantly advanced this area of research pertaining to *S. punctillum* and has provided practical information that can be applied to future research.

The induction of diapause prior to cold storage may aid survival in storage (e.g. Tauber *et al.*, 1993) and the factors that induce diapause provide an insight into the processes involved in cold storage techniques. Therefore, further experiments in this study investigated the onset of diapause in *S. punctillum* by measuring oviposition rate with decreasing photoperiods. It was shown that a reduction in photoperiod resulted in a decrease in mean oviposition rate. Results suggested that newly eclosed *S. punctillum* adults were more sensitive to short day-lengths of 12L: 12D compared with 16- to 18-day-old adults. The information provided in this study regarding cold storage and the factors affecting diapause are important to the establishment of techniques to ensure effective rearing of *S. punctillum* and the subsequent administration of efficient pest management programmes.

10.3 Releasing *Stethorus punctillum* in the glasshouse

Putman (1955) stated that *S. punctillum* was not attracted to spider mites on broad bean leaves. Subsequent research by Colburn and Asquith (1970) reported an attraction of *Stethorus punctum* to *P. ulmi* and *P. ulmi*-infested apple leaves. However, the apparatus they used allowed both sight of, and direct contact with, the mites. It has since been observed that *Stethorus* spp. are able to locate rare prey patches (Hull *et al.*, 1977; Congdon *et al.*, 1993). For example, Congdon *et al.* (1993) reported that *Stethorus punctum picipes* was able to locate small prey patches in red raspberries.

Based on these results, they suggested that the key components of this predator-prey interaction were dispersal and searching ability, rather than a numerical response.

In my study, *S. punctillum* was shown to display directed movement towards odours emitted from the host plant, *V. faba* and a mixture of odours from *V. faba* and the host mite, *T. urticae*. Beetles tended to show an increased turning rate in response to host odours and increases in distance walked and angular velocity were detected in response to odours from the host plant plus host mite. The indication is that this kind of searching behaviour displayed by *S. punctillum* would enhance the location of spider mite infestations, which are often patchy on groups of plants intermittently spread throughout a glasshouse (e.g. Hussey and Scopes, 1985). The inclusion of a highly mobile predator, which has the ability to locate spider mites when released at distances of more than a few centimetres from sites of infestations, has obvious merits when applied to crops in vast areas of glasshouse.

Previous reports have suggested the successful establishment of *S. punctillum* on glasshouse crops (e.g. Raworth, 1990; Rott and Ponsonby, 2000ab). In the present study it was shown that *S. punctillum* was successful in the upper, humid part of a cucumber crop under glasshouse conditions. *P. persimilis* often fails in this area (D. Ponsonby, personal communication) and therefore *S. punctillum* would be an excellent alternative to the predatory mite or a possible supplement to it in biocontrol programmes. Spider mite numbers were shown to rapidly decrease in the presence of *S. punctillum* and *F. acarisuga* in this study. It appears that *S. punctillum* is particularly suited to conditions typical of the middle part of the growing season under glass, when temperatures are high and spider mites often show a peak in numbers.

10.4 Recommendations for further research

When development of *S. punctillum* was compared with that of *P. persimilis* it was shown that the coccinellid had a much slower rate of development. However, in order to understand the full implications of these results, it is also necessary to take into account predation rates and mortality rates for both spider mite predators. This should be evaluated in further work.

Further research would be required to evaluate the effect of fluctuating temperatures and prey consumption on daily and total egg lay in *S. punctillum*. Further work is also indicated to determine the adult longevity, total lifetime fecundity and oviposition rate of *S. punctillum* in a wider range of temperatures.

During the course of experimentation, eggs of *S. punctillum* that were noticeably smaller than 'normal' eggs were observed. However, it was not determined whether this reduced size affected egg viability. Further research is required to investigate this.

Future research should concentrate on securing a suitable method of cold storing *S. punctillum*. Factors highlighted in the research presented here, such as the importance of the physiological state of beetles prior to storage and the effect of photoperiod on diapause, could be used as a platform for further research. Previous research into the cold storage ability of coccinellids has been based on months in storage, rather than days, and thus it is suggested that future research of *S. punctillum* should involve longer time periods in cold storage. Subsequent work should also establish the effectiveness of beetles previously cold stored in controlling spider mites in a crop.

Here it was shown that a reduction in photoperiod resulted in a decrease in mean oviposition rate. However, results were variable. Further work is indicated to evaluate the effect of age on the photoperiodic response of *S. punctillum* and the factors involved in diapause by *S. punctillum* in nature. Future research could concentrate on developing the proposal of exploiting diapause in cold storage techniques. Further experiments would be required to evaluate *S. punctillum* adults reared under short day-lengths as larvae, for incidence of diapause as a cumulative sensitivity to diapause induction has been exhibited in the pre-imaginal and adult stages of, for example, *C. bipustulatus* (Hodek, 1996a). Time constraints prevented the evaluation of this in my study.

In experiments using a four-arm olfactometer, *S. punctillum* was shown to orientate towards odours from *V. faba* and *V. faba* plus *T. urticae*. The behaviour of *S. punctillum* was significantly modified in odour fields containing odours from *V. faba* plus *T. urticae*. Future work is indicated to evaluate other possible processes involved (e.g. visual cues) in prey location by *S. punctillum*.

As a natural enemy in glasshouses, *S. punctillum* would encounter crops such as tomatoes, cucumbers and peppers. It has been shown that rearing history of *P. persimilis* affected olfactory responses by it to different host plants (Dicke *et al.*, 1990; Takabayashi and Dicke, 1992). Research into the olfactory responses displayed by *S. punctillum* could be expanded to include common glasshouse crops such as tomatoes and cucumbers. Furthermore, evaluation of the effect of rearing history on the olfactory responses by the coccinellid is indicated.

The inadvertent establishment of *F. acarisuga* in a six week semi-field trial prevented the evaluation of *S. punctillum* alone in reducing spider mite pest numbers. It is possible that *S. punctillum* predated on *F. acarisuga* causing a reduction in numbers of the cecidomyiid, or prey levels may have decreased past the threshold density for further development of *F. acarisuga*. Thus a subsequent decrease in levels of this predator was observed between weeks three and five. Further research to clarify possible interactions between the two predators and to determine threshold densities of spider mites required to support populations of *F. acarisuga* and *S. punctillum* is indicated.

Previous research has shown the potential of *S. punctillum* as a spider mite predator for inclusion in biological control programmes. This study aimed to expand on previous research and provide realistic information for successful pest management in glasshouses using *S. punctillum*. It has been demonstrated that *S. punctillum* not only tolerates a wide range of temperatures typical to the glasshouse environment, but also has the ability to withstand low temperatures typically used in the cold storage of biocontrol agents. The behavioural responses of *S. punctillum* to host odours are an extremely interesting addition to our current knowledge of this predator. This discovery provides a reasonable explanation of the possible reasons why *S. punctillum* is able to locate rare patches of spider mites in the glasshouse.

In glasshouses, the use of *S. punctillum* will be suited to the beginning of the growing season when spider mite distribution is patchy. As the growing season progresses, spider mite numbers will rapidly increase with temperature and the development of *S. punctillum* has been shown to closely match that of *T. urticae* from 20-30 ^{0}C. Towards the end of the growing season when plant quality is low and spider mite numbers show

a decline, there is an indication that *S. punctillum* is able to continue development in contrast to *F. acarisuga*, which showed a decline in density in this study.

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