Establishment potential of non-native glasshouse biological control agents, with emphasis on *Typhlodromips montdorensis* (Schicha) (Acari: Phytoseiidae) in the UK

by

Ian Stuart Hatherly

A thesis submitted to The University of Birmingham
for the degree of DOCTOR OF PHILOSOPHY

School of Biosciences
The University of Birmingham
September 2004

Abstract

Typhlodromips montdorensis is a non-native predatory mite used for control of red spider mite and thrips, but is not yet licensed for use in the UK. Current legislation requires that non-native glasshouse biological control agents may not be introduced into the UK without a risk assessment of establishment potential outside of the glasshouse environment. This work focuses on the application of a recently developed protocol to assess the establishment potential of *T. montdorensis* in the UK. Further, the use of alternative prey outside the glasshouse by, *Macrolophus caliginosus* is examined, and interactions between *Neoseiulus californicus*, *Typhlodromus pyri* and *T. montdorensis* are investigated.

Laboratory results demonstrate that *T. montdorensis* has a developmental threshold of 10.7°C, lacks cold tolerance and is unable to enter diapause when tested under two different regimes. Field studies indicate that no reproduction occurs within the field in winter (November to March) and 100% mortality of eggs, larvae and adults occurs within two weeks of release during this period. It is suggested that *T. montdorensis* would be a 'safe candidate' for introduction as a glasshouse biological control agent in the UK as outdoor establishment is unlikely to occur.

Macrolophus caliginosus is able to feed and reproduce on *Trialeurodes vaporariorum*, *Myzus persicae* and *Aleyrodes proletella* although performance (lower fecundity and longevity) is reduced on the latter prey source. Both *N. californicus* and *T. pyri* were able to feed on larval stages of each other but when given a mixed diet, showed a preference for *Tetranychus urticae* over their phytoseiid prey. *Neoseiulus californicus* showed a preference for *T. urticae* over *T. montdorensis*, whereas the latter species showed no preference between *T. urticae* and *N. californicus*.

This work has identified a robust experimental protocol for predicting the establishment potential of non-native biological control agents outside the glasshouse and has begun to develop a further experimental system for assessing the possibility of non-native biological control agents feeding on alternate hosts outside the glasshouse.

Acknowledgements

I would like to thank my supervisor Jeff Bale for his help and advice over the past three years and enabling me to travel to conferences in Canada and Australia, and CSL for funding this project.

I am grateful to Richard GreatRex, Syngenta Bioline for supplying mites and pollen whenever they were needed. Roger Worland, BAS for letting me use the DSC. Barbara Russel for helping to culture and count mites for the first 18 months of my work. Andy for some statistical genius, Emma H for two very funny years in our last, yet 'beautiful' student house and the office crew: Alison, Kat, Phil, Sarah P and Tim.

Also thanks to all the people responsible for many memorable nights in faithful Staff House (and the beer out of plastic glasses is still pretty horrible even after a jug or two) that were needed to relieve the stresses of PhD life: Angela, Chris, Clare, Deryn, Emma F, Joe, Nathalie, Rav, Sam and Sarah WJ.

Finally thanks Steph for being there and just being you.

Table of Contents

Hatherly, I. S., Bale, J. S., Walters, K. F. A., and Worland, M. R. (2004). Thermal biology of *Typhlodromips montdorensis*: implications for its introduction as a glasshouse biological control agent in the UK. *Entomologia Experimentalis et Applicata*, **111,** 97-109.

Hatherly, I. S., Bale, J. S. and Walters, K. F. A. (2004). U.K. winter egg survival in the field and laboratory diapause of *Typhlodromips montdorensis*. *Physiological Entomology,* **in press**.

List of tables

List of figures

List of Equations

List of Photographs

Chapter 1: Introduction

1.1 History of Pest Control

The first recorded insecticide was sulphur used by the Sumerians to control insects and mites around 2500 BC (Dent, 2000). The first evidence of cultural control was discovered around 1500 BC and the first example of biological control was the use of predatory ants in citrus groves to control caterpillar pests over 1000 years ago (Huffaker *et al.*, 1971). The first successful commercial example of classical biological control dates back to 1888 when the Vedalia beetle was used to control the cottony cushion scale, *Icerya purchasi* (Maskell) (Homoptera: Margorodidae) in California (van Driesche and Bellows, 1996). Despite these cultural and biological efforts to control insect pests, man's recent history of pest control has been predominately chemical. This is primarily due to the discovery of organochlorine insecticides such as DDT (dichlorodiphenyl trichloroethane). DDT, developed in 1939, was cheap to produce, offered a high level of persistence with broad spectrum activity, low mammalian toxicity and produced virtually no plant damage (Dent 2000). The success of DDT led to the advent of other similar chemicals such as aldrin, hexachlorocyclohexane (HCH) and dieldrin. In consequence, other pest control methods became insignificant as farmers relied solely on chemicals.

It was not until 1962 when Rachel Carson published her book 'Silent Spring' that the devastating effects of the continued use of chemicals on the environment emerged. Concerns about rates of chemical breakdown, toxicity of chemicals to non-target organisms, accumulation of chemicals in the food chain, as well as problems of insects becoming resistant to chemicals such as DDT, changed the view that chemicals provided the panacea for pest control. In consequence, alternative control measures such as pheromone traps, male sterile techniques and microbial insecticides such as *Bacillus thuringiensis* were developed and used commercially. In addition, plant breeding has been used to combat insect attack. More recently, advances in transgenic technology are providing a new approach to pest management, but public opinion about the use of transgenic crops is still very much divided. By contrast

biological control is considered an environmentally safe and sustainable option and therefore continues to attract great interest (Howarth, 1991).

1.2 Pest control in glasshouses

Commercial glasshouse crop production has been established since the end of the 19th century (Wittwer and Castilla, 1995). In 1988, the total area of the world covered in glasshouses was only 150000 ha, of which biological control was applied to only 8000 ha (van Lenteren and Woets, 1988). The area covered by glasshouses by the year 2000 had risen to 300000 ha of which biological control was employed on 15000 ha (van Lenteren, 2000). It was originally thought that it would not be possible to use natural enemies in glasshouses. Only high value vegetables such as tomatoes and peppers and ornamental plants are grown in glasshouses, making production very expensive. Thus cost accompanied by the high cosmetic standard demanded by the consumer has meant that pest damage can not be tolerated and therefore chemical pest control in glasshouses has remained the preferred option. However, biological control has proved possible in glasshouses due to the uniqueness of the environment. Preceding the cropping season the glasshouses are pest free and may remain so for some time into the growing season. Only a limited number of pests will invade the glasshouse, for example, the glasshouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) and the two spotted spider mite, *Tetranychus urticae* (Koch) (Acari: Tetranychidae). The constant conditions in a glasshouse may favour the pest due to rapid generation times, but these constant conditions make predictions on pest development and natural enemy interactions more reliable than in the field.

The first example of a biological control agent used in a glasshouse was *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae), a parasitoid *of T. vaporariorum*, which was first noticed in glasshouses in 1926 (Speyer, 1927). This parasite was mass produced in Britain and shipped to other European countries and other parts of the world (van Lenteren and Woets, 1988).

As with pest control in field crops, interest in biological control halted due to the production of inexpensive organochlorine insecticides during and after the second

world war. Pest control in tomato production is only 2% of the overall cost, therefore using chemical control has not been a limiting factor (van Lenteren, 1995). However, early signs of resistance in *T. urticae*, pesticide residue concerns and high costs of new pesticide registration have led to the revival of interest in biological control in Britain and subsequently in other parts of the world. Since the 1970s the list of natural enemies used for biological control has steadily lengthened and, due to ever growing interest in pest control within glasshouses, will continue to do so. Integrated pest management is becoming an important control strategy in glasshouses; already 5% of the world's glasshouse area is under IPM and this could increase to 20% over the next decade (van Lenteren, 2000).

1.3 Phytoseiid mites

Phytoseiid mites such as *Phytoseiulus persimilis* (Athias-Henriot) (Acarina: Phytoseiidae) are free living, terrestrial, aggressive predators of small insects and a number of phytophagous mites (Chant, 1985). Phytoseiid bodies consist of the gnathosoma (which includes the chelicera and palps) and the idiosoma (which bears the legs). All phytoseiid mites have five developmental stages: egg, larva, protonymph, deutonymph and adult. The larval stage of phytoseiid mites has three pairs of legs, whereas all the other motile stages have four. Males are smaller than females and transfer spermatophoral material into the spermatheca of the female during copulation (Chant, 1985).

1.3.1 Biological control with phytoseiid mites

In the late 1950s it emerged that *P. persimilis* was efficient at reducing *T. urticae* numbers (Dosse, 1959). Nevertheless in mainland Europe researchers were not impressed with the performance of *P. persimilis* under commercial conditions; however in Britain it was put into commercial practice (Hussey and Bravenboer, 1971). Since then, *P. persimilis* and other phytoseiid mites have been successfully used as glasshouse biological control agents. The majority of studies on predacious mites have focussed on whether the rate of mites population growth allows them to compete with spider mite outbreaks. In the last 30 years the realisation that phytoseiids can regulate pest mites at low densities has led to more recent studies

investigating the ability of phytoseiids to persist at low prey densities. Strategies include survival when starved, alternative foods and cannibalism (McMurtry and Croft, 1997).

Despite the success of predatory mites such as *P. persimilis* and *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) there is still great potential for research into new mite species that can be used as biological control agents. *Phytoseiulus persimilis* has become such a successful biological control agent due to a thorough knowledge of the plant/pest/predator relationship (Caltagirone, 1981). A possible target to achieve the same success is *Typhlodromips montdorensis* (Schicha) (Acari: Phytoseiidae). Recent work in Australia has demonstrated that it has potential for thrips and mite control (Steiner, 2002; Steiner and Goodwin, 2002a; Steiner *et al.*, 2003b) and further work will determine whether it could be an effective alternative to the current commercially produced predatory mite species.

1.4 Alien pests

1.4.1 Introductions into the UK

Glasshouse crop production is now largely reliant on biological control and therefore there is a major drive to screen more insects for their potential as biological control agents. In the UK most natural enemies imported for biological control originate from countries with a warmer climate. Despite a number of exotic species being introduced into the UK, only 0.8% of UK insects are thought to be non-native (van Lenteren and Loomans, 2000).

In theory, the introduction of non-indigenous biological control agents into UK glasshouses is a safe option as there will not be any interaction with the outside world. However, insects will always overcome their physical boundaries and then interact with their surroundings (Howarth, 1991; Simberloff and Stiling, 1996a; Simberloff and Stiling, 1996b; Thomas and Willis, 1998). To date, introductions of alien species into the UK have mainly been accidental. They generally involve insects associated with plants or plant products, which are hidden within the plant, for example *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). Some introductions

however are intentional such as the predatory bug *Macrolophus caliginosus* (Chen and Walker) (Hemiptera: Miridae) to control *T. vaporariorum* (Bale and Walters, 2001).

It has generally been assumed that any individuals escaping from the glasshouse will die out, as the temperatures experienced during UK winters have been regarded as a natural barrier, stopping the establishment of non-native species. However, recently *N. californicus*, which was assumed to be unable to survive a British winter, has established in parts of the UK (Jolly, 2000).

1.4.2 Impact of alien insects on UK flora and fauna

The environmental impact of introducing biological control agents into the UK is difficult to assess. However, exotic species are the possible cause of 40% of species extinctions worldwide (Caughley and Gunn, 1996). Research into potential biological control agents seldom includes data collected on the likely effects of introduced species on the surrounding ecosystem (Elliott *et al.*, 1996; Howarth, 1991). Data gathered is likely to concern only economically important species and thus a lot of post-release problems will never be uncovered as they are not economically significant or of public interest (Lynch and Thomas, 2000; Simberloff and Stiling, 1996a; Simberloff and Stiling, 1996b). Often, biological control programmes are started, but fail. Only 10-20% of classical biological control agents ever become established in the areas where they were released (Beirne, 1985; Harris, 1988). However, if these programmes are abandoned, an unknown amount of damage to the environment may have already been caused.

1.5 Impacts of biological control introductions

1.5.1 Species extinctions

Research has shown that removal of species can result in unexpected changes to other ecosystem components (Zavaleta *et al.*, 2001). At least 100 non-target species have been driven to extinction by the introduction of biological control agents (Howarth,

1991). Although evidence for these effects is often scarce and weak, two examples should be mentioned.

In the 1950s the rosy wolf snail, *Euglandia rosea* (Ferussac) was introduced onto the Hawaiian Islands to control the previously introduced Giant African Land Snail *Achantina fulica* (Bowdich) (Funasaki *et al.*, 1988). *Euglandia rosea* subsequently caused the extinction of several snails endemic to Hawaii, for example *Achatinella mustelina* (Meghels) (Gastropoda: Pulmonata), the Oahu Tree Snail. *Euglandia rosea* was then introduced onto Moorea in 1977 and subsequently contributed to the extinction of seven endemic snail species (Clarke *et al.*, 1984; Murray *et al.*, 1988).

A further non-target extinction caused by a biological introduction is that of the large blue butterfly, *Maculinea arion* (Linnaeus) (Lepidoptera: Lycaenidae) in the UK. To control the introduced European rabbit, *Oryctolagus cuniculus* (Linnaeus) (Lagomorpha: Leporidae) populations, a myxoma virus was introduced as a control agent. *Maculina arion* develops in nests of the ant *Myrmica sabuleti* (Meinart). When rabbit grazing decreased due to the myxoma virus, conditions became unfavourable for the ant, leading to the butterfly having far fewer nesting sites and becoming extinct (Moore, 1989; Ratcliffe, 1979).

The above examples demonstrate that extinctions may be more likely to occur on islands (Howarth, 1991). This emphasises the care that must be taken when introducing non-native species into the UK.

1.5.2 Native ecosystem disruptions

There are over 20 known cases of introduced biological control agents shifting from their original intended host to a non-target species (Secord and Kareiva, 1996). Host shifting has been reported in micro-organisms (Murdoch *et al.*, 1985) and insect species (Funasaki *et al.*, 1988; Hokkanen and Pimentel, 1989; Pimentel *et al.*, 1984; Samways, 1988). Host shifts rarely lead to extinction, but may alter the population structures of other organisms and disrupt native ecosystems, threatening biodiversity (van Lenteren and Loomans, 2000). Four examples of how biological control introductions have disrupted native ecosystems are outlined below.

The coconut moth, *Levuana iridescens* (Bethune-Baker) (Lepidoptera: Zygaenidae) was eradicated from Fiji in the 1940s by the introduced tachinid fly *Bessa remota* (Aldrich) (Diptera: Tachinidae). Despite the eradication of *L. iridescens*, *B. remota* still occurs and therefore must be attacking 'non-target' lepidoptera (Roberts, 1986; Russel, 1986). It is thought that the native moth, *Heteropan dolens* (Druce) (Lepidoptera: Zygaenidae) has also become extinct due to the introduction of *B. remota* on Fiji (Roberts, 1986).

More recently it is thought that the native parasitoid of the sugarcane borer, *Diatraea saccharalis* (Fabricius) (Lepidoptera: Pyralidae) in Florida, *Apanteles diatraeae* (Muesebeck) (Hymenoptera: Braconidae), has been displaced and possibly driven to extinction by the introduced parasitoid *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae). *Apanteles diatraeae* could not be collected from *D. saccharalis* in 1984-1985 (Bennett, 1993).

The parasitoid *Microctonus hyperodae* (Loan & Lloyd) (Hymenoptera: Braconidae) was introduced into New Zealand in 1982 against the weevils *Sitona discoideus* (Gyllenhal) (Coleoptera: Curculionidae) and *Listronotus bonariensis* (Kushel) (Coleoptera: Curculionidae), both pests of lucerne *Medicago sativa* (Linnaeus). Parasitism rates of 40% and often much higher were recorded in three non-target weevil species (Barratt *et al.*, 1997).

A final example illustrates how the community structure of seven native coccinellids in three different crops was altered in America due to the establishment of the non-native Seven spot lady beetle, *Coccinella septempunctata* (Linnaeus) (Coleoptera: Coccinellidae). Populations of the two spotted lady beetle *Adalia bipunctata* (Linnaeus) (Coleoptera: Coccinellidae) were reduced 20 times and populations of the transverse lady beetle *Coccinella transversoguttata* (Faldermann) (Coleoptera: Coccinellidae) were reduced by up to 32 times (Elliott *et al*., 1996).

1.5.3 Non-target impact database

Recently, a database constructed of non-target effects in classical and inundative biological control of insects showed that 87 classical introductions have led to

recorded non-target effects, 17 of which have included population reductions. These numbers are small, but this is likely to be because a relatively low proportion of studies directly assess the non-target effects of classical biological control introductions (Lynch and Thomas 2000). It is thought that the minor non-target effects outweigh the major ones by nine to one, but up to 11% of introductions have had serious population consequences for non-target species. However, a large number of introductions were made over 100 years ago before priorities had changed to take account of environmental impacts and therefore some of the non-target effects of earlier introductions have to be accepted. Despite this, some governmental agencies will still risk introducing polyphagous biological control agents such as the multicoloured lady beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) in the USA, if a substantial economic payoff or socio-economic urgency is involved (Lynch and Thomas 2000).

1.6 Regulation of Biological Control Introductions

Since the problems that non-native species can cause to native ecosystems have been recognised, legislation is being implemented in different parts of the world to attempt to regulate biological control introductions.

1.6.1 Legislation

It is well documented that exotic species can cause serious and permanent damage to native ecosystems (Bale and Walters, 2001; Howarth, 1991; Mach, 2000; Simberloff and Stiling, 1996a; Simberloff and Stiling, 1996b). The Convention on Biological Diversity (Article 8(h)) forms the body of worldwide legislation, whilst within Europe, Council Directives on the Conservation of Wild Birds, the Conservation of Natural Habitats and the Conservation of Wild Fauna and Flora form the legislation (DETR, 1997). In addition, codes of practice have been issued by the Food and Agriculture Organisation (FAO) and the International Union for Conservation of Nature (IUCN) (DETR, 1997). When searches to find new biological control agents are conducted, agencies such as the European Biological Control Laboratory in Montpellier (France), the International Organisation for Biological Control of

Noxious Animals and Plants in Zurich (Switzerland) and the Commonwealth Institute of Biological Control in Trinidad (West Indies) assist and oversee quarantine procedures.

In addition to international regulation a number of countries have their own additional legislation. In Australia, New Zealand and Hawaii the guidelines for introducing non-native biological control agents are so strict that hardly any natural enemies can now be introduced (van Lenteren *et al.*, 2002). Australia has been at the forefront of providing guidance for the introduction of non-native biological control agents and introduced the Australian Biological Control Act in 1984 (Simberloff and Stiling, 1996b). In New Zealand the Hazardous Substances and New Organisms Act from 1996 deals with biological control introductions (Barratt *et al*., 1999) and in the US the Endangered Species Act is the primary environmental legislation (Lockwood, 1999). Within the European Union, Britain regulates the introduction of non-native biological control agents by the Wildlife and Countryside Act, 1981 (DETR, 1997). The use of indigenous biological control agents is not regulated. Norway does not allow the import of any organism that can not be proven to be native to the country itself. Sweden and Austria also have a formal registration procedure for all biological control agents. Switzerland was the first country to make registration of biological control agents compulsory (Blum *et al.*, 2003). Despite these regulations, less than 10% of all countries have any form of regulation involving the import of non-native biological control agents (van Lenteren *et al.*, 2002).

1.6.2 Pre-release requirements

Countries often allow the release of non-native biological control agents if a licence is granted by the governing body. In the USA scientific protocols that are over 100 years old are still used (Carey *et al.,* 1996). In New Zealand, a precautionary approach is adopted in which the risks associated with the introduction of a biological control agent have to be weighed against the potential economic benefits (Barratt *et al.*, 1999). The pre-release requirements in Australia are the most extensive where detailed environmental impact studies are required prior to releasing a non-native biological control agent (Delfosse, 1988). In addition, the public are told about possible introductions and are able to voice their opinion (Waage and Greathead,

1988). In both New Zealand and Australia host range testing prior to release of new species is common (van Driesche and Hoddle, 1997). In many other countries, screening of potential biological control agents under quarantine is now also routine (Follett et al., 2000). In Britain, licence applications are usually submitted by small commercial companies and granted by DEFRA, but will be refused if the possible effects of an introduced species on the environment are not clear. Licences are granted after advice from members of the Advisory Committee on Releases into the Environment (ACRE) (DETR, 1997). In 1998 a research project lasting four years on 'Evaluating Environmental Risks of Biological Control Introductions into Europe' (ERBIC) began. This project was funded by the European Union (EU) and aimed to review and examine the environmental impact of past biological control introductions and to issue guidelines for further introductions in Europe (Lynch and Thomas, 2000; van Lenteren *et al.*, 2003). General frameworks for risk assessment methodologies for biological control agents are often proposed and the most recent suggests that information on the establishment potential of a biological control agent, its dispersal abilities, its host range and its direct and indirect effects on non-targets need to be integrated (van Lenteren *et al.*, 2003).

The current regulations and the introductions of new regulations to make the introduction of biological control 'safer' for the environment have their critics. It is argued that the biological control industry may be adversely affected due to increased costs of obtaining a licence for the release of a biological control agent (Ehler, 1999; Messing, 1999). Testing all the non-target species possibly at risk by the introduction of a non-native species would be vastly expensive (Waage and Greathead, 1988) and the control agents may then be rejected (Stanley and Julien, 1998). In the past much of the non-target testing has been done on species of economic importance rather than all species potentially at risk (Thomas and Willis, 1998). However, selecting an extensive list of taxa for host range testing is impractical and therefore a range of non-target arthropods for host range testing must be selected carefully to include as many indicative species as possible (Sands, 1998). It is important that the correct balance is found for pre-release protocols. It is hoped that current work will result in a harmonised registration procedure that does not prohibit the biological control industry and will result in the pre-selection of safe natural enemies (van Lenteren *et al.*, 2002).

1.6.3 Release protocols

There is general agreement that some form of legislation governing the introductions of non-native biological control agents is needed (Howarth, 1991; Messing, 1999; Orr *et al.*, 1999; Waage and Greathead, 1988). However, researchers and experts disagree on the exact form of the pre-release protocols, some claiming the existing protocols are satisfactory (Carruthers and Onsager, 1993; van Lenteren and Manzaroli, 1999). Others claim pre-release protocols should include host specificity testing (Cory and Myers, 2000; Howarth, 1991; Orr *et al.*, 1999; Simberloff and Stiling, 1996a; Simberloff and Stiling, 1996b), cost benefit analyses (Lockwood, 1993; Simberloff and Stiling, 1996a; Simberloff and Stiling, 1996b), climatic tolerance estimates (Goldson *et al.*, 1992), assessment of environmental impacts from the introduction of non-native biological control agents (Orr *et al.*, 1999) and studies in the organism's native range to determine its distribution in its country of origin (Stanley and Julien, 1998). It is also argued that in some cases biological control release regulations are too strict, but in other cases too slack, offering little consistency to the system (Cory and Myers, 2000).

1.6.4 Post-release studies

Too often has the evaluation of possible negative non-target effects of introducing biological control agents been hindered by the lack of post-release monitoring (Cory and Myers, 2000). Post-release studies determine the effectiveness of control, the rate and direction of movement of the biological control agents and what factors influence this, variation in host selection, how the biological control agent interacts with other plants and insects, and thus the effect it is having on the food web and the ecosystem as a whole (Thomas and Willis, 1998). It is vital that a licence for the release of a biological agent is only granted if extensive post-release studies are undertaken. In Britain, licences will only be renewed if post-release data is presented in the licence renewal request by the company (DETR, 1997).

1.6.5 Host range testing

The main task of a risk assessment for the introduction of a non-native biological control agent is to show that the natural enemy will only attack target pest species and not other species outside the glasshouse environment. Testing hundreds of non-target species is not a viable option and the behaviour of the biological control agent in its environment can not always be predicted accurately and therefore absolute safety can never be guaranteed (van Lenteren and Loomans, 2000). The number and range of non-target taxa to be tested must therefore be selected carefully (Sands, 1998).

To gain a better understanding of which non-target hosts may be attacked by non-native biological control agents, several authors have discussed appropriate host specificity tests (Follett *et al.*, 2000; Loomans *et al.*, 2002; Sands, 1997; Sands, 1998; Stanley and Julien, 1998). Most recently, a sequential test by the EU-ERBIC project has been proposed (van Lenteren *et al.*, 2002). The basic outline of such a sequential test can be summarised as follows:

1. Initially it is important to determine whether the biological control agent in question will attack a non-target organism. The non-target species would be placed in a small cage/dish with the control agent. If the non-target species was not attacked at any of its life stages then testing could be ended. If attacks did occur then a 'no choice' behavioural test could be undertaken.
2. A 'no choice' behavioural test would determine at what rate the non-target pest is attacked by the biological control agent. In this case the non-target pest is again placed in the vicinity of the biological control agent and encounter and attack rates over time are recorded and compared with the response of the control agent to the target pest.
3. If the non-target species is attacked in the 'no choice' behavioural test then a similar test should be performed where the biological control agent is presented with both the target and the non-target pest at the same time. If in this choice test the non-target species is attacked frequently then the same test should be undertaken within a larger arena to simulate a semi-natural situation.

4. In this scenario a large cage would include multiple host plants with target and non-target hosts on them. If the non-target hosts are readily attacked then a high risk of non-target effects is posed.
5. The final step of host range testing would be to conduct a field test. However, this is only possible if the biological control agent can not establish in the target area.

In addition to the choice and no-choice tests it would be valuable to study the developmental times of the biological control agents on both the target and non-target host. It could also prove useful to supplement traditional studies that determine host range by rearing large quantities of predators on a variety of potential food sources, with 'new' techniques such as enzyme linked immunosorbent assay (ELISA) that can be used to determine the gut contents of predators (Memmott, 1999) collected directly from the field.

If development of the biological control agent proved possible on non-target taxa the benefits of biological control of a pest would have to be weighed against the possible detrimental effects on the ecosystem as a whole (Sands, 1997; Sands, 1998). Similarly, if the biological control agent had a narrow host range then development on some non-target species may be acceptable (Sands, 1997). A possible target for host-range testing in the UK is the non-native predatory bug *M. caliginosus* as it has been observed outside the glasshouse environment. It could therefore be important to determine what species it may be feeding on at times when its glasshouse prey of whitefly is not available.

In addition, if an established non-native species in the UK such as *N. californicus* can survive on alternate prey, then it would be of interest to examine how *N. californicus* will interact outside the glasshouse with native predatory mites such as *Typhlodromus pyri* (Scheuten) (Acari: Phytoseiidae).

1.6.6. Post-release adaptation

Despite post-release control it is clear that insects are able to evolve quickly due to their rapid generation times and can then adapt to their new environment. Introduced biological control agents will often be free of selection pressures that were

experienced in their native range and new selection pressures may force the insects to evolve after they have been introduced (Ehler, 1998). Evolution of the introduced species will inevitably lead to host shifting, the probability of this event increasing over time. Further, as climate changes globally, insects are likely to respond (Bale *et al.*, 2002) and may become more or less suited to their new environments. However, evolution is not testable (Follett et al., 2000) and this may be a small price to pay, if pre-release studies on the biology of the species are so extensive that post-release effects on native ecosystems are minimised and therefore biological control continues further, minimising the use of pesticides in the environment. Biological control may pose some risks, but with other control methods such as chemical control, the risks are often perceived as being greater (van Lenteren and Loomans, 2000). For each possible exotic biological control agent, the risks of non-target impacts have to be weighed against the overall benefit of an introduction (Hopper, 1998).

1.6.7 Importance of risk assessment

As insects are likely to escape from the glasshouse, their impact on native flora and fauna must be assessed prior to their release as biological control agents. The temperature regime in the UK primarily governs the insect's developmental period, reproduction and survival, which will ultimately contribute to the distribution and abundance of the insect (Bale and Walters 2001). Therefore, whether a non-native insect that escapes from the glasshouse is capable of surviving a UK winter and possibly establishing due to a lack of natural enemies, is an important feature to assess (van Lenteren and Loomans, 2000). For an alien insect to survive it needs a sufficient thermal budget above the developmental threshold temperature to complete its life cycle, reproduce, and be able to survive the winter either, as a non-diapausing lifestage or in diapause (Bale and Walters, 1997). Should temperature regimes favour the non-indigenous species, then its ability to find and utilise food sources and its ability to avoid predation (if present) must be examined (Tauber *et al*., 1986).

1.6.8 Compiling a risk assessment

To date, companies submitting licence applications stress that the low winter temperatures in Britain are likely to prevent the establishment of any introduced

tropical non-native biological control agent and therefore licences should be routinely granted. Companies realise that often an exotic insect introduced for biological control that can not survive outside a glasshouse does not have to meet as many safety criteria as an insect that can survive in its new environment (van Lenteren and Loomans, 2000). Companies are therefore keen to provide evidence for a degree of cold susceptibility in the target species. However, investigations into the cold tolerance of the target species are often incomplete, if undertaken at all. It is therefore important that a quantitative protocol based on laboratory and field derived indices of insect cold tolerance is developed to have a clearer idea of the overwintering potential and success of non-native biological control agents in the UK (Bale and Walters, 2001).

Protocols for predicting aphid population numbers based on winter temperatures and aphid thermal biology data have already been explored. Studies on the numbers of overwintering eggs of *Aphis fabae* (Scopoli) (Hemiptera: Aphididae) can be used to predict population numbers on bean plants in Britain in the following summer (Way *et al.*, 1981). Similar studies on *Rhopalosiphum padi* (Linnaeus) (Hemiptera: Aphididae) can predict summer occurrence of the aphid in Finland (Leather, 1983). Timing of the spring migration in *Myzus persicae* (Sulzer) (Homoptera: Aphididae) can be determined on the basis of the effect of winter on aphids and resulting aphid mortality (Bale *et al.*, 1988). Climatic data combined with insect cold hardiness studies can increase the power of insect population forecasting models (Chen and Walker, 1994; Howling *et al.*, 1993). These studies indicate that the level of overwintering of pests can have an effect on their subsequent abundance.

1.6.9 UK risk assessment protocol

In the past DEFRA have granted licences for the UK release of non-native biological control agents based on biological information provided by the applicant. This was likely to be the commercial company wishing to release the target organism. The data provided usually included some information on the developmental threshold and host range of the species. Often however, no information on the cold tolerance of the target biological control agent has been available. Some climatic comparisons between the hosts' natural habitat and the UK were also occasionally included. The climatic

comparisons often led to the assumption that species of tropical and subtropical origin would not be able to survive through a UK winter. However the recent local establishment of *N. californicus* (Jolly, 2000) and the regular sightings of *M. caliginosus* (Hart *et al.*, 2002b) outside the glasshouse environment have cast a doubt over this theory.

A recent study aimed to develop a series of experiments that could be used as a primary screen to quantify the outdoor establishment potential of species that are likely to be used for glasshouse biological control in the UK (Hart *et al.*, 2002b). The protocol assesses the thermal biology of the target species and investigates its outdoor survival during winter. This system has been tested on *M. caliginosus* (Hart *et al.*, 2002b), *N. californicus* (Hart *et al.*, 2002a), the predatory beetle *Delphastus catalinae* (Horn) (Coleoptera: Coccinellidae) (Tullett, unpublished data) and the parasitoid *Eretmocerus eremicus* (Rose & Zolnerowich) (Hymenoptera: Aphelinidae) (Tullett *et al.*, 2004). Part of this study will apply the same protocol to *T. montdorensis* with a view to identifying those areas that should be used to produce a rapid and reliable assessment procedure for biological control companies applying for licences.

1.7 Insects at low temperature

Temperature is regarded as the most important environmental variable in determining the distribution and success of insects. The severity of winter and the degree of cold tolerance exhibited by the insect will ultimately determine the size of the population surviving the winter (Bale, 1987).

1.7.1 Developmental Threshold

For all insects there is a certain temperature below which no development takes place, known as the developmental threshold. The developmental threshold not only varies between species, but also between individuals and different life stages of a particular species (Hart *et al.*, 1997). Development at constant increasing temperatures usually shows a characteristic pattern (Figure 1), where developmental rate is approximately linear within a certain temperature range (zone B) and non-linear above and below this range (zones A and C).

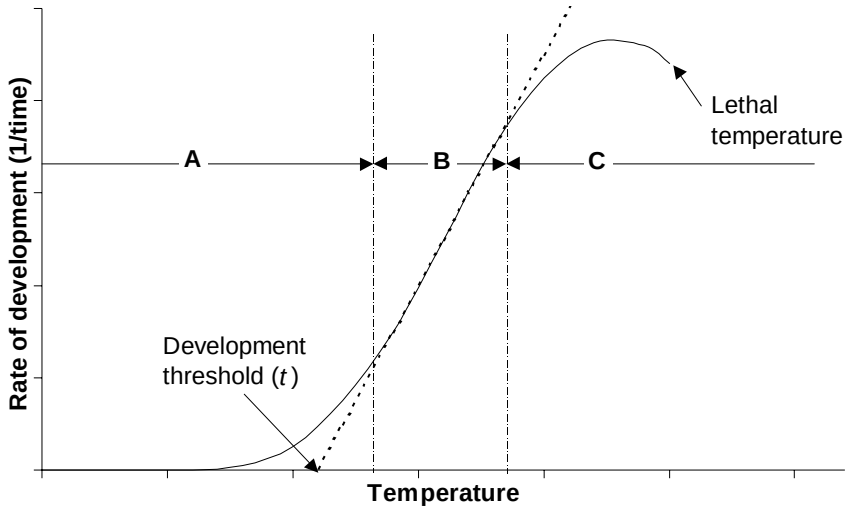


Figure 1: Relationship between the rate of insect development and temperature (after Campbell *et al.*, 1974).

Temperatures within zone A will usually result in high mortality, but the deleterious effect of high temperatures in zone C will usually only arise if temperatures remain high (within zone C) for extended periods of time (Campbell *et al.*, 1974). A brief exposure of an insect to temperatures within zone C will usually not result in death. The upper temperature limit is known as the upper lethal temperature (Lamb, 1992) and *vice versa* for the lower limit.

The linear relationship in zone B can be represented as a straight line. Extrapolation to the x- axis where the rate of development is zero, using simple linear regression with Equation 1 gives the developmental threshold (t).

$$R(t) = bT - a$$

Equation 1: Relationship between rate of development (R) and rearing temperature (T) (Lamb, 1992)

An estimate of the developmental threshold is calculated as –a/b (Equation 2) where a and b appear as coefficients in Equation 1.

$$t = -a/b$$

Equation 2: Calculation of the developmental threshold (t) (Campbell *et al.*, 1974)

The statistical accuracy of the developmental threshold can be enhanced by calculating the standard error of t (Equation 3).

$$S.E.\ (t) = y/b\ \sqrt{s^2/Ny^2 + [S.E.\ b/b]^2}$$

Equation 3: Calculation on the standard error of the developmental threshold (Campbell *et al.*, 1974). Where s^2 is the residual mean square of y and $\bar{y}$ is the sample mean.

1.7.2 Thermal Budget

The thermal budget (K) is the number of day degrees above the threshold required to complete one generation and is calculated from the developmental threshold and measured in Celsius degree-days (ºd) (Campbell *et al.*, 1974). In Equation 4, K is the reciprocal of the gradient (b) of the straight line (Equation 1).

$$K = 1/b$$

Equation 4: Calculation of the thermal budget (degree day requirement) (Campbell *et al.*, 1974)

The standard error of K can be calculated (Equation 5).

$$S.E.\ (K) = S.E.\ b/b^2$$

Equation 5: Calculation of the standard error of the thermal budget (degree day requirement) (Campbell *et al.*, 1974)

Alternatively the thermal budget can be calculated from the developmental threshold (Equation 6).

$$D \times (R-t)$$

Equation 6: Calculation of the thermal budget from the developmental threshold (Bale and Walters, 2001)

Where *R* is the rearing temperature, *t* the developmental threshold and *D* is the number of days required to complete development at the rearing temperature (R). If an insect took 50 days to develop from egg to adult at 15ºC (R) and had a developmental threshold of 10ºC (D) then the thermal budget would be 250 day degrees (i.e. 50 x (15-10)).

It is important to reiterate that the developmental threshold is calculated using simple linear regression, which assumes that the sample variances are equal. However, as exposure temperatures increase the variances often follow suit. Therefore weighted linear regression can be used to overcome the problem of unequal variances (Draper and Smith, 1981). Hart *et al.,* (1997) have also used the cumulative thermal time method to further analyse development.

1.7.3 Voltinism

The number of generations per year, termed voltinism, can be calculated from the thermal budget. When temperature data are available for a particular area over a certain time period the voltinism can be calculated for any species once the developmental threshold and the thermal budget have been determined (Campbell *et al.*, 1974; Wagner *et al.*, 1984). Equation 7 shows how to calculate the degree-days available on a set day; the voltinism over a specified time period is calculated from the sum of the degree days available for each day in the specified time period and the thermal budget required by the species (Equation 8).

$$^{o}d = \text{mean daily temperature} - t$$

Equation 7: Degree days available per day.

$$\text{Voltinism} = \sum {}^{o}d/K$$

Equation 8: Calculation of voltinism over a specified time period.

1.7.4 Limitations to the developmental threshold calculations

Developmental threshold is determined by linear regression and this can be an inaccurate estimate. To increase the accuracy of this estimate large numbers of individuals (>50) would have to be reared at each temperature and checked frequently each day (Campbell *et al.*, 1974). However, for use in the field far less individuals are needed (10-20) at each temperature as the estimates of time to adult (K) and the threshold (t) are highly negatively correlated. This results in a small positive error in t being corrected by a similar negative value in K. This leads to the predicted developmental rates not being affected except when temperatures are slightly over the developmental threshold (by 1-2ºC) (Campbell *et al.*, 1974). Further, the fewer temperatures used to calculate *t* the greater the inaccuracy, especially if temperatures close to the lower developmental threshold are not used. Using linear regression when the relationship between development and temperature is not linear, tends to lead to underestimates of the developmental rates at low temperatures (Lamb, 1992). In this case, non-linear methods could be used to calculate the thermal budget, but usually the use of linear methods is adequate to estimate developmental parameters.

1.8 Insect cold hardiness

1.8.1 What is cold hardiness?

Cold hardiness refers to the ability of an organism to survive low environmental temperatures (Leather *et al*., 1993). The freezing temperature of an individual insect or species can vary considerably, depending on a range of factors such as its prior

experience to low temperatures or the presence or absence of ice nucleating agents such as dust particles in the gut. These particles once within the insect digestive tract become potential nucleators when in contact with water (Salt, 1961). Often, feeding insects are less cold hardy than non-feeding insects that have evacuated their gut contents. However the use of the 'gut clearing' hypothesis to explain lower cold tolerance in feeding insects compared with non-feeding individuals has been challenged (Baust and Rojas, 1985).

Insect cold tolerance is assessed by measurements such as the supercooling point (SCP), the lethal temperature over a fixed time (LTemp) and the lethal time at a fixed temperature (LTime).

1.8.2 The supercooling point

Supercooling is the phenomenon by which water and aqueous solutions remain unfrozen below their melting point. The supercooling point (SCP) refers to the temperature at which spontaneous freezing occurs in a supercooled liquid. The SCP of pure water can be as low as -40ºC and the difference between the SCP and MP of a system is known as the supercooling capacity (SCC) (Zachariassen, 1985). A cooling rate of 1ºC min^{-1} is often used to determine SCPs, although varying the cooling rate may affect the SCP (Salt, 1961). A slower rate (0.1ºC min^{-1}) may be used, as a cooling rate of 1ºC min^{-1} would seldom be experienced in natural environments (Kelty and Lee, 1999).

The SCP is identified by an exotherm registered during the cooling which is caused by the latent heat of crystallisation. Supercooling points can be used to determine the capacity of an insect to be cooled and its reaction to freezing. From this the strategy for winter survival of the species can be determined, for example, whether it is a freeze tolerant or freeze intolerant species. Supercooling points should not be used as sole indicators of insect cold hardiness as a large proportion of insects may die before reaching their SCP (Bale, 1987; Chen *et al.*, 1991; Chen *et al.*, 1990; Nedved, 1993; Somme, 1996), and it is therefore advisable to look at other aspects of insect cold hardiness.

1.8.3 Lethal times and temperatures

Temperatures above the SCP of an insect can still lead to death by acute exposure. These lethal temperatures can be determined by exposing the insect to a series of sub-zero temperatures above the SCP for a set period of time before re-warming to a favourable temperature. When an insect is kept at a set temperature above the SCP for a set period of time it may die of chronic exposure. These lethal times are determined by exposing insects to a series of temperatures for set time periods and then assessing mortality. The relationship between temperature or time (the dose) and mortality is often represented by a sigmoid curve and the curve can be linearised by the use of Logit or Probit transformations (Finney, 1971; Fry, 1993). These two methods are very similar and can be used to provide estimates of the lethal doses (either temperature or time) required to kill for example 10, 50 or 90% of the sample population (LD_{10}, LD_{50}, LD_{90} respectively).

1.8.4 Strategies of insect cold hardiness

The formation of ice within an insect is potentially lethal. Ice may denature macromolecules and cause dehydration of cells due to water freezing out of solution, which causes cell plasmolysis (Baust, 1973; Salt, 1961). Insects have developed a series of strategies to deal with the problem of ice formation. Traditionally, if an insect was able to survive ice formation within its body it was described as freeze tolerant and if ice formation proved lethal it was classified as freeze intolerant (Salt, 1936; Salt, 1961). However, death of an insect may be due to prolonged exposure to unfavourable temperatures, resulting in an accumulation of cryogenic injuries eventually causing pre-freeze mortality (Bale, 1991). Other factors, which may contribute to the death of insects during winter are predation by other species and starvation, through lack of food or through depletion of accumulated fat reserves. Therefore, other low temperature related processes such as pre-freeze mortality should be taken into account when considering cold hardiness. In consequence, a further classification has been suggested where the terms freeze avoiding, chill tolerant, chill susceptible, and opportunistic survival replace the traditional class of freeze intolerance (Bale, 1993). The class of freeze tolerant insects is maintained. Additionally it has been suggested that Bale's (1993) classification replacing the class

of freeze intolerance could also be applied to freeze tolerant species (Klok and Chown, 1997). Recent work has also suggested that freeze tolerant insects can be subdivided into four groups: partially freeze tolerant, moderately freeze tolerant, strongly freeze tolerant and freeze tolerant with a low SCP (Sinclair, 1999).

Recently a further alternative classification has been suggested (Nedved, 2000). Objections raised by Nedved (2000) about the past classification are (Nedved, 1998; Nedved, 2000):

1. The existence of freeze avoiding insects has not been proved. It is an assumption that has not been explicitly tested.
2. Freeze tolerance encompasses two variables; time and temperature and therefore can not be viewed as one. Mortality in the frozen state increases with increasing time spent frozen and with decreasing temperature (Somme, 1982; Zachariassen, 1985)
3. No novel mechanism of injury is involved in the opportunistic survival class and therefore such species can not be separated from others. The distinction of the opportunistic survival class has been based on quantitative rather than qualitative features.

There are three causes of low temperature mortality: cold shock, freezing of body fluids and accumulation of chill injuries. Nedved's (2000) classification is based on the presence or absence of each of the three causes rather than on the mechanisms involved in avoiding injury and eight classes of cold tolerance are proposed which include some sub-classes according to quantitative parameters of survival (Nedved, 2000). Despite these debates on the classification of cold hardy insects, the traditional view of freeze tolerance and freeze intolerant species is still the most commonly used, demonstrating a reluctance to end the use of this dichotomy (Sinclair, 1999). In fact Ramlov (1998) believes that the division between the traditional freeze tolerant and freeze intolerant classification is sufficient. Naming an insect as freeze tolerant should be enough to describe it and future researchers can then consult the literature for a description of the actual freeze tolerance of the insect (Ramlov, 1998).

The attributes of the main categories of insect cold hardiness are discussed below.

1.8.5 Freeze tolerance

Salt (1961) defined insects as freeze tolerant if they could survive the formation of internal ice. It has generally been accepted that this freezing occurs extracellularly to prevent lethal intracellular freezing (Leather *et al*., 1993), but Yi and Lee (2003) have recently reported that some fat body cells can survive freezing in the gall fly *Eurosta solidaginis* (Fitch) (Diptera: Tephritidae). Freeze tolerant insects can contain all three of the main biochemical components involved in insect cold hardiness; Ice nucleating agents (INAs), cryoprotectants and Thermal hysteresis proteins (THPs) (section 1.8.11). Freeze tolerant insects usually freeze between -5 and -10ºC (Zachariassen, 1985). Once a freeze tolerant insect is frozen it can tolerate much lower temperatures and when warmed, will survive and continue normal development.

Freeze tolerance has also been further divided into a number of categories. In *E. solidaginis* intracellular freezing occurs whereas in the New Zealand Alpine Weta, *Hemideina maori* (Orthoptera: Stenopelmatidae) intracellular freezing is avoided (Sinclair and Wharton, 1997). Both these insects are classed as freeze tolerant as both can tolerate freezing. Sinclair (1999) identifies partially freeze tolerant species which survive ice formation in the body until the SCP, but then die. An example is the cranefly *Tipula paludosa* (Meigen) (Diptera: Tipulidae) (Todd and Block, 1995). Moderately freeze tolerant insects are those that freeze at high temperatures, but die at temperatures less than 10°C below their SCP. The woodroach *Cryptocerus punctulatus* (Scudder) (Dictyoptera: Cryptocercidae) can survive 205 days frozen at -10°C (Hamilton *et al.*, 1985) and is an example of a moderately freeze tolerant species (Sinclair, 1999). Strongly freeze tolerant species are insects that survive considerably below their SCPs (Sinclair, 1999). An example is the hoverfly *Syrphus ribesii* (Linnaeus) (Diptera: Syrphidae) (Hart and Bale, 1997). The final sub-division of freeze tolerant insects described by Sinclair (1999) can have extremely low SCPs (-25°C or lower) and can survive freezing just below their SCP. These insects are known as freeze tolerant with a low SCP, for example, the beetle *Pytho deplanatus* (Coleoptera: Pythidae) with a SCP of -54°C which survives freezing to -55°C (Ring, 1982).

1.8.6 Freeze avoidance

Freeze avoiding insects can not survive freezing, but will survive in very high numbers as long as temperatures remain above their SCP. An example is the goldenrod gall moth, *Epiblemma scudderiana* (Clemens) (Lepidoptera: Tortricidae) (Rickards *et al.*, 1987). It can supercool to -38ºC, but freezing would be lethal as a high percentage of body water would be converted to ice allowing no time for compensatory and protective responses by cells (Storey and Storey, 1996). A further example of a freeze avoiding species is the autumnal moth, *Epirrita autumnata* (Borkhausen) (Lepidoptera: Geometridae) in Scandinavia (Nilssen and Tenow, 1990).

1.8.7 Chill tolerant

In this group moderately and highly chill tolerant species have been identified. The Antarctic mite, *Alaskozetes antarcticus* (Michael or Dalenius) (Acari: Podacaridae) has a SCP of around -30ºC; however 27% of the population die after 100 days at -20ºC (Cannon, 1987). This mite can be described as highly chill tolerant. In the wild, due to the buffering effects of snow cover, *A. antarcticus* needs only to survive temperatures of around -15ºC that are present for several months of the year (Davey *et al.*, 1992). An example of a moderately chill tolerant species is the beech weevil *Rhynchaenus fagi* (Linnaeus) (Coleopteran: Curculionidae). It has a SCP of approximately -25ºC, but less than 30% of the population can survive for more than 50 days at -15ºC. However, in the wild *R. fagi* seldom needs to withstand temperatures lower than -5ºC, so winter survival is possible in high numbers (Bale, 1991).

1.8.8 Chill susceptible

Chill susceptible insects can survive at low temperatures below their developmental threshold, but die after brief (for example 1 min) exposure to high sub-zero temperatures (Bale, 1993). Aphids are often classed as chill susceptible. *Myzus persicae* for example, has a SCP of -25ºC, but will die after being exposed for 1 min at -5 to -15ºC without freezing (Bale *et al.*, 1988).

1.8.9 Opportunistic survival

Insects classed as opportunistic survivors can not survive temperatures below their developmental threshold and are often of tropical or sub-tropical origin (Bale, 1993). They survive by seeking favourable habitats and sites when the opportunity arises (Bale, 1996) such as the housefly, *Musca domestica* (Linnaeus) (Diptera: Muscidae) where 97.5% of larvae die at 5ºC (Somme, 1961) and 90% of pupae die after 4 days at 0ºC (Coulson and Bale, 1990).

The work in the present study will help determine the cold hardiness characteristics of *T. montdorensis*.

1.8.10 Key biochemical components concerned with cold hardiness

Insects either avoid freezing by supercooling of their body fluids or develop a tolerance of freezing. The biochemical and physiological mechanisms involved are diverse. A summary of the biochemical mechanisms used by freeze tolerant and freeze avoiding insects is given in Figure 2. Freeze tolerant insects can utilise INAs, polyols and antifreeze proteins, whereas freeze intolerant species use only the latter two components to survive low temperatures.

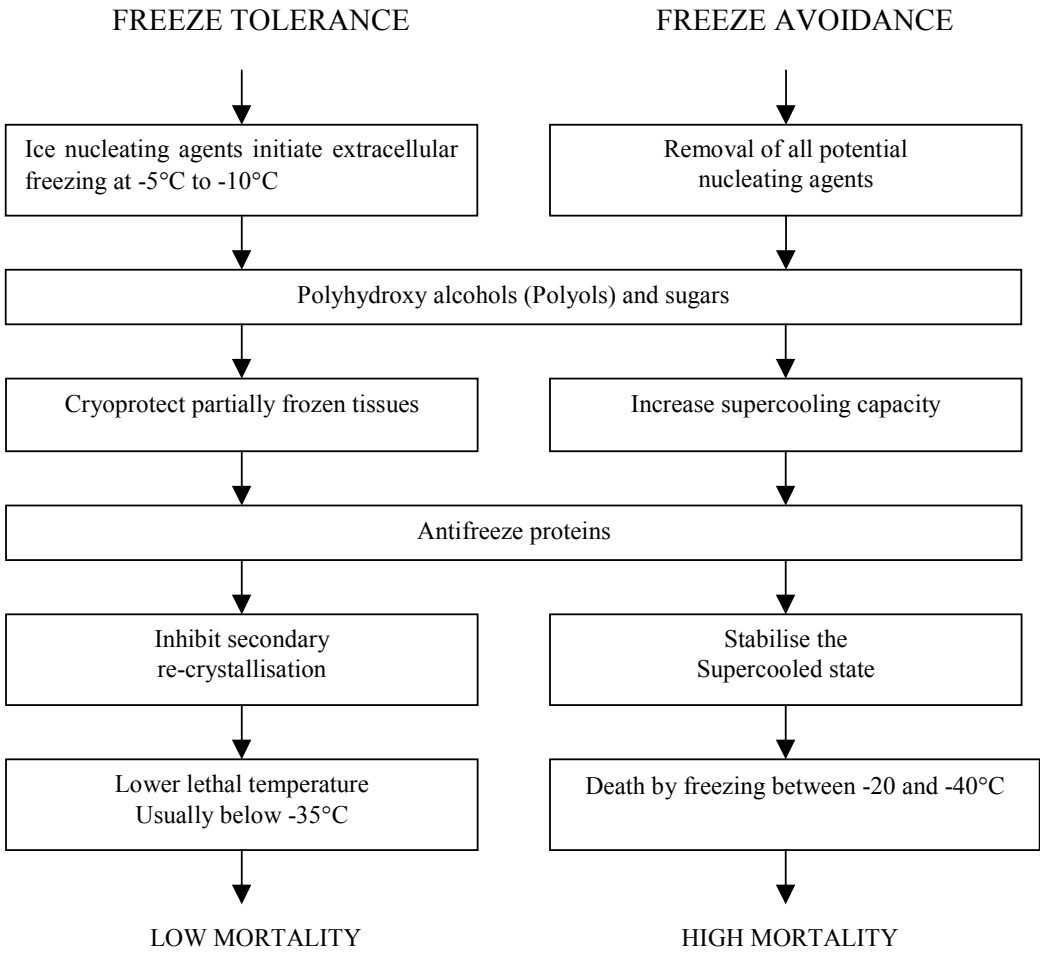


Figure 2: Biochemical mechanisms of cryoprotection utilised by freeze tolerant and freeze avoiding insects (Bale, 2002).

1.8.11 Biochemical agents utilised by freeze tolerant species

Ice formation is promoted by the presence of INAs such as food, proteins and other particles such as dust (Salt, 1961). The synthesis of INAs elevates the freezing temperature of extracellular areas, promoting the movement of water from intracellular areas to re-establish the osmotic equilibrium and thus reduce the risk of intracellular freezing (Duman, 2001; Duman and Horwath, 1983; Zachariassen and Hammel, 1976). INAs are found in freeze tolerant insects and were first identified in the haemolymph of *Eleodes blanchardi* (Blaisdell) (Coleoptera: Tenebrionidae), the freeze tolerant darkling beetle (Zachariassen and Hammel, 1976). INAs so far identified are proteins (Zachariassen, 1982; Zachariassen, 1985) and are generally produced in the haemolymph, for example in freeze tolerant Coleoptera, Diptera and Hymenoptera (Zachariassen, 1982). INAs play a vital role in assisting the survival of

freeze tolerant insects during extracellular ice formation. *Dendroides canadensis* (Latreille) (Coleoptera: Pyrochroidae) and *Cucujus clavipes* (Fabricus) (Coleoptera: Cucujidae) beetle larvae are freeze tolerant during the winter when INAs are present, but lose the freezing state in summer when INAs are absent (Duman, 1984; Horwath and Duman, 1984). An exception is the beetle *Phyllodecta laticollis* (Suffrian) (Coleoptera: Chrysomelidae) which remains freeze tolerant all year round (van der Laak, 1982).

Sugars and polyhydroxy alcohols (polyols) act as cryo-protectants for partially frozen tissues in freeze tolerant insects and usually, like INAs, are produced in late autumn and are absent during the summer. Polyols and sugars can protect freeze tolerant insects from cryogenic injuries by stabilising protein structure, reducing transmembrane water fluxes, reducing the rate of ice formation and spread and maintaining cell volumes above a critical threshold (Baust, 1973; Baust, 1982). Two of the most common polyols associated with freeze tolerant insects are glycerol and sorbitol while sucrose and trehalose are common sugars (Baust, 1973; Somme, 1964; Zachariassen, 1985).

Thermal hysteresis proteins (THPs), also known as antifreeze proteins (AFPs), were first found in marine Antarctic teleost fish (DeVries, 1971). Thermal hysteresis proteins act by lowering the freezing point of water in the absence of any significant effect on the melting point (Barrett, 2001; Duman, 2001). This produces a difference between the freezing and melting point known as thermal hysteresis (DeVries, 1986), the magnitude of which is dependent on the activity and concentration of the particular THP present (Wu and Duman, 1991).

In insects, the main site of THP synthesis and storage is thought to be the fat body (Easton and Horwath, 1994). As with cryo-protectants and INAs, expression of THPs is a seasonal occurrence and in *D. canadensis* for example, it is under photoperiodic control (Duman *et al*., 1991). In freeze tolerant insects THPs may protect from injuries caused by early or late frosts before INAs are active and the insects are not fully cold-hardened (Duman, 1982; Duman, 2001). THPs may lower the internal freezing point and prevent ice re-crystallisation that may cause cryogenic injuries (Barrett, 2001; Duman, 2001; Knight and Duman, 1986). Thermal hysteresis proteins

extracted from insects and put into solution have demonstrated that the antifreeze effect increases with decreasing crystal size (Zachariassen, 1982).

1.8.12 Biochemical agents utilised by freeze intolerant/avoiding species

Freeze intolerant species are killed by freezing and thus must remove or mask all potential ice nucleating agents. Simultaneously, polyols such as glycerol, sorbitol, mannitol and ethylene glycol (Bale, 1989; Morrisey and Baust, 1976; Salt, 1961) and sugars such as fructose, glucose and trehalose (Bale, 1989) are synthesised. These components reduce the supercooling point in freeze intolerant insects (Duman, 1982; Young and Block, 1980; Zachariassen, 1985). These cryoprotectants may also reduce enzyme activity to decrease energy expenditure during the winter (Zachariassen, 1985). The production of THPs in freeze intolerant insects lowers the internal freezing point and stabilises the supercooled state (Kristiansen *et al.*, 1999; Zachariassen, 1985). To date, all identified THP producing mites are freeze intolerant (Duman, 2001).

1.9 Diapause

1.9.1 Diapause induction

Diapause is a form of dormancy or hibernation among insects and acarines. Its onset, maintenance and termination are characterised by a number of morphological, behavioural, physiological and biochemical features (Tauber and Tauber, 1976). Diapause induction has been studied in a number of mite species (see Overmeer, 1985a; Veerman, 1992; Denlinger, 2002 for reviews). Previous studies have shown that diapause occurs only in mated females and the most striking characteristic of the diapause state is that no eggs are produced (Overmeer, 1985a). Activity and feeding in diapausing females tends to be reduced or ceases entirely (Hoy and Flaherty, 1975; Morewood and Gilkeson, 1991; van Houten *et al.*, 1988). Diapausing females often appear paler and flatter than non-diapausing females (Morewood and Gilkeson, 1991; Overmeer, 1985a; Veerman, 1992). Under close inspection, the body cavity contents of diapausing mites appear granular; this is thought to be due to accumulation of lipids as energy reserves (Morewood and Gilkeson, 1991). Additionally, diapausing

mites may seek shelter and their longevity often increases compared with non-diapausing mites (Veerman, 1992).

Photoperiod is the primary cue for diapause induction in phytoseiid mites and has been demonstrated for 15 phytoseiid species (Veerman, 1992). Diapause only occurs in species exposed to short day lengths and generally only in individuals that have been exposed to diapause inducing conditions in their juvenile stages (Morewood, 1993; Veerman, 1992). However, some species for example *Amblyseius potentillae* (Garman) (Acari: Phytoseiidae), can enter diapause as adults after being reared in non-diapause inducing conditions (van Houten, 1989). In most diapausing mites the developmental stages prior to the stage where diapause expression occurs is where the actual induction of diapause occurs (Veerman, 1992). In *Amblyseius cucumeris* (Oudemans) (Acari: Phytoseiidae) diapause was most frequent when the mites were reared in diapause-inducing conditions before the eggs even hatched, the embryonic stages of *A. cucumeris* therefore being most sensitive to diapause inducing conditions (Morewood and Gilkeson 1991). In contrast, in *A. potentillae*, sensitivity to diapause inducing conditions was greatest in protonymphs (van Houten and Veenendaal, 1990). Some of the critical photoperiods required for diapause induction in several mite species are listed in Table 1.

Temperature also influences diapause induction and the length of the critical photoperiod may change with varying temperatures. For example, diapause in *Amblyseius andersoni* (Chant) (Acari: Phytoseiidae) and *A. cucumeris* can be induced even when they are kept in constant darkness, providing the temperatures are optimal for induction (Veerman, 1994). Temperature may act independently as a diapause-inducing stimulus or may modify the photoperiodic response (Veerman, 1992). Diapause may be prevented entirely if temperatures remain above 20ºC (Hoy and Flaherty 1975; Morewood and Gilkeson 1991).

Relative humidity (r.h.) may also play a role in diapause induction. A low r.h. (35%) during diapause induction resulted in a shorter diapause duration in *A. potentillae* than if mites were subjected to a high r.h. (75%) during diapause induction (Van Houten and Veenendaal, 1990).

Table 1: Recorded critical photoperiodic ranges of phytoseiid mites

Species	Critical Photoperiod (h)	Reference
Metaseiulus (Typhlodromus) occidentalis	10-12	(Hoy and Flaherty, 1975; James, 1988)
Typhlodromus pyri	12.5-13.5	(Fitzgerald and Solomon, 1991)
Amblyseius potentillae	14.5	(van Houten and Veenendaal, 1990)
Amblyseius cucumeris	12.45	(Morewood and Gilkeson, 1991)
Neoseiulus fallacis	11.75-12	(Rock *et al.*, 1971)

1.9.2 Diapause termination

To terminate diapause certain specific developmental stages within diapause must take place (Veerman 1992). Diapause duration and intensity are species specific and the intensity of response to external stimuli varies (Tauber *et al*., 1986). The rate of diapause development is influenced by photoperiod and temperature and the intensity of the diapause state decreases during late winter when conditions slowly become more favourable (Veerman 1992). Diapause does not terminate when favourable conditions occur, but will often require a certain number of day degrees to accumulate (Mansingh, 1971). Some insects such as the flesh fly, *Sarcophaga bullata* (Parker) (Diptera: Sarchphagidae) move from a state of diapause to a post-diapause developmental stage when conditions become more favourable (Tauber *et al*., 1986). Physiologically the insects will appear identical, but the two stages can clearly be distinguished at the molecular level (see Denlinger, 2002 for review).

1.9.3 Cold hardiness and diapause

In some insects diapause appears to be an essential prerequisite for successful cold acclimation and overwintering (Denlinger, 1991; Slachta *et al.*, 2002), although a

seasonal increase in cold hardiness without a diapause trait is also common (Fields *et al.*, 1998; Young and Block, 1980). Diapausing pupae of the cabbage white butterfly, *Pieris brassicae* (Linnaeus) (Lepidoptera: Pieridae) can survive for up to 30 days at -15ºC, whereas non-diapausing pupae die after 14 days at -5ºC (Pullin and Bale, 1989). In contrast there is no difference in SCP between diapause and non-diapause induced pupal and pre-pupal cabbage fly, *Delia radicum* (Linnaeus) (Diptera: Anthomyiidae) (Kostal, 1993). Historically, it is likely that the ability of insects to successfully move through different climates has involved both diapause and cold tolerance and these may have evolved in tandem or independently (Pullin, 1996).

Studies on some predatory mite species have shown that during cold winters diapausing females overwinter, whereas males and juveniles die. Some species may lack a diapause response and overwinter without diapausing (Morewood, 1993; Overmeer, 1985a). Previous studies on $LTemp_{50}$ values of *A. potentillae* showed that survival times at sub-zero temperatures were three times higher in non-acclimated diapausing mites than in non-acclimated, non-diapausing *A. potentillae*. Survival times of diapausing mites acclimated at 4ºC were much greater than non-acclimated diapausing mites. However, survival times of acclimated non-diapausing mites were only slightly longer than non-acclimated non-diapausing mites, indicating the increased cold hardiness and acclimation ability in diapausing compared with non-diapausing mites (van der Geest *et al.*, 1991). Cold hardiness in *Amblyseius umbraticus* (Chant) (Acari: Phytoseiidae) and *Phytoseius finitimus* (Ribaga) (Acari: Phytoseiidae) is greater in diapausing than in non-diapausing females, further demonstrating the advantage of diapausing mites compared with non-diapausing mites in winter (Overmeer, 1985a).

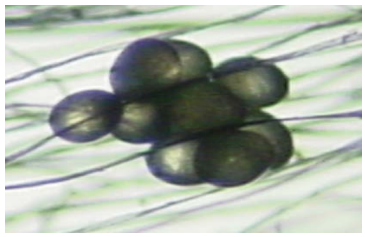
Results such as the above demonstrate the need to investigate the possibility of a diapause trait in *T. montdorensis* as, if present, this may increase the likelihood of survival through UK winters outside glasshouses.

1.10 Photographs

Photographs 1-12 are of various species studied during the present work and of arenas used to rear mites.

Photograph 1:
Typhlodromips montdorensis (larval)

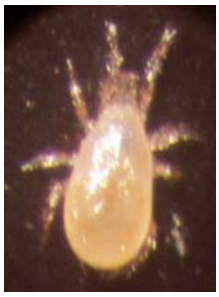
Photograph 2:
Typhlodromips montdorensis (female)



Photograph 3:
Typhlodromips montdorensis (eggs)

Photograph 4:

Neoseiulus californicus (female)

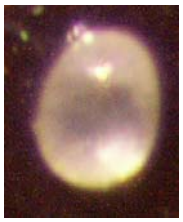


Photograph 5:

Typhlodromus pyri (female)

Photograph 6:

Neoseiulus californicus (egg)



Photograph 7:

Typhlodromus pyri (egg)

Photograph 8:

Macrolophus caliginosus (4th instar female)

Photograph 9:

Macrolophus caliginosus (adult) male)

Photograph 10:
Slide adapted to study individual mites

Photograph 11:
Thread for oviposition, cotton water source and pollen for rearing mites

Photograph 12:
Tetranychus urticae on Dwarf French beans

1.11 Objectives

The objectives of this work were to:

1. Investigate the establishment potential of *T. montdorensis* in the UK by studying its developmental, thermal biology and field survival, and determine and use the developmental threshold of *T. montdorensis* to predict its potential voltinism in the UK.

2. Compare data obtained for *T. montdorensis* with other non-native biological control agents previously subjected to the experimental protocol.

3. Investigate other factors affecting the establishment of non-native biological control agents in the UK such as alternate food sources outside the glasshouse environment and the possibility of competition between non-native and native mite species outside the glasshouse.

Chapter 2: Developmental and low temperature biology of *Typhlodromips montdorensis*

2.1 Biology of *Typhlodromips montdorensis*

Typhlodromips montdorensis (Photographs 1-3) was first described in 1978 from New Caledonia, an island under French Law 1500 km off the East Coast of Australia (Schicha, 1979) and then also reported in Queensland (Australia), Fiji and Tahiti (Schicha, 1987). It is a sub-tropical species and in its natural habitat the minimum temperature encountered is generally above 8°C (Steiner and Goodwin, 2002a). Until recently it was in the genus *Amblyseius*, but has now been reclassified as knowledge of taxonomic relationships has grown. *Amblyseius* and *Typhlodromips* can be distinguished from each other by an erect proximal seta on Tarsus I present in *Amblyseius*, but absent in *Typhlodromips* (Beard, 2001). Taxonomic studies of *T. montdorensis* are available (Beard, 2001; Schicha, 1979).

Typhlodromips montdorensis is reported to feed on eriophyid mites, *Tetranychus urticae* (Schicha, 1979; Schicha, 1987), broad mite *Polyphagotarsonemus latus* (Banks) (Acarina: Tarsonemidae), tomato russet mite *Aculops lycopersici* (Massee) (Acarina: Eriophyidae) and other small arthopods (Steiner and Goodwin, 2002a) on common bean, strawberry, cucumber and tomato. In Australia *T. montdorensis* is being studied as a potential field biological control agent of thrips and tests have yielded successful control of Western flower thrips, *Frankliniella occidentalis* and onion thrips *Thrips tabaci* (Lindeman) (Thysanoptera: Thripidae) on cucumber and strawberries (Steiner, 2002; Steiner and Goodwin, 2002b). *Typhlodromips montdorensis* also maintains good population levels when feeding on *T. urticae* (Steiner and Goodwin, 2002b) and can survive and reproduce when fed solely on pollen such as cumbungi (cattail) pollen (*Typha sp*) (Steiner and Goodwin, 2002a; Steiner *et al.*, 2003b).

A range of tests on the sensitivity of *T. montdorensis* to various commercial insecticides have been done and shown that products such as Pirimor®, Tilt® and

Bravo® have low toxicity to *T. montdorensis*. Sulphur is of intermediate toxicity and Vertimec®, Pyrethrum® and Vapona® are highly toxic (Steiner and Goodwin, 2002a). Information gathered on thrips control by *T. montdorensis* on various crops concludes that good control is reached on strawberry, cucumber, peppers, tomatoes and gerbera either by the predator alone, or with a small application of a low toxicity insecticide (Steiner and Goodwin, 2002a).

Recent work on *T. montdorensis* concluded that it had a minimum temperature for development of about 11ºC and colonises crops rapidly at temperatures above 20ºC. Lifetime fecundity is around 53 eggs and it has a sex ratio of more than 65% female. Consumption of first stage thrips can be as high as 20 per day. It does not enter diapause under normal glasshouse conditions and relative humidity for egg hatch must be around 70% (Steiner and Goodwin, 2002a; Steiner *et al.*, 2003b).

In Europe *T. montdorensis* is currently being tested and marketed as Amblyline-M® by Syngenta Bioline™. To obtain a licence for its release in the UK a risk assessment concerning its establishment potential outside UK glasshouses must be compiled. A thorough risk assessment is crucial as *N. californicus* was granted a licence for release in UK glasshouses on the basis that it was unlikely to survive through winter, but has subsequently become established in the south-east and west of England (Jolly, 2000). After examining the evidence provided in the original licence application and considering the situation concerning the unexpected establishment of *N. californicus*, the Advisory Committee on Releases to the Environment (ACRE) decided not to allow the release of *T. montdorensis* in glasshouses (ACRE, 2001) until further research had been done. A permit for the release of *T. montdorensis* in Canada is also currently being sought (Goodwin and Steiner, 2002). Furthermore, at the present time, small scale commercial releases have been undertaken in Finland and some trials have also been done in Spain, France, Holland and Denmark (R. GreatRex, pers. comm.).

2.2 Aims

The aims of this chapter were to:

1. Determine the developmental threshold of *T. montdorensis* and predict its potential voltinism in the UK.

2. Investigate laboratory low temperature biology of *T. montdorensis*.

3. Investigate possible laboratory induction of diapause in *T. montdorensis*.

4. Undertake field survival and reproduction experiments and draw comparisons with laboratory data.

2.3 Mite rearing

The following rearing methods were used to provide predators and prey for all experiments during the present study. Temperatures throughout all experiments were recorded using Tinytalk® dataloggers (Gemini, UK) and light regimes were monitored using HOBO Onset® lightloggers (Tempcon, UK).

2.3.1 Tetranychus urticae

Tetranychus urticae were reared on Dwarf French beans (Photograph 12), *Phaseolus vulgaris* (Linnaeus) (Fabaceae) at 25ºC, 18:6 LD and a relative humidity of approximately 50-70%. Bean plants were grown in a separate growth room (20ºC, 18:6 LD) and 4-6, 20 cm tall plants were fed into the *T. urticae* culture daily. Plants were watered every two days. Unused plants were removed from the culture to reduce problems with thrips that occasionally infested the plants. The developmental time of thrips is slightly longer than that of *T. urticae* and therefore by removing old plants rapidly they could be largely contained. Heavily infested leaves from old plants were picked off and placed onto younger plants to maintain a healthy stock of *T. urticae*.

2.3.2 Typhlodromips montdorensis

Typhlodromips montdorensis were obtained from Syngenta Bioline, UK (Amblyline-M®) and reared under quarantine conditions at 25ºC, 18:6 LD and 70-75% r.h. at the University of Birmingham, UK. High r.h. is important to ensure a high level of egg hatch (Steiner *et al.*, 2003b). Mites used in experiments reared under the above regime were classed as non-acclimated. Mites used to detect any acclimation capacity were held for 7 days at 10ºC, 12:12 LD and classed as acclimated. This regime was selected to be consistent with previous use of this protocol (Hart *et al.*, 2002a; Hart *et al.*, 2002b) and was not intended to produce fully acclimated mites, but to detect whether there was any acclimation ability in the species. The culturing method was adapted from Overmeer (1985b). Mites were placed on black ceramic tiles (13 x 17 x 0.8 cm) using a fine artist's paintbrush, on a sponge block (6 x 14 x 4 cm) submerged in water in a plastic box (17 x 25 x 9 cm). The water level was maintained just below the level of the tile. OecoTak® A5, (Oecos, UK), a non-drip insect trapping adhesive which does not set or dry was applied to the outer edge of the tiles and provided a physical barrier for the mites, which they did not attempt to cross. A 0.5 cm wide, 10 cm long piece of cotton wool connected the tile to the water as a moisture source. Lids with holes (14 x 8 cm) covered in muslin were placed on the plastic boxes. Strands of cotton thread (2 cm long) frayed at both ends were placed on the tiles to provide an oviposition site for the females (Photograph 11). *Typhlodromips montdorensis* were fed on Cumbungi (cattail) pollen (*Typha sp.*) and *T. urticae* eggs and nymphal stages reared on *P. vulgaris*, which were brushed onto the tiles daily using a size 12 paintbrush. Both pollen and *T. urticae* were used as food; although pollen is known to be an adequate food source (Steiner *et al.,* 2003b), it does not contain β-carotene found in *T. urticae*, which is required as part of a balanced diet.

New culture tiles were set up every 4 weeks when debris prevented mites and any new eggs being clearly seen. The mites were transferred from the old to the new tiles.

2.3.3. Studying individual mites

To study individual mites, a piece of filter paper from which 16, 4 cm^2 squares had been removed was placed on a tile (Photograph 10), thus creating 16 'arenas' in which individual mites could be contained and studied. Each arena was separated from the neighbouring arena by 1 cm of filter paper, and to provide an impassable barrier between the arenas, a 0.5 cm wide strip of OecoTak® was applied to the upper and lower surfaces of the filter paper. Along the length and width of the tile, excess filter paper (10 cm long) was suspended in water, so keeping the filter paper around each arena moist. The mites were able to reach the water without getting stuck to the OecoTak®. As before, the tiles were placed on foam blocks, in water-filled boxes with a ventilated lid.

2.4 Influence of temperature on development of *Typhlodromips montdorensis*

It is often assumed that non-native biological control agents of tropical or sub-tropical origin lack the necessary cold hardiness to over-winter and establish in the UK outside the glasshouse environment. To gain a better understanding of the thermal biology of *T. montdorensis*, developmental biology experiments were conducted at a range of temperatures to determine the developmental threshold and together with the available day-degrees, the number of possible generations per year.

2.5 Methods

Developmental times of non-acclimated *T. montdorensis* were studied on individual arenas in incubators at 10, 15, 20, 25, 27, 30 and 35ºC, 18:6 LD (n = 30 at each temperature. Each arena was checked every 12 h to record the date of egg hatch and time (days) for development to larval, protonymph, deutonymph and adult stage. A thin sprinkle of pollen was the only food source provided as it was assumed that feeding the mites on pollen alone would be sufficient (Steiner and Goodwin, 2002a; Steiner *et al.*, 2003b). By not feeding the mites with *T. urticae* it was possible to keep the arenas sufficiently clean so that cast skins shed during nymphal moults could be seen and thus the time of moulting through successive life stages determined. Developmental times were log transformed and differences between temperatures and

life stages analysed by Two-way Analysis of Variance (ANOVA) and then by pairwise comparisons using Tukey's honestly significant difference (HSD) method. The data were also analysed by simple and weighted linear regression (Draper and Smith, 1981). The developmental threshold was estimated by extrapolation of the linear relationship between development and temperature (over the middle temperature range), and the thermal budget (day-degree requirement) determined by taking the reciprocal of the slope (Campbell *et al.*, 1974). The developmental threshold and day-degree requirements were then used in combination with temperature data provided by the School of Geography, Earth and Environmental Sciences at the University of Birmingham to estimate the likely number of generations per year under outdoor conditions.

Additionally, the development of 30 non-acclimated *T. montdorensis* fed on *T. urticae* was compared with the development of 30 individuals fed on pollen at 25ºC to determine if differences in diet affected developmental time. The data were analysed by a T-test.

2.6 Results

2.6.1 Effect of food sources on rate of Typhlodromips montdorensis development

There was no difference in developmental time (± SE) from egg hatch to adult in *T. montdorensis* fed on pollen or *T. urticae* at 25ºC ($T_{1,58}$ = 0.732, P > 0.05). Mean time from egg hatch to adult was 4.8±0.09 and 4.7±0.17 days for *T. montdorensis* fed on pollen and *T. urticae* respectively.

2.6.2 Developmental time

At 10ºC, 16% of individuals hatched after 15-20 days, two moulted to protonymphs and all were dead by day 30. At 35ºC, 36% of individuals hatched, 50% of which moulted to protonymphs; all individuals were dead by day six. In consequence, these incomplete data were omitted from subsequent analyses. At 30ºC the developmental time from egg to adult was slower than at 27ºC. The 30ºC data were therefore also omitted from the analysis to estimate the developmental threshold. The mean number

of days (± SE) to complete each development stage and the total mean time for development from egg to adult at different temperatures are shown in Table 2. Developmental time was significantly more at 15 than at 20, 25 and 27°C for total developmental time from egg to adult ($P < 0.01$). Similarly, developmental time was more at 20 than at 25°C ($P< 0.01$) and at 25 than at 27°C ($P < 0.01$); the optimum temperature for development in the present study was therefore 27°C. In addition, time until egg hatch and time spent as larvae was significantly more at 15 than at 20, 25 and 27°C ($P< 0.01$) and more at 20 than at 25 and 27°C ($P < 0.01$). Time spent as proto- and deutonymphs was significantly longer at 15 than at 20, 25 and 27°C ($P < 0.01$) and at 20 than at 25 and 27°C and at 25 than at 27°C ($P< 0.05$).

Table 2: Effect of temperature on developmental time (mean days ± SE) for each stage of *Typhlodromips montdorensis*

Temperature (°C)	Egg hatch	Larvae	Protonymph	Deutonymph	Total time egg-adult
15	7.1 +/- 0.22	2.9 +/- 0.02	5.8 +/- 0.07	6.3 +/- 0.06	22.1 +/- 0.6
20	4.5 +/- 0.13	1.9 +/- 0.13	3.6 +/- 0.22	2.9 +/- 0.16	12.9 +/- 0.34
25	2.7 +/- 0.09	0.9+/- 0.08	2.0 +/- 0.14	1.9 +/- 0.16	7.5 +/- 0.16
27	2.4 +/- 0.09	0.9 +/- 0.07	1.5 +/- 0.12	1.5 +/- 0.12	6.3 +/- 0.12
30	2.7 +/- 0.09	0.9 +/- 0.10	1.9 +/- 0.13	2.5 +/- 0.17	8.0 +/-0.24

2.6.3 Developmental threshold and thermal budget

Developmental rate from egg to adult of *T. montdorensis* was plotted against temperature (Figure 3). The lower developmental threshold for *T. montdorensis* was 10.7 and 10.3ºC when estimated by simple ($R^2 = 0.925$) and weighted ($R^2 = 0.932$) linear regression respectively (Equations 1 and 2). The relationship between developmental rate (1/days) and temperature is approximately linear between 10 and 27ºC. For development from egg to adult, *T. montdorensis* required a thermal budget of 105.3 degree-days (1/0.0095) and 108.7 degree days (1/0.0092) above the developmental threshold using simple linear and weighted linear regression respectively (Equations 4 and 6). Using weighted linear regression results in a lower estimate of developmental threshold for *T. montdorensis* than when using simple linear regression. The day-degree requirement per generation is higher using simple

linear regression due to the slope reduction with the lower developmental threshold (Hart *et al.*, 2002b).

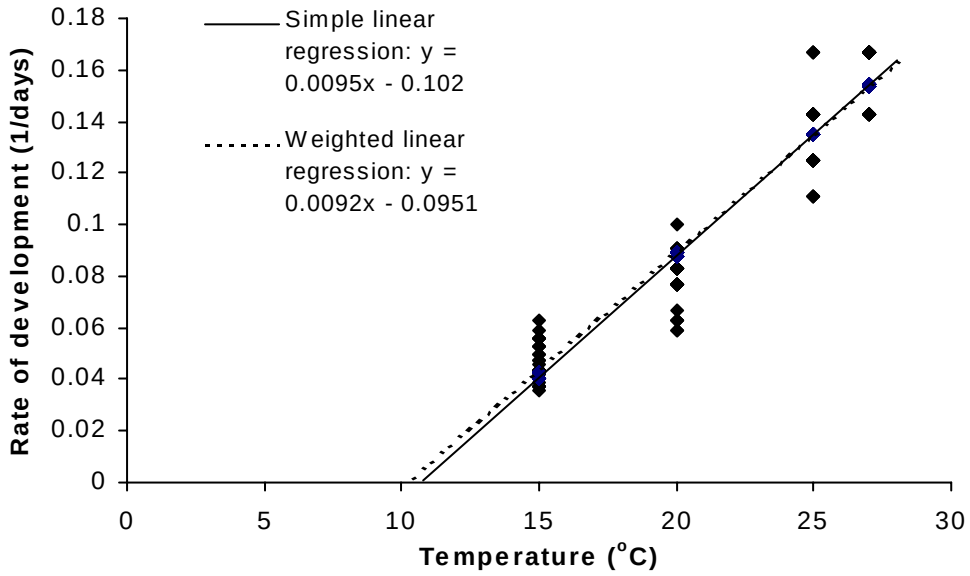


Figure 3: Development of *Typhlodromips montdorensis* from egg to adult at varying temperatures, with lines fitted by simple (R^2 = 0.925) linear regression, y = 0.0095x - 0.102 (solid line) and weighted (R^2 = 0.932) linear regression, y = 0.0092x - 0.0951 (dotted line).

2.6.4 Number of possible yearly generations of Typhlodromips montdorensis in the UK

The mean number of day degrees available in each year from 1986-2000 as calculated from available temperature data (Equation 7) is shown, together with the theoretical number of generations of *T. montdorensis* possible each year over a 15 year period, based on the number of available day degrees using simple linear regression (Equation 8) (Table 3) and weighted linear regression (Table 4). Between the beginning of April and the end of September the mean number of possible generations for 1986 to 2000 was determined to be five and six compared with 0.34 and 0.41 for the corresponding autumn and winter period for estimates using simple and weighted linear regression respectively (October – March).

Table 3: Theoretical number of generations each year between April and September and October and March from 1986 – 2000 of *Typhlodromips montdorensis* in Birmingham, UK using simple linear regression (values in brackets refer to actual number of generations possible).

Year	Available ^{o}d per year	Max. no. of generations per year	Available ^{o}d Apr-Sept	Max. no. of generations Apr-Sept	Available ^{o}d Oct-Mar	Max. no. of generations Oct-Mar
1986	450.5	4.3 (4)	410.5	3.9 (3)	40	0.38 (0)
1987	513.5	4.9 (4)	493.1	4.7 (4)	20.4	0.19 (0)
1988	492.6	4.7 (4)	468.4	4.4 (4)	24.2	0.23 (0)
1989	766.2	7.3 (7)	726.1	6.9 (6)	40.1	0.38 (0)
1990	740.3	7.0 (7)	665.5	6.3 (6)	74.8	0.71 (0)
1991	654.3	6.2 (6)	621.9	5.9 (5)	32.4	0.31 (0)
1992	650.7	6.2 (6)	643	6.1 (6)	7.7	0.07 (0)
1993	500.4	4.8 (4)	492.3	4.7 (4)	8.1	0.07 (0)
1994	626.3	6.0 (6)	588.6	5.6 (5)	37.7	0.36 (0)
1995	841.1	8.0 (8)	768.6	7.3 (7)	72.5	0.68 (0)
1996	617.1	5.9 (5)	581.8	5.5 (5)	35.3	0.33 (0)
1997	730.2	6.9 (6)	676.2	6.4 (6)	54	0.51 (0)
1998	621.5	5.9 (5)	586.8	5.6 (5)	34.7	0.33 (0)
1999	695.1	6.6 (6)	664.1	6.3 (6)	30.9	0.29 (0)
2000	638.8	6.1 (6)	613.6	5.8 (5)	25.2	0.24 (0)
Mean	**635.9**	**6.1 (6)**	**600**	**5.7 (5)**	**35.9**	**0.34 (0)**

Table 4: Theoretical number of generations each year between April and September and October and March from 1986 – 2000 of *Typhlodromips montdorensis* in Birmingham, UK using weighted linear regression (values in brackets refer to actual number of generations possible).

Year	Available ^{o}d per year	Max. no. of generations per year	Available ^{o}d Apr-Sept	Max. no. of generations Apr-Sept	Available ^{o}d Oct-Mar	Max. no. of generations Oct-Mar
1986	511.9	4.7 (4)	463.5	4.2 (4)	48.4	0.44 (0)
1987	576.2	5.3 (5)	548.5	5.0 (5)	27.7	0.25 (0)
1988	561.8	5.1 (5)	530.2	4.8 (4)	31.6	0.29 (0)
1989	837.6	7.7 (7)	785.2	7.2 (7)	52.4	0.48 (0)
1990	820.4	7.5 (7)	729	6.7 (6)	91.4	0.84 (0)
1991	715.8	6.5 (6)	674.3	6.2 (6)	41.4	0.38 (0)
1992	719.8	6.6 (6)	706.2	6.5 (6)	13.5	0.12 (0)
1993	563.7	5.2 (5)	550.2	5.0 (5)	13.5	0.12 (0)
1994	697.8	6.4 (6)	646.3	5.9 (5)	51.5	0.47 (0)
1995	914.7	8.4 (8)	830.2	7.6 (7)	84.4	0.77 (0)
1996	682.6	6.3 (6)	637.7	5.8 (5)	44.9	0.41 (0)
1997	802.3	7.4 (7)	743.4	6.8 (6)	58.8	0.54 (0)
1998	694.3	6.4 (6)	648.8	5.9 (5)	45.5	0.41 (0)
1999	773.2	7.1 (7)	732.2	6.7 (6)	40.9	0.37 (0)
2000	710.9	6.5 (6)	674.9	6.2 (6)	35.9	0.33 (0)
Mean	**705.6**	**6.5 (6)**	**660**	**6.0 (6)**	**45.4**	**0.41 (0)**

The estimates of developmental threshold and voltinism indicate that from 1986-2000 an average of six generations would be possible per year in the Midlands region of the UK when using both simple and weighted linear regression. Development of *T. montdorensis* is likely to be confined to periods between April and September of each year. Between five and six generations may be possible during this time period. Data from 1986-2000 shows that temperatures above the developmental threshold of *T. montdorensis* are reached only between mid April and the end of September (Figure 4). Therefore *T. montdorensis* would have to survive 6 months of the year at temperatures below its developmental threshold and some exposures to temperatures below -7°C. To test whether *T. montdorensis* is able to do this, studies on its thermal biology and cold tolerance are necessary.

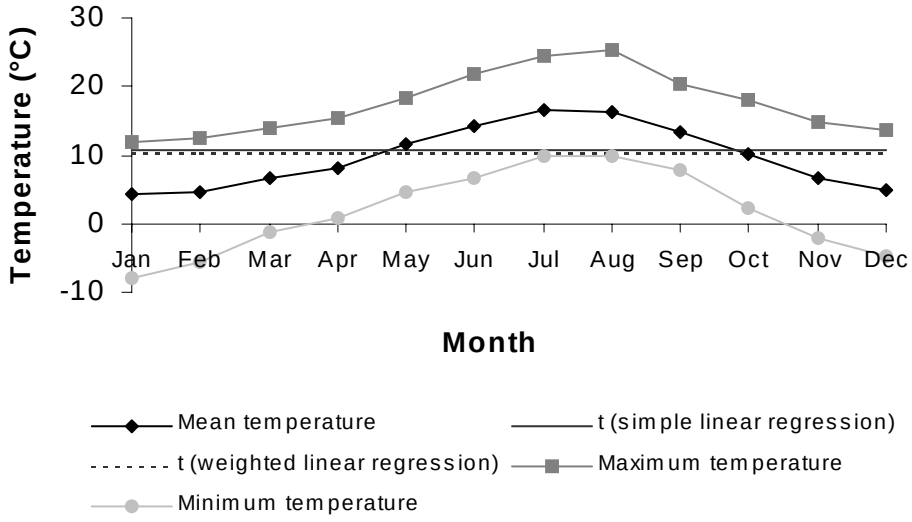


Figure 4: Average maximum, mean and minimum temperatures recorded for each month from 1986-2000 in Birmingham, UK. Developmental threshold (t) when calculated using simple- and weighted linear regression shown.

2.7 Low temperature biology of *Typhlodromips montdorensis*

Measurement of the supercooling point can be used to determine an insect's response to low temperature cooling and to freezing. From this its strategy for winter survival, for example whether it is freeze tolerant or freeze intolerant, can be determined, at least in a preliminary way. Temperatures above the SCP of an insect can still

sometimes lead to death and therefore further studies on laboratory survival rates both to brief exposures to low temperatures and to longer term exposures at a set temperature are sometimes required. From this, the lethal temperature (LTemp) and the lethal period of time (LTime), required to kill a certain proportion of a population can be assessed.

2.7.1 Survival assessment

Survival after a cold hardiness experiment is best assessed by the ability of the individual insect to reproduce (Baust and Rojas, 1985). However, due to large sample sizes and limited rearing resources, this was not feasible in this study. Therefore, in all low temperature laboratory experiments adults were deemed alive if they responded to a tactile stimulus directly after the experiment and after a further 24 and 48 h. Larval *T. montdorensis* were deemed alive if they successfully moulted to the next stage of their life cycle (protonymph) within 8 days of the end of the cold exposure. One experiment also investigated reproductive output after cold exposure.

2.8 Methods

Unless otherwise stated, for all of the following experiments adult females and larvae were used to identify any differences in cold tolerance between different life stages of *T. montdorensis*. Unless otherwise stated, four groups of mites were used: non-acclimated females, non-acclimated larvae, acclimated females and acclimated larvae. Each group was subjected separately to a variety of treatments. Non-acclimated females had moulted from the deutonymph stage 24 h prior to being used, and larvae were less than 12 h old. Acclimated females and larvae had been held at 10°C for 7 days and were thus slightly older than non-acclimated mites.

2.8.1 Supercooling points of Typhlodromips montdorensis

Supercooling points (SCP) of *T. montdorensis* were measured by a differential scanning calorimeter (DSC) at the British Antarctic Survey, Cambridge, UK. Due to the small size of *T. montdorensis* it was not possible to use the T thermocouples described by Bale *et al*., (1984) to measure SCPs. Supercooling points were measured

for each of the four experimental groups (n = 20 for each group). Five mites from any one treatment were placed in a small aluminium pan (40 µl), pressure sealed and placed inside the DSC. The mites were cooled at 1ºC min^{-1} to their SCP (onset of freezing exotherm). The pans were then re-opened and checked for survivors.

2.8.2 Lower lethal temperatures

By determining the lower lethal temperature of *T. montdorensis* it is possible to get an indication of its laboratory cold tolerance and by comparing the data to the SCPs, the degree of pre-freeze mortality can be determined. Fifty mites of each of the four groups were placed individually into size 3 Beem capsules (Agar Scientific Ltd, UK). The mites were exposed singly within each replicate as their rapid movement made it difficult to load more than one individual into a capsule. Ten capsules were placed in each of five boiling tubes and suspended in a low temperature programmable alcohol bath (Haake F8-C50), (Haake, Germany). After being transferred from 25ºC and held at 15ºC for 15 min (this temperature was selected as it represented a more realistic outdoor temperature than the rearing temperature of 25ºC), non-acclimated mites were cooled at 1ºC min^{-1} to a range of temperatures between 15 and -15ºC. Acclimated mites were cooled from 10°C (acclimation temperature). After being held at the required minimum exposure temperature for 1 min, the non-acclimated mites were re-warmed to 15ºC and the acclimated mites to 10ºC, both at 1ºC min^{-1}. A control sample of 30 females and 30 larvae was placed individually in Beem capsules and held in the alcohol bath at 15ºC for 1 h, equivalent to the maximum exposure time of the treated groups, to ensure that the Beem capsules and the vibrations made by the alcohol bath were not having a deleterious effect on the mites. The capsules containing the treated and control mites were then opened and placed in individual arenas so that each mite could emerge and feed on the pollen provided. The mites were held at 15 and 10ºC, 18:6 LD for non-acclimated and acclimated mites respectively, and survival recorded after 24 and 48 h.

The results were assessed using Probit analysis (Finney, 1971) (section 2.8.4) to estimate the temperature required to kill 10, 50 and 90% of the population ($LTemp_{10,50,90}$). Additionally, the larval control population and an acclimated larval

control population were reared to adulthood. Developmental times were compared with the survivors of a non-acclimated and an acclimated population both of which had been exposed to their $LTemp_{50}$ and then also reared to adulthood. The developmental times of both acclimated and non-acclimated populations of 30 mites each were log transformed to ensure a normal distribution and compared to their respective controls by One-way ANOVA.

2.8.3 Lower lethal time

Lower lethal time experiments complement the lethal temperature work, by investigating the response of *T. montdorensis* to temperatures likely to be experienced in the field over a longer time period. As in the lethal temperature experiments the four individual groups of *T. montdorensis* were placed into size 3 Beem capsules, with the addition of a strip of moist filter paper (1 cm) to avoid desiccation. Batches of ten capsules were placed in separate boiling tubes and a cotton wool bung was placed in the top of each tube. The four groups of 15 boiling tubes were suspended in an antifreeze solution (20%) to reduce temperature fluctuations inside the incubators around the tubes to a minimum and initially held at 10ºC for 30 minutes to overcome possible mortality due to cold shock. Then each group of mites was held in three separate treatments: 5, 0 or -5°C. At 24 h intervals three boiling tubes from each treatment group were removed and held for 30 min at 0 then 5 and then 10ºC to overcome possible mortality due to heat shock. The mites from each treatment were then placed onto individual rearing arenas where survival was assessed after 48 h at 15ºC. A control sample of 30 mites in individual capsules was maintained at 15ºC and survival assessed every 7 days. The results were analysed using Probit analysis to calculate the time required to kill 10, 50 and 90% of the individuals at each temperature ($LTime_{10,50,90}$).

2.8.4 Probit analyses

A Probit analysis tool from MINITAB v 13.32 was used to analyse the lethal temperature and lethal time results as this allowed the inclusion of control data. All temperature data had to be log transformed before analysis and all negative values were ignored as required by Probit.

2.9 Results

2.9.1 Supercooling points

No differences were detected in supercooling points between female and larval acclimated and non-acclimated *T. montdorensis* ($F_{3,78} = 0.44$, $P > 0.05$). The mean and range of supercooling points for each treatment are shown in Table 5. All treated individuals were dead after freezing. An example of the type of data obtained from the DSC is shown in Figure 5. Each peak on the DSC trace represents a SCP for a mite.

Table 5: Mean (± SE) and range of supercooling points of non-acclimated and acclimated female and larval *Typhlodromips montdorensis*.

Mite group	n	Mean +/- SE (°C)	Range (°C)
Non-acclimated females	20	-24.1 +/- 0.61	-19.7 to -28.2
Acclimated females	20	-22.4 +/- 0.48	-20.5 to -25.9
Non-acclimated larvae	20	-24.3 +/- 0.38	-21.4 to -28.2
Acclimated larvae	20	-22.6 +/- 0.26	-20.8 to -24.6

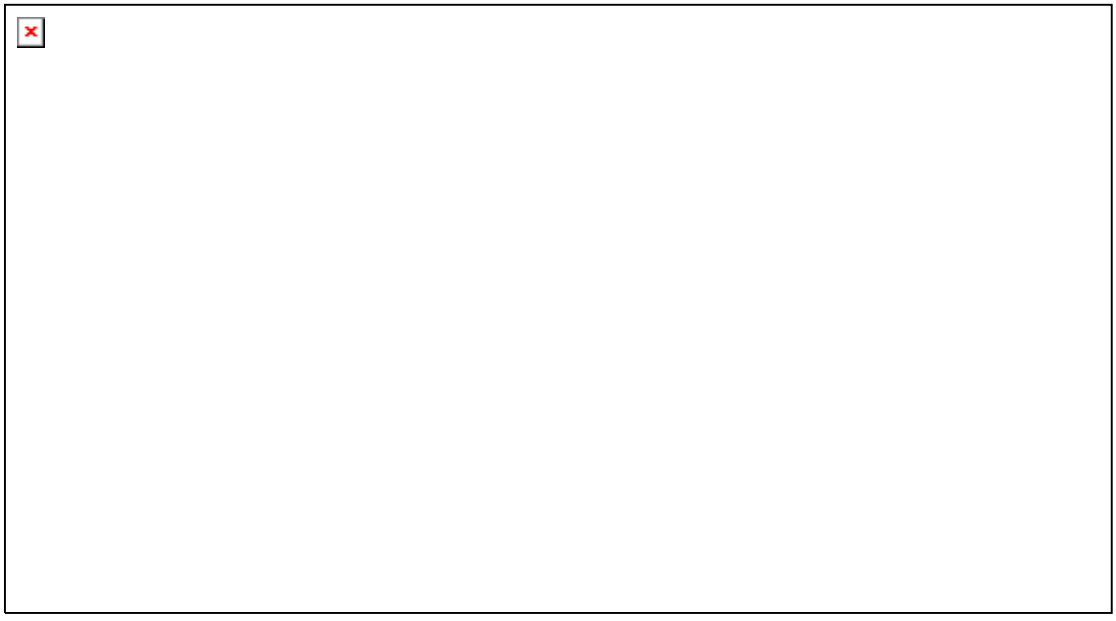


Figure 5: An example of a DSC graph to determine SCPs of acclimated *Typhlodromips montdorensis* larvae.

2.9.2 Lower lethal temperature

No female or larval individuals from control populations died after exposure at 15°C for 60 min. The lethal temperatures for 10, 50 and 90% ($LTemp_{10, 50, 90}$) mortality of acclimated and non-acclimated female and larval *T. montdorensis* are shown in Figure 6. Acclimated females survived at significantly lower temperatures at $LTemp_{10 \text{ and } 50}$ than acclimated larvae and non-acclimated females and larvae (indicated by non-overlapping fiducial limits) (Hart *et al.*, 2002a; Hart *et al.*, 2002b). Acclimated larvae survived significantly lower temperatures at $LTemp_{90}$ than the other three treatments and acclimated females survived at lower temperatures compared with non-acclimated populations at $LTemp_{90}$.

There was no difference in developmental time from egg to adult between the control population after exposure to 15°C for 60 min as larvae and survivors from the population exposed to the $LTemp_{50}$ of -6.7°C ($F_{1,58} = 1.74$, $P > 0.05$). Mean developmental times from egg to adult were 7.2 and 7.4 days for control and $LTemp_{50}$ population respectively. Similarly, there were no differences in developmental times between the acclimated control population after exposure to 15°C for 60 minutes as larvae and the acclimated experimental population after exposure to the $LTemp_{50}$ of -8°C ($F_{1,58} = 2.44$, $P > 0.05$). Mean developmental times from egg to adult were 7.4 and 7.7 days for control and $LTemp_{50}$ population respectively. Females of all four mite groups oviposited forming viable populations. Even after exposures to their $LTemp_{90}$, some individuals of both acclimated and non-acclimated populations reached adulthood and laid viable eggs.

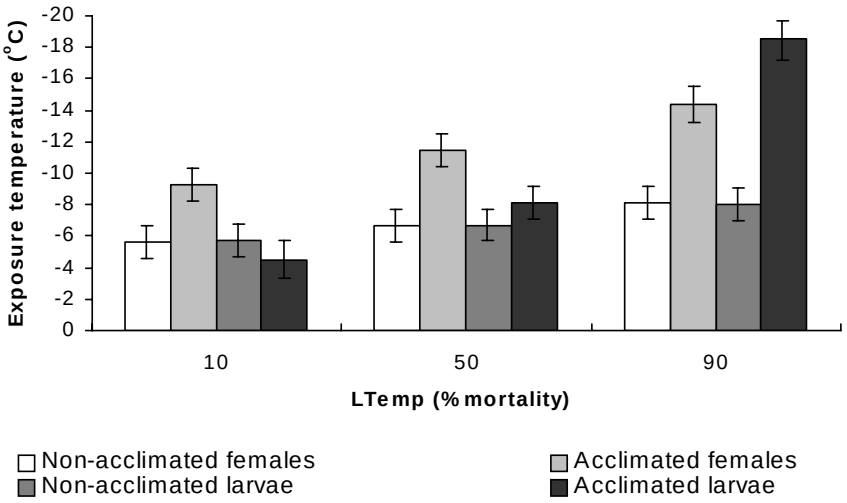


Figure 6: LTemp $_{10,\ 50}$ and $_{90\%}$ mortality (± 95% fiducial limits) of non-acclimated and acclimated female and larval *Typhlodromips montdorensis* after cooling at 1°C min to exposure temperature.

2.9.3 Lethal times

The lethal times for 10, 50 and 90% mortality ($LTime_{10,50\ 90\%}$) at -5, 0 and 5°C of acclimated and non-acclimated female and larval *T. montdorensis* are shown in Figure 7. At -5°C there was no difference between treatments at the $LTime_{10}$ and $LTime_{50}$ (indicated by overlapping fiducial limits). At the -5°C $LTime_{90}$ acclimated females survived significantly longer than non-acclimated larvae (indicated by non-overlapping fiducial limits). There were no significant differences in survival times for the remaining three mite groups. At 0°C acclimated females survived longer than non-acclimated larvae at the $LTime_{10}$ and at the $LTime_{90}$ acclimated larvae survived longer than non-acclimated females and larvae. At 5°C non-acclimated females and acclimated females and larvae all survived longer than non-acclimated larvae at the $LTime_{10}$. Both female populations survived significantly longer than the larvae at the $LTime_{50}$ and $LTime_{90}$ and acclimated larvae survived significantly longer than non-acclimated larvae at the $LTime_{50}$. Acclimated females survived significantly longer than non-acclimated females at the $LTime_{90}$, but there was no difference between the larval treatments at this level.

A control population of 60 adult and larval *T. montdorensis* was placed at 5°C with water and food provided. The maximum survival time for individuals used in LTime experiments was 20 days and 100% mortality was observed in the control population after this time, suggesting death was due to the cold stress and not to desiccation or starvation.

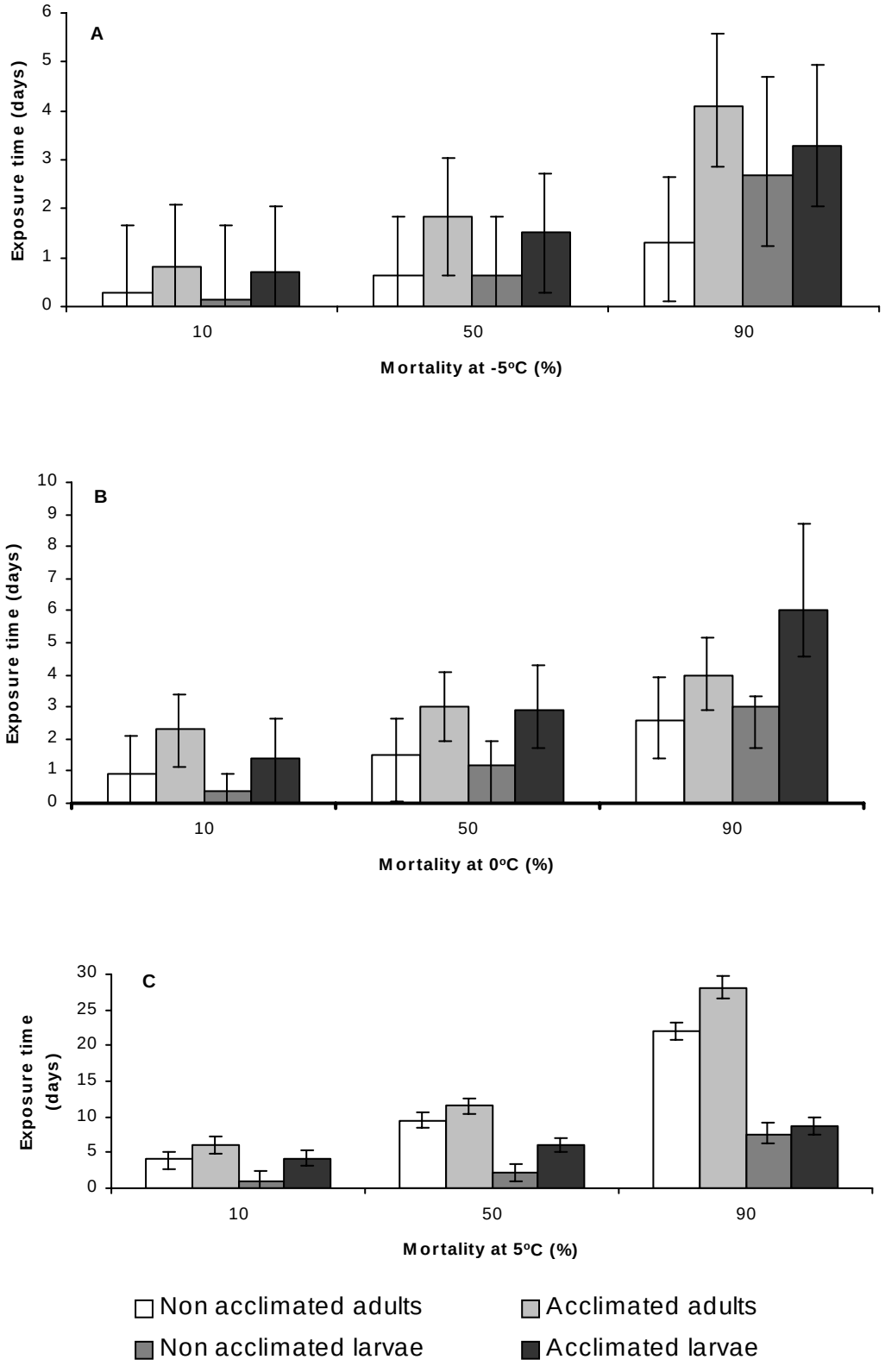


Figure 7: LTime $_{10,\ 50}$ and $_{90\%}$ mortality (± 95% fiducial limits) of non-acclimated and acclimated female and larval *Typhlodromips montdorensis* at -5 (A), 0 (B) and 5°C (C) (NB: different scales of axis).

2.10 Diapause

As discussed in the introduction, the ability of a mite to enter diapause may aid its winter survival outside the glasshouse. Therefore the current strain likely to be used for biological control in the UK (if a licence is granted) was subjected to tests to determine its response to conditions known to induce diapause in *N. californicus* (Hart *et al.*, 2002a).

2.11 Methods

Sixty *T. montdorensis* eggs that had been laid at 25ºC, 18:6 LD were placed on a rearing stage at 18ºC, 6:18 LD (regime 1). A 6:18 LD regime was selected to take all critical day lengths into account as it would elicit a strong response if a diapause trait was present within the sampled individuals (Hoy and Flaherty, 1975). Also, previous work using *N. californicus* resulted in 96% of individuals entering diapause at 6:18 LD (Jolly, 2000). Pollen and *T. urticae* were supplied as food sources as β – carotene present within the latter food is often necessary for some phytoseiid species to diapause (Overmeer, 1985a; Overmeer *et al.*, 1989). Hatched eggs were left to develop to adult and then for a further two days to allow time for mating. Mated females were then transferred to individual rearing arenas with a frayed piece of black cotton thread (0.5 cm long) as an oviposition substrate and fed pollen and *T. urticae*. The females were examined every 24 h for possible oviposition and the proportion of females ovipositing and the number of eggs laid by each female was recorded. If the females had not laid eggs after two weeks at 18ºC, 6:18 LD they were transferred to 25ºC, 18:6 LD and if oviposition occurred after a lag period of 4 days, then the mites were considered to have been in diapause (Jolly, 2000). The results had indicated that all females had oviposited before transfer to 25ºC, 18:6 LD, nevertheless they were still placed in the optimal rearing regime to allow comparisons in egg production between other studies. Any eggs laid by first generation females under diapause-inducing conditions were kept under the same regime and reared to adulthood and mated, becoming the second generation.

The same procedure was repeated for 60 eggs laid at 25ºC, 18:6 LD except that these were kept at 21ºC, 11:13 LD (regime 2) to attempt to induce diapause. Females were

kept at this regime for their entire oviposition period and not transferred to 25ºC as was done for individuals from regime 1. The 21ºC, 11:13 LD temperature and light regime was selected to correspond with typical conditions found in New Caledonia (the place of origin of *T. montdorensis*) during the cool season, which is from April to August. Average temperatures during this period are 20-21ºC (Chazeau, pers comm) and the light regime varies from approximately 11:13 to 13:11 LD throughout the year in Noumea in the south of the island (Chappell, pers. comm.). It was hoped that by using this regime and regime 1, any possible diapause trait could be detected in *T. montdorensis*.

For both experimental light regimes a control sample of 40 gravid females was left at 25ºC, 18:6 LD to detect the proportion of egg laying females in a population not reared under diapause inducing conditions. The number of eggs recorded per day for the control population and the first and second generation of individuals reared under both the induction regimes were log transformed and compared by a One-way ANOVA in MINITAB v 13.32.

2.12 Results

The number of eggs laid per female per day reared under two putative diapausing inducing regimes for one and two generations and reared under non-diapause inducing conditions (control) are shown in Figures 8 (regime 1) and 9 (regime 2). For both experimental regimes oviposition in the control population began on day 1 after females had mated and egg numbers peaked at day 6 (2.3 eggs/female) and then decreased and oviposition ceased at day 21. In regime 1 females reared from egg to adult (first generation) produced eggs at a slower rate than the control population and peaked on day 13 (2.3 eggs/female) one day after transfer to 25°C. The eggs laid by the first generation females before transfer to 25°C were reared to adulthood under regime 1 (second generation) and produced slightly fewer eggs than the first generation, but a similar daily laying pattern was observed. Egg numbers peaked on day 15 (1.7 eggs/female), one day after being transferred from regime 1 to 25°C. All of the control females oviposited. The total mean numbers (± SE) of eggs laid per female were 19.4 ± 1.16, 18.2 ± 0.73 and 20.5 ± 1.68 for the first and second generation reared under regime 1 and the control population respectively and there

were no differences between the number of eggs laid by the control population and the first and second generation of individuals reared under regime 1 ($F_{2,66} = 0.26$, $P > 0.05$).

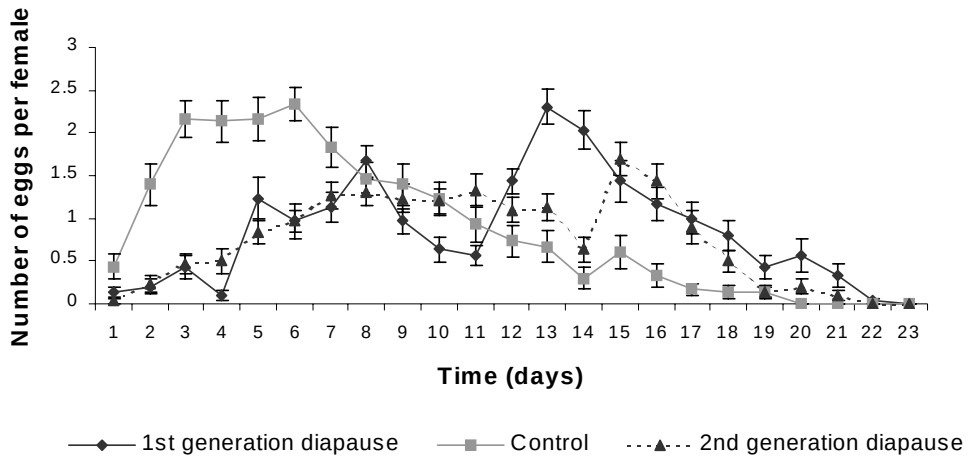


Figure 8: Number of eggs laid (± SE) per female *Typhlodromips montdorensis* per day under control conditions (25°C, 18:6 LD) and for females reared for 1 and 2 generations at 18°C, 6:18 LD (regime 1).

First and second generation females reared under regime 2 (21°C, 11:13 LD) (Figure 9) began oviposition on day 1 after mating. Oviposition in first generation females peaked on days 3,4 and 5 (2 eggs/female) and on day 6 for second generation females (2 eggs/female). Oviposition ceased after 21 and 19 days for first and second generation females respectively. The mean number (± SE) of eggs laid per female were 18.1 ± 1.41, 18.3 ± 1.4 and 20.1 ± 1.66 for the first and first generations reared under regime 2 and the control population, respectively and there were no differences between the number of eggs laid by the control population and the first and second generation of individuals reared under regime 2 ($F_{2,63} = 0.09$, $P > 0.05$).

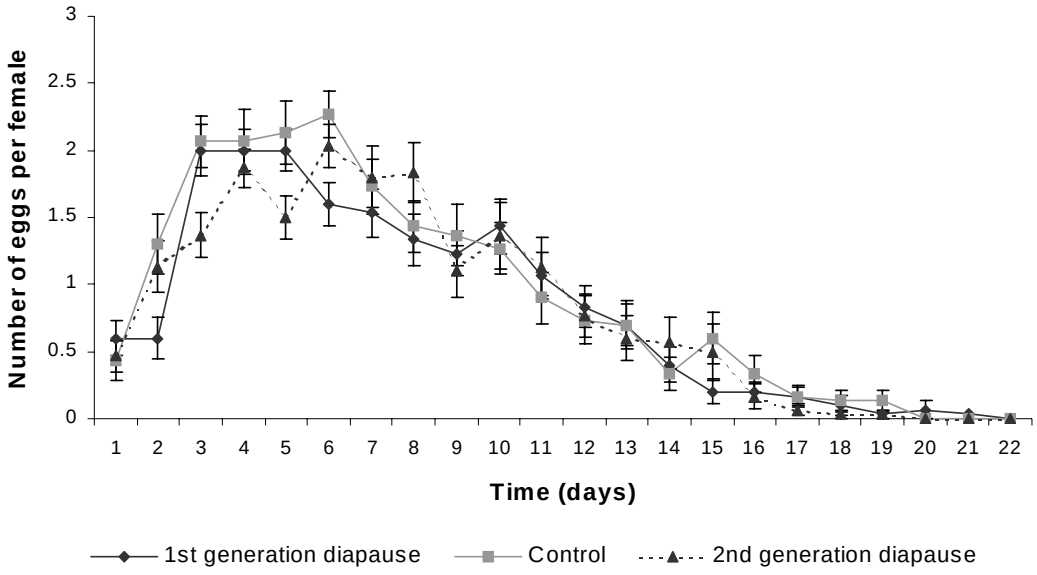


Figure 9: Number of eggs laid (± SE) per female *Typhlodromips montdorensis* per day under control conditions (25°C, 18:6 LD) and for females reared for 1 and 2 generations at 21°C, 11:13 LD (regime 2).

2.13 Field survival

The objective of the field exposures was to determine how well *T. montdorensis* could survive outside the glasshouse depending on when individuals escaped from a glasshouse environment and the ability of the species to respond to fluctuating field temperatures. The laboratory data suggests that *T. montdorensis* lacks cold tolerance, but is still likely to show some survival in the field. The first field experiment was conducted in early winter (October 2002) when temperatures were predicted to be constantly above 0°C. The second field experiment was set up in mid-winter (January 2003) when the temperature was likely to be more stressful for *T. montdorensis*. If *T. montdorensis* could survive for a few months during a mid-winter escape then it could in theory re-colonise the glasshouse and move between the two environments. In contrast, if *T. montdorensis* only survived for a short period then potentially it would not re-colonise the glasshouse and would die out.

Additionally, field experiments were conducted throughout the winter for one and two week periods to attempt to identify if there were temperature regimes in the field where 100% mortality was probable. Should *T. montdorensis* be able to survive in the

field then its ability to reproduce and form a viable field population also had to be determined. Further, does the slight (but insignificant) increase in laboratory survival in acclimated populations compared with non-acclimated populations also give acclimated populations an advantage in the field? Insects escaping from the glasshouse would be non-acclimated; however, if they escaped in early winter they might undergo a natural acclimation in the field before the temperatures became too low for development, reproduction or survival.

2.14 Methods

2.14.1 Survival assessment

Individuals in all field experiments were scored as alive if they responded to a tactile stimulus after being left at 15°C for 1 h after the field exposure.

2.14.2 Adult and larval field exposures

Each of the four mite groups (acclimated adults, larvae, non-acclimated adults, larvae) was placed individually in glass vials (3.5 x 2.5 cm), on a layer of 0.5 cm of agar (2%), (Oxoid Ltd, UK) with a circular piece of filter paper (2 cm in diameter) resting on the agar which provided a moisture source. Each vial was sealed with a ventilated plastic lid covered in 75 μm muslin, (Lockertex, UK). The 'unfed' treatment was with no prey or other food. The 'fed' treatment included the addition of a leaf disc (0.5 cm in diameter) with *T. urticae* and pollen *ad libidum*. Food was provided in the fed treatment as it was believed that if no diapause was possible in *T. montdorensis* then field survival may be increased by the addition of food. Ten vials of each of the four mite groups of each treatment were placed in a box, which was sealed except for four ventilation holes covered in muslin in the box side (3 cm in diameter).

Between 3 and 20 boxes, depending on the experiment, were then placed in a sheltered field location (1 m apart) at the University of Birmingham and covered with a plastic tray (45 x 35 cm) which provided protection from direct sunlight. A data logger was placed inside each box to record the temperature. Experimental times from October to November 2002, December 2002 to early February 2003 and mid February to March 2003 were selected as these represented early, mid and late winter

respectively. One trial was conducted every 7 days from October 2002 to February 2003 (weekly trial). By selecting the entire winter period a wide variety of temperature regimes were experienced. In the weekly field experiments using acclimated and non-acclimated female and larval mites, all three batches of ten vials of each treatment were collected after 7 days between October 2002 and February 2003. Short term field experiments, using non-acclimated mites only, were carried out every one to two weeks from December 2002 to February 2003. Three boxes each containing 40 vials (10 each of fed females and larvae and 10 each of unfed females and larvae) were taken at random from the field after 7 and 14 days and mortality (%) recorded. One 4-6 week trial involved non-acclimated females and larvae from October to November 2002 and one four to six week trial involved acclimated females and larvae, from February to March 2003. In both of these trials three boxes each containing 40 vials (10 each of fed and unfed females and larvae of the respective group) were taken at random every 7 days and mortality (%) recorded. In the 4-6 week trials, fed mites still in the field were transferred into fresh vials with fresh food at week three. The mortality of the field populations was tested for a normal distribution and analysed using a general linear model (ANOVA) and differences between treatments were compared using Tukey's HSD. Data from the 7 day field experiments were collated to predict the temperatures required to cause 10, 50 and 100% field mortality of *T. montdorensis*.

A control sample of 30 non-acclimated larval mites in individual vials was placed in an incubator at 25°C, 18:6 LD and mite development observed to ensure that the experimental set up in each vial was not deleterious to them.

2.14.3 Egg field survival

Between December 2003 and February 2004 *T. montdorensis* eggs were placed in the field. Five *T. montdorensis* eggs that had been laid at 25°C, 18:6 LD and were less than 12 h old were placed in a vial, with agar and filter paper as before and put into the field in sealed boxes. The procedure was repeated for batches of eggs laid at 25°C, 18:6 LD and then held at 10°C for 7 days (acclimated eggs). Seven separate one week field trials were conducted and each week six boxes were placed in the field containing five vials each with five eggs. After 7 days, all vials from each box were

collected, the number of eggs retrieved was recorded and the eggs were placed on individual rearing stages at 25°C, 18:6 LD and checked every 12 h to see if a larva emerged (egg mortality). A control sample of 100 eggs was left in the laboratory at 25°C, 18:6 LD and the proportion of larvae emerging from the eggs was recorded.

2.14.4 Fecundity of Typhlodromips montdorensis in the field

Five third instar and five newly moulted adult *T. montdorensis* reared at 25°C, 18:6 LD were placed in separate glass vials (3.5 x 2.5 cm), on a layer of 0.5 cm of agar (2%) with a circular piece of filter paper (2 cm in diameter) resting on the agar, a piece of frayed black cotton (1 cm long) as an oviposition substrate, a large leaf disc (1 cm in diameter) with *T. urticae* and pollen, and sealed with a ventilated plastic lid covered in 75 μm muslin. Six consecutive field trials from December 2002 to March 2003 were conducted and for each trial six boxes containing three vials each were sealed except for four ventilation holes in the side of the boxes and egg production was recorded after two weeks. A control sample of six replicates of three vials each was left at 25°C, 18:6 LD for two weeks to ensure the experimental set-up was not deleterious to the mites.

The inclusion of a treatment with third instar *T. montdorensis* ensured that one moult followed by mating would have to be completed in the field for oviposition to be possible. The treatment with adult *T. montdorensis* meant that only mating was required in the field for oviposition to be possible and it was known that some individuals had recently mated in the laboratory. Egg production in the control was compared with the two field treatments.

2.15 Results

2.15.1 Adult and larval field exposures

For the duration of the field exposures populations of 60 *T. montdorensis* were left at 10°C for five week periods with a moisture source and food provided. Survivorship after five weeks was above 90% suggesting that death of *T. montdorensis* in the winter months after five weeks in the field was due to cold and not related to ageing.

Field mortality of fed and unfed female and larval *T. montdorensis* examined after 7 and 14 days (short term trials) during mid-winter from December 2002 to February 2003 is shown in Table 6 together with mean, maximum and minimum field temperatures. In the first (6 Dec 2002) and third (21 Jan 2003) trials, 100% mortality was recorded after 14 days with mean temperatures over the 14 day period of 3.1 and 3.3°C in the two trials respectively. In the second (7 Jan 2003) and fourth (11 Feb 2003) trials, 100% mortality was reached after 7 days and fed females survived significantly longer than unfed larvae after one week ($P = < 0.05$) and unfed females survived longer than fed ($P < 0.05$) and unfed larvae ($P < 0.01$). Mean temperatures over the 7 days were 0.2 and 2°C in the two trials respectively. All control mites reached adulthood in experimental vials in the laboratory.

Table 6: Mortality (± SE) of fed (FF) and unfed (FU) female and larval (LF and LU) *Typhlodromips montdorensis* after 7 and 14 days in the field and temperatures recorded during the exposure periods.

Date into field	Mite group/ treatment	Mortality (%) 7 days	14 days	Mean, max and min temperatures (°C) Days 1-7	Days 8-14	Mean temperatures (oC) Days 1-14
	FF	55 +/- 12.6	100 +/- 0			
06/12/02	FU	57 +/- 15.9	100 +/- 0	3.1, 7.5, -0.2	3, 4.9, -0.4	3.1
	LF	87 +/- 6.2	100 +/- 0			
	LU	86 +/- 7.3	100 +/- 0			
	FF	100 +/- 0	100 +/- 0			
07/01/03	FU	100 +/- 0	100 +/- 0	0.2, 5.7, -3.0	5.6, 7.5, 1.8	2.9
	LF	100 +/- 0	100 +/- 0			
	LU	100 +/- 0	100 +/- 0			
	FF	26 +/- 7.4	100 +/- 0			
21/01/03	FU	17 +/- 11	100 +/- 0	5.2, 9.3, 0.8	1.5, 6.4, -2.7	3.3
	LF	56 +/- 2.9	100 +/- 0			
	LU	65 +/- 2.2	100 +/- 0			
	FF	100 +/- 0	100 +/- 0			
11/02/03	FU	100 +/- 0	100 +/- 0	2, 10.6, -1.8	2.7, 8, -2.8	2.3
	LF	100 +/- 0	100 +/- 0			
	LU	100 +/- 0	100 +/- 0			

Field mortality rates of non-acclimated female and larval *T. montdorensis* with and without the provision of prey recorded during early winter are shown in Figure 10 and the corresponding field temperatures in Figure 11. Mortality of all four treatments increased with time. After 7 days there was no difference between treatments and mean mortality was 10% ($F_{3,33} = 1.97$, $P > 0.05$). After 14 days mortality for unfed

females and larvae was over 60% and significantly higher ($F_{3,33} = 36.85$, $P < 0.01$) than for fed females and larvae (15%). After 21 days, mortality of unfed females and larvae was over 75%; and after 28 days over 90% and significantly higher ($F_{3,33} = 16.84$, $P < 0.01$) than for fed populations (55% and 70% for 21 and 28 days respectively). After 35 days, 100% mortality was recorded in unfed populations and 98% mortality in fed populations, a non-significant difference ($F_{3,33} = 1.57$, $P > 0.05$). The mean, maximum and minimum temperatures during the experimental period were 10, 28 and 0.8°C respectively.

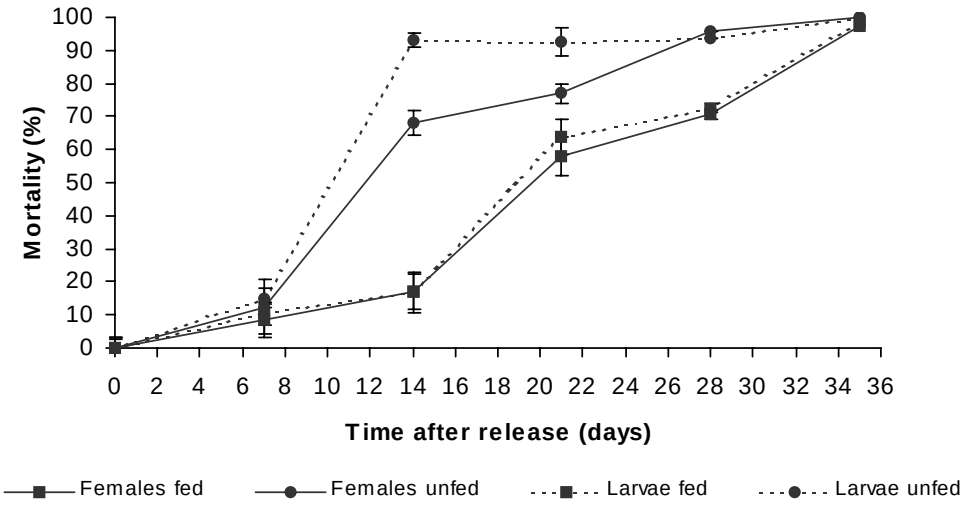


Figure 10: Mortality (± SE) of non-acclimated female and larval *Typhlodromips montdorensis* with and without prey in field from 1 October 2002 to 5 November 2002.

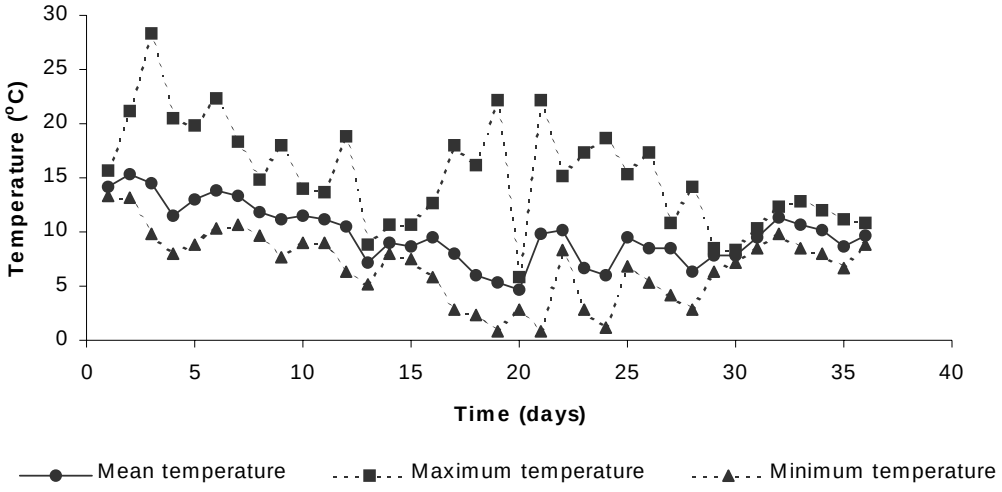


Figure 11: Mean, maximum and minimum field temperatures experienced by *Typhlodromips montdorensis* in field from 1 October 2002 to 5 November 2002.

Field mortality rates of non-acclimated female and larval *T. montdorensis* with and without the provision of prey recorded during November and December 2002 are shown in Figure 12 and the corresponding field temperatures in Figure 13. Mortality of all four treatments increased with time. There were no differences in survival time between the four treatments with individuals collected after 7 ($F_{3,6} = 4.75$, $P > 0.05$), 14 ($F_{3,6} = 0.9$, $P > 0.05$), 35 and 42 days. Significant differences in survival times were recorded in individuals collected after 21 and 28 days respectively ($F_{3,6} = 7.07$ and 5.82, $P < 0.05$). In both cases fed females and fed larvae survived significantly longer than unfed larvae ($P < 0.05$). In unfed larvae 100% mortality was recorded after 28 days and after 35 days in the remaining populations. The mean, maximum and minimum temperatures during the experimental period were 5.6, 11.2 and 0.4°C respectively.

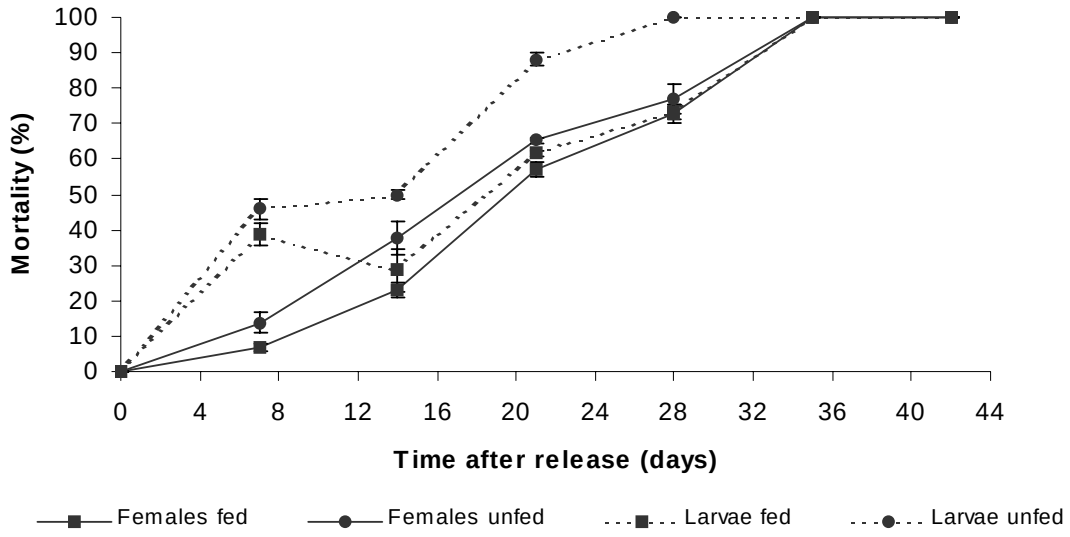


Figure 12: Mortality (± SE) of non-acclimated female and larval *Typhlodromips montdorensis* with and without prey in field from 7 November 2002 to 19 December 2002.

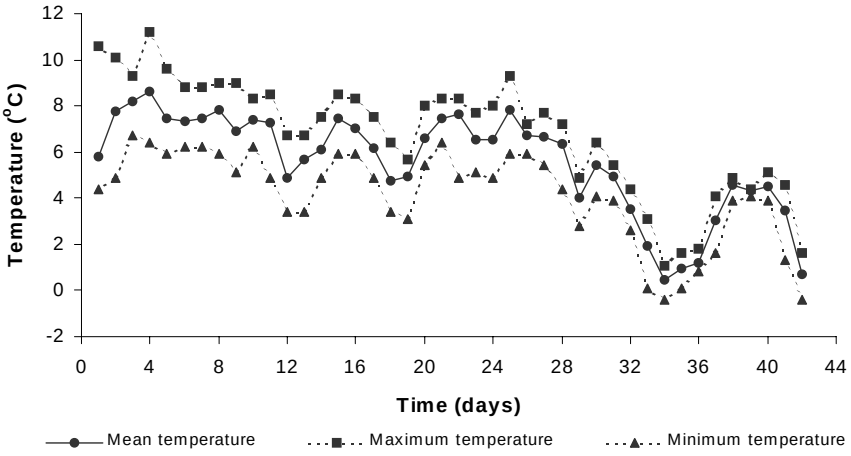
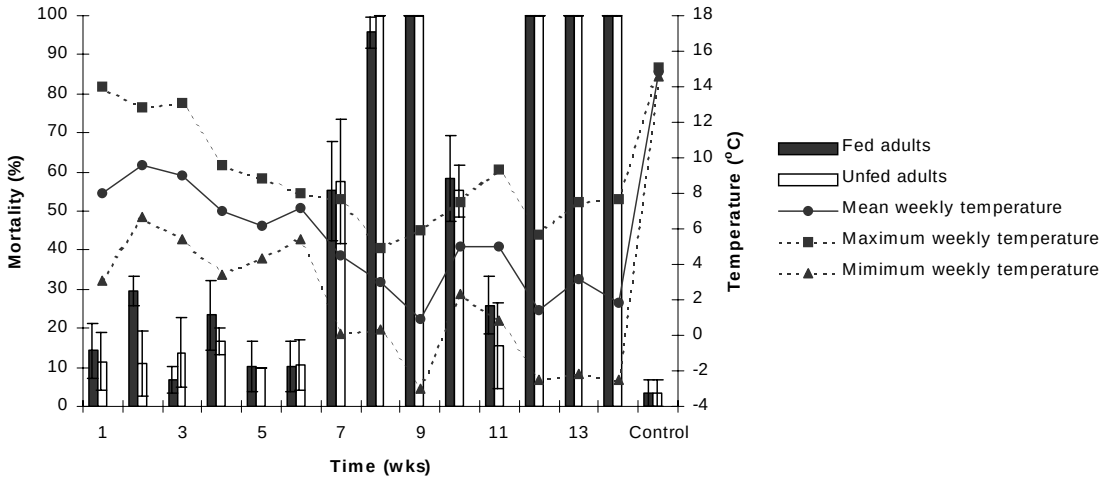


Figure 13: Mean, maximum and minimum field temperatures experienced by *Typhlodromips montdorensis* in field from 7 November 2002 to 19 December 2002.

Weekly field mortality rates recorded for 14 weeks from October 2002 to February 2003 and the corresponding mean, maximum and minimum temperatures are shown in Figures 14 A and B respectively. Female weekly mortality was below 30% for the first six exposure periods with a mean temperature above 5°C. There were no differences in survival times between treatments exposed in weeks 1, 7, 8, 9, 12, 13 and 14. There were significant differences in survival times between treatments exposed in weeks 2, 4, 5, 10 and 11 ($F_{3,6}$ = 13.1, 12, 11.1, 13.8 and 14, $P < 0.01$) and

in weeks 3 and 6 ($F_{3,6}$ = 6.33 and 5.87, P < 0.05). Significant differences in survival times between treatments are shown in Table 7. For fed and unfed females 100% mortality was recorded in exposure weeks 9, 12, 13 and 14 where mean temperature was below 3°C. A similar situation was observed in fed and unfed larvae although mortality was slightly higher in larvae than in females. Only 5% of the control populations of both female and larval fed and unfed *T. montdorensis* died and mortality rates of fed and unfed populations in the field were very similar.

A



B

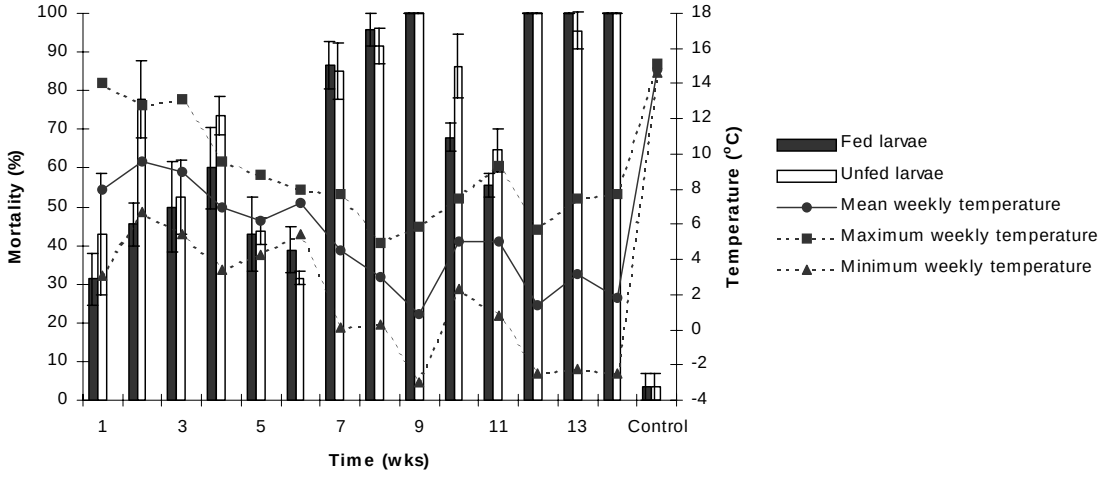


Figure 14: Mortality (± SE) of fed and unfed female (A) and larval (B) *Typhlodromips montdorensis* over a one week exposure period. Weeks 1-8 (22 October 2002 to 17 December 2002) 9-14 (7 January 2003 to 18 February 2003).

Table 7: Significant differences in survival time between treatments of *Typhlodromips montdorensis*. FA = fed female, FL = fed larvae, UA = unfed female, UL = unfed larvae.

Weeks	Significantly longer survival	P value
2+10	FA > UA	< 0.01
	UA > UL	< 0.01
3	FA > FL	0.05
4	FA > UL	0.05
	FL > UA	0.05
	UA > UL	< 0.01
5	FA > FL & UL	0.05
	FL > UA	0.05
	UA > UL	0.05
6	FA > FL	0.05
	FL > UA	0.05
11	FA > FL & UL	0.05
	UA > UL	< 0.01

High mortality rates of *T. montdorensis* appear to be caused by a combination of mean temperatures below the developmental threshold and some exposures to a minimum temperature well below the developmental threshold. Data obtained during the week long exposures were collated in order to predict the mean and minimum temperatures required in combination to cause 10, 50 and 100% field mortality of female and larval *T. montdorensis* (Figure 15). The mean temperatures for 10 and 50% mortality were similar; 50% mortality is likely to occur if the minimum temperature is approximately 2°C lower than the minimum temperature likely to cause 10% mortality (5.5°C). Field mortality of *T. montdorensis* is likely to be 100% when the mean temperature is approximately 3°C with some sub-zero exposures.

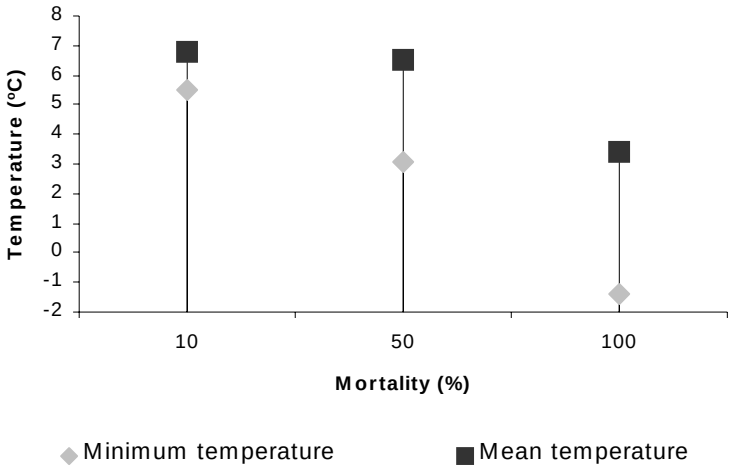


Figure 15: Mean and minimum temperatures when combined predicted to cause 10, 50 and 100% field mortality of female and larval *Typhlodromips montdorensis*.

2.15.2 Acclimation

Field mortality rates of acclimated fed and unfed females and larvae recorded in late winter from February to March 2003 (4-6 week trial), together with the field temperatures recorded for the same period, are shown in Figures 16 and 17 respectively. High mortality rates (100% in fed larvae, >85% in fed females and unfed females and larvae) were observed by day 7 and 100% mortality was recorded in all populations by day 14. There were no differences in survival times between any of the mite groups ($F_{3,6} = 1.08$, $P > 0.05$). The mean, maximum and minimum temperatures throughout the first 14 days of the exposure were 2.1, 10.1 and -2.7°C respectively.

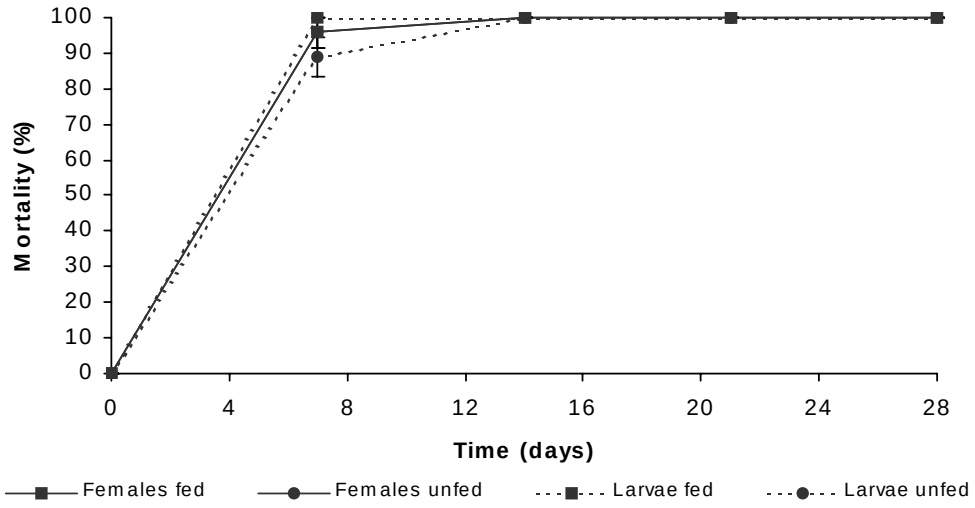


Figure 16: Mortality (± SE) of acclimated female and larval *Typhlodromips montdorensis* with and without prey in field from 13 February 2003 to 13 March 2003.

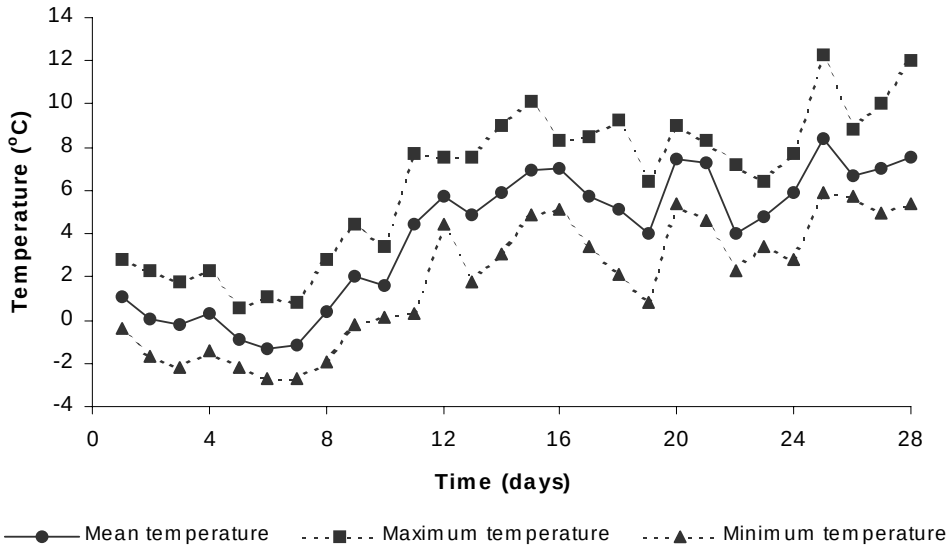


Figure 17: Mean, maximum and minimum field temperatures experienced by acclimated *Typhlodromips montdorensis* in field from 13 February 2003 to 13 March 2003.

2.15.3 Egg viability in the field

For both non-acclimated and acclimated experiments (data pooled as results are identical) the number of eggs retrieved from the field, the resulting egg mortality and the mean, maximum and minimum temperatures in each of the seven field experiments and the laboratory control trial are shown in Table 8. All field trials produced 100% egg mortality; survival in the control trial was 96%.

Table 8: Field egg mortality of *Typhlodromips montdorensis* and the mean, maximum and minimum field temperatures (°C).

Date of release	Eggs recovered from field (%)	Egg mortality (%)	Mean, max and min temperatures (^{o}C)
08/12/03	100	100	5.5, 8, 3
15/12/03	100	100	3.7, 9, -0.2
05/01/04	100	100	6.2, 10.4, 2.6
12/01/04	100	100	4.9, 9.8, 0.1
19/01/04	100	100	3.9, 8.4, -1.9
26/01/04	100	100	7.7, 12.4, -1.2
02/02/04	100	100	6.8, 10, 2.8
Control	100	4	20.2, 21, 19.6

2.15.4 Field reproduction

Adult *T. montdorensis* mortality and the number of eggs produced and their viability over six, 14 day trials from December 2002 – March 2003 and the temperatures recorded are shown in Table 9. Only four eggs were produced in total over all six trials compared with 224 eggs produced by the control sample in a single trial. Control egg mortality was low at 7% compared with 100% recorded in each field trial. Mean temperatures throughout the trials ranged from 2.5 to 7°C. There was no difference between egg numbers produced by individuals placed in the field as deutonymphs instars and newly moulted adult females, suggesting no development and mating was possible in the field.

Table 9: Adult mortality (± SE), egg production and egg mortality of *Typhlodromips montdorensis* in six 14 day field trials.

Week	Adult mortality (%)	Egg production	Egg mortality (%)	Mean, maximum and minimum temperatures (°C)
1	85 +/- 3	3	100	5.5, 9.8, -1.9
2	54 +/- 3.9	0	0	4.1, 6.4, 1.8
3	100 +/- 0	0	0	2.5, 7.5, -2.7
4	100 +/- 0	0	0	5, 7.5, -1.4
5	48 +/- 5.5	1	100	7, 16, 1.3
6	65 +/- 6.6	0	0	6.6, 14.8, 0.8
Control	3 +/- 3.3	224	7	24.7, 25.3, 24

2.16 Discussion

This discussion deals with the data on the developmental threshold, laboratory cold tolerance and field survival of *T. montdorensis*. The data will help to investigate the biology of *T. montdorensis* and enable a prediction on its establishment potential outside the glasshouse environment in the UK. Throughout, a comparative analysis with *N. californicus* will be included. It was previously thought that the sole distribution range of *N. californicus* was around the Mediterranean region, and it was therefore granted a licence on the grounds that is could not survive outdoors through a UK winter. However, subsequently it has emerged that there are UK, USA and Spanish strains of *N. californicus* (Jolly, 2000). Due to this recent establishment of *N. californicus* in the UK a more precautionary approach has been adopted. *Typhlodromips montdorensis* has not been granted a licence for release in the UK. Comparing *T. montdorensis* directly with *N. californicus* should allow a more accurate overall prediction of the establishment potential of *T. montdorensis* outdoors in the UK. Where appropriate, comparisons with other mite and insect species will also be given.

The optimum temperature for *T. montdorensis* development in the present study was 27ºC and the mean total time from egg to adult was 6.3 days. At 25ºC total development took 7.5 days. In recent work on *T. montdorensis*, development was also observed between 15 and 30ºC with time to first oviposition ranging from 27 days at 15ºC to 6 days at 30ºC (Steiner and Goodwin, 2002a) and development completed

within 7 days at 25ºC (Steiner *et al.*, 2003b). The 30ºC developmental times were omitted from the analyses in the present study, as they were above the optimum developmental temperature. However, development from egg to adult was completed at this temperature. *Typhlodromus pyri* developed from egg to adult in 13 to 16 days at 20ºC (Hayes and McArdle, 1987), 6.6 days at 27ºC (Duso and Camporese, 1991) and 7 days at 25ºC when fed on *Panonychus ulmi* (Koch) (Acari: Tetranychidae) (Zhang and Croft, 1994). *Typhlodromalus lailae* (Schicha) (Acari: Phytoseiidae) completed its lifecycle in 6 days at 25ºC (Steiner *et al.*, 2003a). These results are comparable to *T. montdorensis*, suggesting similar temperature responses between related species. Work on *N. californicus* revealed that development from egg to adult at 25ºC took 8.1 days (Hart *et al.*, 2002a), 5.8 days (Castagnoli and Simoni, 1991) or 4.4 days (Gotoh *et al.*, 2004). The discrepancies between *N. californicus* developmental times at the same temperature were attributed to strain differences and slight variations in methodology (Gotoh *et al.*, 2004; Hart *et al.*, 2002a). However, in light of the results obtained on overall cold tolerance of *T. montdorensis* in the present study when compared with similar studies on *N. californicus*, the developmental threshold of *N. californicus* is likely to be below that of *T. montdorensis*.

The relationship between developmental rate and temperature is linear from 10 to 27ºC for *T. montdorensis*. Temperatures and developmental rate relationships outside this range are likely to be curvilinear, but this data set does not justify the use of a non-linear model (Lamb, 1992; Wagner *et al.*, 1984). A linear approximation is acceptable as long as the temperatures considered are in the linear region (Higley *et al.*, 1986), and in the present study this was the case. Using weighted linear regression (Hart *et al.*, 1997) results in a lower estimate of developmental threshold for *T. montdorensis* than when using simple linear regression. The day-degree requirement per generation is higher using simple linear regression due to the slope reduction with the lower developmental threshold (Hart *et al.*, 2002a). In the present study the weighting towards the temperatures with the least variation alters the developmental threshold by 0.4ºC. Due to this minimal difference, voltinism was calculated using the results obtained from the simple linear regression and a day-degree requirement of 105.3 day degrees per generation was estimated. The failure of any development past the protonymph stage at 10ºC indicates that the developmental threshold of 10.7ºC for *T. montdorensis* is realistic. A developmental threshold of 11ºC has also previously

been recorded for *T. montdorensis* (Steiner and Goodwin, 2002a). Estimates by simple linear regression of developmental thresholds of *N. californicus* and day-degrees per generation have ranged from 10.3ºC and 86.2 (Gotoh *et al.*, 2004) to 9.9ºC and 123.5 (Hart *et al.*, 2002a) and 9ºC and 90 (Castagnoli and Simoni, 1991) respectively. These estimates suggest that *T. montdorensis* requires slightly higher temperatures to develop than *N. californicus*. If no maximum temperature for development is calculated for a species it is likely that the day-degree requirement may be overestimated, but not by much if the daily maximum temperatures in the UK are below the developmental maximum (Higley *et al.*, 1986). In the present study the maximum developmental temperature for *T. montdorensis* was between 30 and 35°C and therefore above UK maximum daily temperatures (Figure 4). No development occurred at 35ºC.

The laboratory data on *T. montdorensis* confirms that like all invertebrates it possesses some cold tolerance, the extent of which will determine the survival outside the glasshouse environment. *Typhlodromips montdorensis* did not survive temperatures below its freezing point (SCP) and therefore is not freeze tolerant. Mean supercooling points were -24.1, -24.3, -22.4, and -22.6°C for non-acclimated females and larvae and acclimated females and larvae respectively. Non-acclimated individuals survived slightly lower temperatures than acclimated individuals. Although this was surprising it may have been because acclimated individuals were stressed during acclimation and also were 7 days older (the acclimation period) than non-acclimated individuals. Pre-freeze mortality was evident (Bale, 1987), as all individuals died during the lethal temperature experiments before their SCP was reached. *Neoseiulus californicus* had mean SCPs of -21.6 and -22.2°C for female non-acclimated and acclimated individuals (Hart *et al.*, 2002a). These are comparable with *P. persimilis* and *A. cucumeris* with SCPs of -22.5 and -20.7°C respectively for non-acclimated mites (Morewood, 1992). Using SCP data alone is generally not a reliable measure of cold hardiness (Bale, 1987; Bale, 1996). The supercooling points recorded in the present study were very similar to those obtained for other mite species, supporting the view that SCP data alone should not be used as a reliable indicator of cold hardiness.

The lethal temperature is a valuable indicator of *T. montdorensis* pre-freeze mortality; however, lethal time data generally best represents naturally occurring cold stress due to interaction with exposure time. In the present study *T. montdorensis* mortality increased from 10 to 90% between -5.6 and -8.1ºC for non-acclimated females and between -5.7 and -8ºC for non-acclimated larvae. Acclimation for 7 days at 10ºC increased survival at lower temperatures for both females and larvae, in contrast to the SCP data, where non-acclimated individuals survived lower temperatures. Female *N. californicus* survived temperatures of -15.1ºC for non-acclimated mites and -19.3ºC for acclimated mites and SCP's were -22.2°C and therefore only a maximum of 7ºC lower than the $LTemp_{50}$ (Hart *et al.*, 2002a), suggesting that some individuals were dying close to their SCP. In contrast, the SCP of *T. montdorensis* was up to 16ºC lower than the $LTemp_{50}$.

Non-acclimated *N. californicus* survived for 8 days at -5 and 15 days at 0ºC and acclimated *N. californicus* for 11 and 26 days at -5 and 0ºC respectively. At 5ºC survival was up to 3 months for acclimated *N. californicus* (Hart *et al.*, 2002a). In contrast in the present study, non-acclimated females and larval *T. montdorensis* survived for only 2.7 days and 3 days ($LTemp_{90}$ for larvae) at -5 and 0ºC respectively. Even at 5ºC, non-acclimated and acclimated *T. montdorensis* survived for only 22 and 28 days respectively ($LTime_{90}$ for females). During the 0 and -5ºC lethal time exposures, larger errors in the data set were recorded than in the 5ºC exposures. There is no obvious biological explanation for this; however, it is likely that a substantial increase in sample size would have reduced the errors. Nevertheless, there is still a clear trend that survival time decreases with decreasing temperature. The laboratory data on cold tolerance of *T. montdorensis* recorded in the present study suggests that it is less cold tolerant than *N. californicus*. The developmental threshold of *N. californicus* was also lower than that of *T. montdorensis*, which may be related to the fact that *N. californicus* is more cold tolerant than *T. montdorensis*.

The current work also demonstrates that even when a laboratory population of acclimated and non-acclimated *T. montdorensis* has been exposed to a temperature that killed 50% of individuals, there is no difference in developmental time of the survivors compared with a population not subjected to a potentially lethal temperature. In addition, egg production appeared to be comparable for both the

exposed and the control populations. It has often been assumed that individuals that survive low temperature exposure can continue to develop with no apparent detrimental effects. This may be the case for *T. montdorensis*; however, more detailed experiments would have to have been done to confirm this.

For the introduction of possible non-native biological control agents climate models have often been used to study the effect of temperature on development of both the target pest and the proposed biological control agent (Barratt *et al.*, 1997; Barratt *et al.*, 1999; Goldson *et al.*, 1992). In the present study, temperature data from 1986-2000 in combination with developmental data predicted the mean number of generations per year of *T. montdorensis* possible in the West Midlands to be six. All six generations would occur in the summer period between April and September. No complete generation was possible during the remainder of the year. The predicted voltinism for *T. montdorensis* is likely to be a maximum, and micro-climates, food quality and fluctuating temperatures may modify actual field voltinism relative to the laboratory estimates. It is predicted that *N. californicus* can have seven generations a year, one in the winter period and the remaining six in the summer (Hart *et al.*, 2002a). This supports the fact that *N. californicus* was unexpectedly found to over-winter in the UK (Jolly, 2000). On the basis that over a 15 year period there were never sufficient day degrees to allow the development of a generation of *T. montdorensis* during the winter, it is evident that to over-winter successfully *T. montdorensis* must survive until April (before reproduction can occur) to complete its life-cycle.

As overwintering is likely to occur in sheltered locations such as trees or building crevices as shown in other phytoseiids (Broufas *et al.*, 2002; Veerman, 1992), the field trials of *T. montdorensis* concealed in boxes, provide a realistic assessment of actual survival outside the glasshouse. From December 2002 to February 2003, all field populations of non-acclimated *T. montdorensis* died within two weeks regardless of whether or not prey was provided and no non-acclimated or acclimated eggs hatched in the laboratory after being held in the field for 7 days. Non-acclimated female and larval *T. montdorensis* survived for a maximum of 35 days in field trials between October and December 2002, and although the provision of prey significantly increased survival in some trials, the relatively short field survival time

even in early winter suggests that the availability of food will not influence *T. montdorensis* winter survival. In 7 out of 14 weekly field trials there were some significant differences in survival times between treatments, with fed individuals often, but not always, surviving slightly longer than unfed mites. If in some cases prey was the reason for increased survival of *T. montdorensis* then the fact that *T. urticae* has been shown to survive soil sterilisation and remain in unused glasshouses over the winter (van Lenteren, 2000) may help to prolong winter survival *T. montdorensis* slightly.

In the present study there was no difference in *T. montdorensis* developmental time from egg to adult when reared on cattail pollen or *T. urticae*. Although no work was conducted on the effect of the two food sources on *T. montdorensis* reproductive capacity, it has been suggested that pollen alone is a sufficient food source (Steiner and Goodwin, 2002a; Steiner *et al.*, 2003b). At 25ºC, depending on previous diet *T. montdorensis* produced between 2.72 to 3.58 eggs per female per day when fed on first instar thrips larvae and around three eggs when fed on cattail pollen (Steiner *et al.*, 2003b). Work on the predatory mite, *Euseius* (*Amblyseius*) *finlandicus* (Oudemans) (Acari: Phytoseiidae) found development and reproduction on *P. ulmi* and *T. urticae* was possible, however, at a lower rate on *T. urticae* than on apple, cherry and birch pollens (Schausberger, 1992). Similarly, mass rearing of *E. finlandicus* was possible on a variety of pollens (Kostiainen and Hoy, 1994). A further study found that cherry, apricot, peach and walnut pollens were all of high nutritional value for *E. finlandicus* (Broufas and Koveos, 2000). Development of *A. andersoni* was faster on pollen of the daisy, *Mesembryanthemum criniflorum* than on *T. urticae* (Duso and Camporese, 1991). In contrast, *N. fallacis* development was possible on pollen, but *T. urticae* was a better prey source (Pratt *et al.*, 1999). When fed solely on pollen of the daisy, *Malephora crocea* (Jacques), *N. californicus* development was slow and few individuals reached adulthood and no oviposition was recorded (Friese and Gilstrap, 1985). When fed on the spider mite, *Eotetranychus carpini* (Oudemans) (Acari: Tetranychidae) and the gall mite, *Colomerus vitis* (Pagenstecher), (Acari: Eriophyidae) development of *T. pyri* was quicker than when reared on pollen (Duso and Camporese, 1991).

The above examples demonstrate that the effectiveness of pollen as a food source is both pollen type and mite species dependent. Nevertheless, regardless of species, pollen is likely to enhance the stability of a predator population in the absence of the preferred prey. In terms of biological control, dusting plants with pollen is likely to sustain predators early in the cropping season when pest numbers are low, leading to a reduction of prey populations later in the season. In the present study, rearing *T. montdorensis* on cattail pollen was successful and a previous study on *E. finlandicus* also found cattail pollen to be the best option for laboratory rearing (Kostiainen and Hoy, 1994). Despite this, in the current work, where possible, experiments on *T. montdorensis* were conducted on both *T. urticae* and cattail pollen, as this was considered to be the best option. A number of studies (Kostiainen and Hoy, 1994; Schausberger, 1992) have concluded that pollen is a good food source, however the reproductive potential of the mites was assessed over a short time period and not over the reproductive life of adult mites. Further, nutritional histories can affect the efficacy of mites, such as *N. californicus*, in subsequently preying on tetranychids (Castagnoli and Simoni, 1999). Therefore, when biological control companies are mass rearing predatory mites, the long term effects of using pollen as the sole food source need to be considered. The present study shows that if *T. urticae* was in short supply in the glasshouse, then *T. montdorensis* could use pollen as an alternative food source within or outside the glasshouse.

In addition to the maximum field survival of *T. montdorensis* of 35 days, weekly field experiments and resulting temperature mortality predictions show that if mean and minimum temperatures are approximately 3 and below 0ºC respectively, then 100% mortality of fed and unfed female and larval *T. montdorensis* occurs within 7 days. It is often difficult to compare laboratory cold hardiness and field exposures due to the fluctuating temperatures and humidity experienced in the field (McDonald *et al.*, 2000). In the present study, *T. montdorensis* mortality in the laboratory could have been expected to be higher than in the field as laboratory temperatures were constantly below its developmental threshold. In addition, during exposures to temperatures in the field above the developmental threshold, feeding may have been possible and therefore increased survival. However during the current investigation field survival was relatively short. This corresponds with the laboratory survival data where maximum survival at 5ºC of non-acclimated females and larvae was only 22

and 7.6 days respectively. In a previous comparable field trial with *N. californicus* where the minimum temperature did not fall below 0ºC, unfed *N. californicus* survived for up to 100 days and fed *N. californicus* for up to 112 days (Hart *et al.*, 2002a). Further, some *N. californicus* laid viable eggs and nymph development was observed in the field. In contrast, in the present study, 100% mortality was reached after 35 days in the field and no viable eggs were laid during survival experiments or during the reproduction trials. Therefore individual *N. californicus* would not need to survive the whole winter as their progeny could sustain the population until the spring (Hart *et al.*, 2002a), but this is not the case for *T. montdorensis*, as no progeny would be produced until the spring. Often, glasshouse crops are grown for 11 months of the year and there is only a small time period where pests are deprived of host plants (Hart *et al.*, 2002b). Therefore, the fact that *T. montdorensis* never survived for more than 14 days, and often no longer than 7 days in the field in mid-winter is encouraging from a biological control company's point of view as mortality in the field is so rapid that death is likely to occur before glasshouses crops are re-planted. If *T. montdorensis* were able to survive for longer periods in the field it would be possible that it could re-invade the glasshouse at the beginning of the cropping season and thereby move freely between the glasshouse and the outdoor environment. However, the data in the present study suggests that this is unlikely to occur.

Winter temperatures in southern and eastern areas of the UK may be higher than in the Midlands region. However, it is likely that the temperatures experienced in Birmingham during October will be similar to those experienced during December and January in the warmer parts of the UK. Therefore field survival of *T. montdorensis* during October in Birmingham (which was found to be below 35 days) should be a good reflection of expected survival during the colder months of the warmer areas of the UK. For example in St Mawgan in the South West of the UK (Cornwall, 1872E, 641 N) and Eastborne on the South East coast (Sussex, 5611E, 983N) the mean temperatures in January as calculated from the difference between the monthly maximum and minimum temperatures from 1990 to 2000 are 6.3 and 6.2°C respectively. In Sutton Bonington in the Midlands, (Nottinghamshire, 4507E, 3259N) a mean temperature of 6.8°C is recorded in November (Met Office, 2004) two months earlier than in warmer Southern Britain. This suggests that throughout the UK, *T. montdorensis* will die during the winter, be it during early or late winter.

Investigating possible effects of acclimation on winter survival are important as most native species to the UK are likely to undergo a natural acclimation which partly involves lowering their SCP and other thermal thresholds in response to winter (cold hardening). This enables the insects to survive at lower temperatures for longer periods of time. In contrast, mites such as *T. montdorensis* that are native to warmer climates may not be able to naturally acclimate in response to winter conditions. The current investigation demonstrates that *T. montdorensis* has limited acclimation ability in the laboratory, but that its field survival is not increased by this response as 100% mortality was recorded after 14 days in February. Therefore, individuals escaping from the glasshouse in early winter and undergoing a natural acclimation would not be expected to be cold hardier or survive longer than non-acclimated individuals.

A final important conclusion concerning the possible survival of *T. montdorensis* outside the glasshouse environment is that in the present study no diapause was observed in individuals reared for two generations under two different regimes which might have been expected to induce diapause in this species. Although some mite species can overwinter without diapausing (Morewood, 1993; Overmeer, 1985a), diapause may aid survival of *T. montdorensis* in the field. In the present study for regime 1 (18°C, 6:18 LD) oviposition was slightly lower in individuals reared under diapause-inducing conditions than control individuals and in both first and second generation individuals egg laying peaked one day after transfer to the 'optimal' control conditions (25°C, 18:6 LD). The pattern of egg laying was similar to the control populations, but at a slower rate, suggesting that reduced egg numbers were due to the initial lower temperature of regime 1 (and therefore a reduced metabolic rate of *T. montdorensis*), and not due to the individuals actually being in diapause. Oviposition in the control population ceased earlier than in the experimental populations due to the longer time spent at 25°C.

In regime 2 (21°C, 11:13 LD), oviposition began earlier and peaked earlier than in females reared under regime 1. This is likely to be due to the warmer conditions again having an effect on the mites' metabolic rate. In addition, there was no transfer to 25°C for mites reared under regime 2 and oviposition continued to decrease gradually once it had peaked, and ceased after approximately 20 days. Recent work recorded the

first eggs of *T. montdorensis* reared at 25ºC for 8 h and 10ºC for 16 h on day 15 and all females were laying between one and three eggs per female per day by day 17 (Steiner *et al.*, 2003b). In the current work in regime 1, the first eggs were observed on day 1 after adulthood was reached and mating had occurred. All females were laying a maximum of two eggs by day 3, and developmental time from egg to adult in the present study was 13 days at 20ºC. Therefore the results are comparable to those obtained by Steiner *et al.,* (2003b), and the slightly lower mean number of eggs laid per female per day in the present study can be attributed to the lower temperature regime used.

Reintroducing male *T. montdorensis* to the females part way through the diapausing exposure may have increased egg numbers in the present study as a short initial mating period may be inadequate to fertilise the complete egg complement of some females (Steiner *et al.*, 2003b). A similar situation has been observed in *T. lailae* (Steiner *et al.*, 2003a) and *Amblyseius barkeri* (Hughes) (Acari; Phytoseiidae) (Bonde, 1989). Often, diapausing mites appear paler and flatter than non-diapausing mites (Overmeer, 1985; Veerman, 1992), but in the present study this was not the case, as individuals reared under regimes 1 and 2 had the same appearance as the control populations, further suggesting that a diapause trait was not present in the current strain. By rearing two generations of *T. montdorensis* to adulthood, the possibility of different life stages being more or less sensitive to diapause-inducing conditions (Morewood, 1993; Veerman, 1992) was also taken into account. For example, in *A. potentillae* females can enter diapause even when reared in non-diapause inducing conditions as juveniles (van Houten, 1989); in contrast, diapause in *A. cucumeris* was most frequent when the mites were reared in diapause-inducing conditions even before the eggs had hatched (Morewood and Gilkeson, 1991).

It is possible that *T. montdorensis* may be able to enter a summer diapause to overcome any possible negative effects of high temperature or drought. Diapause is common even when not in connection with cold tolerance (Denlinger, 1986; Masaki, 1980). As *T. montdorensis* never encounters winter conditions in its natural environment an inability to enter a winter diapause might be expected. Steiner *et al*., (2003b) found *T. montdorensis* reared under short day conditions of 25ºC for 8h and 10ºC for 16h (and therefore a different regime to the ones in the present study), did

not enter diapause. *Amblyseius cucumeris* and *A. barkeri* both enter diapause under short day conditions, but a non-diapausing strain can be selected for (van Houten *et al.*, 1995). Similarly *N. californicus* can enter diapause; however, this may be strain dependent as 96% of a 'wild' UK strain (evolved from Spanish strain) of *N. californicus* can enter diapause, but only 16% of a Spanish strain and 0% of a US strain showed a similar response (Jolly, 2000). The variation in diapause response between strains of *N. californicus* suggests an increased degree of selection pressure to enter diapause under outdoor conditions in the UK (Hart *et al.*, 2002a). In the present study the strain of *T. montdorensis* originated in New Caledonia. It has since been found in Australia, Fiji and Tahiti (Schicha, 1987). Species that cover a wide range of latitudes can show variations in their developmental thresholds and low temperature survival (Saunders and Hayward, 1998). It is therefore advisable that biological control companies carry out comparative analyses to detect any possible strain differences in developmental thresholds and survival in species that have been collected from a range of climatic origins (Hart *et al.*, 2002b). In the present study, as *T. montdorensis* is confined to a few areas with similar climates, strain differences are less likely than if the species had a broader origin.

The day-degree requirement, together with the field survival data in association with field temperatures recorded during the present work can be used to predict establishment potential in other areas of the UK and other countries by comparing climate data. The potential impacts of climate change on individual organisms can be divided into effects on physiology, phenology, distribution and their ability to adapt to climatic variations (Hughes, 2000). Although climate change is likely to lead to improved winter survival of insects and an extension of favourable summer conditions (Bale *et al.*, 2002), increased climatic variability and changes in precipitation may also leave insects vulnerable to the changing conditions and increase mortality (Coulson *et al.*, 2000). Therefore, even predicting the direct effects of climate change on insects is not straightforward, especially at high latitudes (Sinclair *et al.*, 2003). The effects of global warming are predicted to cause a rise in global surface air temperature of 1 to 3.5°C by 2100 (Houghton *et al.*, 1996). In tandem, increased evaporation in connection with a harsher hydrological cycle may increase precipitation and the frequency of early and late season frosts could also be

influenced (Karl *et al.*, 1997). A rise of 2°C may increase annual voltinism by up to five generations for several insect orders, mites and nematodes. Aphids are expected to increase their annual voltinism most dramatically compared with other insects due to short generation times (Yamamura and Kiritani, 1998).

Periods of unusual winter warmth may be a disadvantage to many insects that are in a quiescent state as their activity may increase without the presence of food and therefore vital energy reserves needed for post-winter survival may be depleted. Therefore, insects that remain active over the winter period may be at an advantage (Harrington *et al.*, 2001). Most insects may be able to move with changing temperatures, but some plants and other more immobile insect species may not be able to do so (Lawton, 1995). Temperature is likely to be the dominant factor governing the possibility of increased establishment potential by non-native species. In the present study the lack of survival in the field throughout the winter suggests that large temperature increases would be needed to enable increased outdoor survival of *T. montdorensis* in winter. In its natural range, minimum temperature is generally above 8ºC (Steiner and Goodwin, 2002a), and therefore well above the lowest temperatures experienced during a UK winter.

To conclude, it is known that in *N. californicus* there is variation in source populations used by biological control companies as breeding stock, particularly with regard to the occurrence of diapause and non-diapause strains. However, this does not appear to be the case for *T. montdorensis* where, to date, only non-diapausing populations have been identified. In the present study, laboratory data shows that *T. montdorensis* lacks cold tolerance and this is supported by field data, where in mid-winter, all populations died within 7-14 days of release. Developmental data in combination with climate data predicts that no generations of *T. montdorensis* are possible between October and April in the UK. In addition, the current strain of *T. montdorensis* is unable to enter diapause and no reproduction was possible in the field over the winter. Therefore, on the basis of the biological information gained in this study on the strain of *T. montdorensis* likely to be used in the UK, this species appears to be a 'safe candidate' for introduction as a glasshouse biological control agent, as establishment in a UK 'outdoor' environment is very unlikely.

Chapter 3: Other factors influencing the establishment potential of non-native biological control agents

3.1 Availability of wild prey

Abiotic factors such as temperature and humidity are important in determining the establishment potential of non-native biological control agents in the UK (Bale and Walters, 2001). If laboratory and field experiments have shown that the abiotic conditions are likely to favour the establishment of the target species then biotic factors also need to be considered if the biological control agent is considered important enough that it may still be released despite abiotic factors suggesting it may establish. The ability of a non-native species to find and utilise food sources outside the glasshouse and its ability to avoid predation (if present) and compete with other species will also affect establishment potential (Bale and Walters, 2001; Tauber *et al.*, 1986). If a number of alternative prey sources prove accessible to the target species then the probability of establishment is greatly increased. Further work may then need to consider the likelihood of the individual species attacking indigenous species of conservation importance.

Although biological control companies are aware that tests on wild prey species are a necessary component of a risk assessment there is as yet no agreed system as to how such tests should be conducted. Alternative host testing for non-native biological control agents can involve sequential testing of various hosts in 'choice' and 'no choice' experiments (see Introduction, section 1.6.5), where a target species is given its target prey in a 'no choice' scenario and then given a variety of prey to determine which species it will select in a 'choice' scenario. However, in some cases it may be more valuable to compare the performance of the candidate biological control agent on its glasshouse host with wild prey species. Outside of the glasshouse the biological control agent may attack prey similar to its glasshouse host and if such species are unavailable it may switch to other species.

Initially, it may be important to determine if a viable predator population can be reared on the wild prey species, and therefore the target biological control agent could be presented with just one food source (wild prey). If a viable population is formed then developmental rates, longevity and reproductive capacities can be determined on the wild prey source at two temperatures, an optimal and sub-optimal temperature, and these can be compared to similar experiments conducted on the glasshouse prey. This would enable direct comparisons between glasshouse and wild prey. At optimal rearing temperatures a biological control agent may be able to survive on a less nutritious food source, but at sub-optimal temperatures; the combined stress of sub-optimal temperature and food may decrease the performance of the biological control agent.

So far in the present study, it has been shown that the investigated strain of *T. montdorensis* is unlikely to become established outside the glasshouse in the UK, thus from a risk assessment perspective it would not be appropriate to use resources in studies on its use of wild prey. In contrast, *Neoseiulus californicus* has established in parts of the UK (Jolly, 2000), and *Macrolophus caliginosus* has been found outside the glasshouse environment in the winter (Hart *et al.*, 2002b). *Neoseiulus californicus* is a predator of *Tetranychus urticae* and *M. caliginosus* feeds primarily on *Trialeurodes vaporariorum*. As *T. urticae* and *T. vaporariorum* are the two most important glasshouse pests in the UK, it was decided to investigate the ability of *M. caliginosus* to utilise wild prey sources. Due to time constraints, similar work on *N. californicus* could not be conducted in the present study.

3.2 *Macrolophus caliginosus*

Mirid bugs were originally considered as plant feeders, however many are also highly predaceous insects (Albajes and Alomar, 1999). *Macrolophus caliginosus* (Wagner) (Heteroptera: Miridae) (Photographs 8 and 9) is a predatory mirid bug first released into the UK in 1995 as a glasshouse biological control agent against *T. vaporariorum* on tomatoes (Sampson and Jacobson, 1999). It originated in the Mediterranean region (Malausa and Trottin-Caudal, 1996) and is reported to have a good resistance to winters in its native climate (Fauvel *et al.*, 1987).

Macrolophus caliginosus has been used primarily for the control of *T. vaporariorum* on tomatoes as the parasitoid *Encarsia formosa* has not always produced economically satisfactory results (Malausa and Trottin-Caudal, 1996). It has also been used to control aphids, caterpillars, thrips and some mite species (Enkegaard *et al.*, 2001; Fauvel *et al.*, 1987; Malausa and Trottin-Caudal, 1996). Although development in most cases is reported to be quickest on *T. vaporariorum*, some work suggests development on thrips is just as successful (Montserrat *et al.*, 2000; Riudavets and Castane, 1998). *Macrolophus caliginosus* is therefore a highly polyphagous predator and is also able to complete a generation by feeding on sap and flowering trusses of tomato plants in the absence of prey (Sampson and Jacobson, 1999).

A possible wild prey target of *M. caliginosus* is the cabbage whitefly, *Aleyrodes proletella* (Linnaeus) (Hemiptera: Aleyrodidae), a relative of *T. vaporariorum*. *Macrolophus caliginosus* is also known to feed on *Myzus persicae* (Foglar *et al.*, 1990; Malausa and Trottin-Caudal, 1996). As there are many common aphids in the UK, *M. persicae* was considered to be a relevant alternative prey to use in experiments. Developmental rates, longevity and reproductive capacities of *M. caliginosus* on its glasshouse host *T. vaporariorum* were therefore compared with its performance when fed on *A. proletella* and *M. persicae*.

3.3 Aims

The aims of this chapter were to:

1. Determine whether *M. caliginosus* will feed on *M. persicae* and *A. proletella*.
2. Compare fecundity and longevity between optimal and sub-optimal growth conditions.
3. Predict how well *M. caliginosus* would survive outside the glasshouse, if its primary food source (*T. vaporariorum*) was unavailable or scarce.
4. Devise a simple experimental protocol to place alongside the system used to assess cold tolerance and overwintering.

3.4 Methods

3.4.1 Rearing of prey species

Trialeurodes vaporariorum were obtained as pupae from Syngenta Bioline and reared on tobacco plants, *Nicotiana tabacum* (Solanaceae) var White Burley at 23°C, 18:6 LD in perspex cages (50 cm high, 45 cm wide) with a muslin top with a relative humidity above 65%. The plants were grown to about 15 cm high and had 4-8 large leaves before being put into the culture. Any black *T. vaporariorum* pupae were removed as these were likely to have been parasitised by *E. Formosa*. *Nicotiana* is a classic re-greening species (Thomas and Stoddart, 1980) and when lower leaves senesced earlier than desired, the plant could reverse this process if part or all of the shoot directly above the yellowing leaves was removed. In addition flower buds were removed to stop the plant bolting, increasing their longevity.

Myzus persicae were obtained from a stock culture held at the University of Birmingham and reared on *N. tabacum* at 23°C, 18:6 LD. Small 5 cm high *N. tabacum* plants were infested with *M. persicae* and new plants were fed into the culture when necessary.

Aleyrodes proletella were collected from an allotment in Birmingham and identified with help from Dr John Martin at the Natural History Museum, London. They were held in cages on brussel sprouts, *Brassica oleracea* (Cruciferae) var Evesham Special at 20°C, 18:6 LD.

3.4.2 Rearing of Macrolophus caliginosus

Macrolophus caliginosus (Macro-C) were obtained from Syngenta Bioline and reared at 25°C, 18:6 LD in quarantine cages (50 cm high, 45 cm wide) on *N. tabacum* leaves infested with *T. vaporariorum*. Fresh plants were fed into the culture every three weeks. During this process, all *M. caliginosus* were collected from the old plants with a pooter and placed onto new plants. The old leaves were stripped off the plant stem and placed in plastic boxes (17 x 25 x 9 cm); emerging *M. caliginosus* nymphs were

then placed back into the main culture. All leaves removed from the main plant stem were kept fresh by wrapping the stem tip in moist cotton wool.

3.4.3 Development of Macrolophus caliginosus

The temperatures selected for developmental work on *M. caliginosus* were 12°C, as this was above the developmental threshold of 8.4°C previously estimated (Hart *et al.*, 2002b) and 24°C, regarded as a suitable temperature for both predator and prey development, but slightly lower than the optimum temperature for development of 25-26°C (Hansen *et al.*, 1999).

To obtain eggs of a known age, 50 *M. caliginosus* adults were placed in a plastic box (17x25x9 cm) with four large *N. tabacum* leaves infested with *T. vaporariorum*. The adults were left for 24 h to oviposit, after which all adult *M. caliginosus* were removed and the leaves were placed at 24°C, 18:6 LD or 12°C, 18:6 LD and observed daily for emerging first instar nymphs. As *M. caliginosus* eggs are laid within the leaf mid rib and stem it was not possible to separate out individual eggs.

Developmental data was collected at 12 and 24°C. First instar nymphs emerging from the stock leaves held at 12 and 24°C were placed individually in glass vials (3.5 x 2.5 cm), on a layer of 0.5 cm of agar (2%) (Oxoid Ltd, UK) with a circular piece of filter paper (2 cm in diameter) resting on the agar which provided a moisture source. In addition, treatments which included a prey source had a 2 cm in diameter leaf disc added to the vial infested with the appropriate prey. Each vial was sealed with a ventilated plastic lid covered in 75 μm muslin (Lockertex, UK). In total, six treatments with *M. caliginosus* were used:

1. *Nicotiana tabacum* leaves infested with *T. vaporariorum* (n = 96).
2. *Nicotiana tabacum* leaves infested with *M. persicae* (n = 79).
3. *Brassica oleracea* leaves infested with *A. proletella* (n = 41).
4. Uninfested *N. tabacum* leaves (control) (n = 30).
5. Uninfested *B. oleracea* leaves (control) (n = 30).
6. Agar and filter paper (control) (n = 30).

Treatments 1-3 were set up to record developmental time of *M. caliginosus* on different prey sources. Treatments 4 and 5 were designed to test whether *M. caliginosus* could survive solely on a leaf diet of the host plants and treatment 6 was used to test whether *M. caliginosus* could survive on agar as a moisture source alone. Sample sizes for treatments 1-3 varied due to *T. vaporariorum* being available in excess with slightly less *M. persicae* available and a more limited supply of *A. proletella*.

Any individual *M. caliginosus* that reached adulthood on their respective treatments were sexed. Females have a keeled shaped abdomen, whilst males have a more slender and longer abdomen. One male and one female were then placed in plastic boxes (17 x 11 x 5 cm) with one infested leaf (10 x 3 cm) of their respective food source. *Macrolophus caliginosus* feeding on *A. proletella* leaves were also given an unifested *N. tabacum* leaf to provide the same oviposition substrate as with the other two treatments. After 24 h the adults were removed and placed on fresh leaves. The 'old' leaves were then kept at either 24 or 12°C to observe any possible emerging *M. caliginosus* first instar nymphs (F1 generation).

The F1 generation nymphs were then reared to adulthood on the original parental prey source and the same procedure repeated to obtain an F2 generation.

To discover if prey source affected fitness of *M. caliginosus*, developmental times, longevity and fecundity were recorded. Developmental times were log transformed and analysed by a General Linear model, and differences within treatments were compared using Tukey's HSD. Data on longevity and fecundity were log transformed and analysed by a One-way ANOVA and differences within treatments were compared by Tukey's HSD.

3.5 Results

3.5.1 Developmental time

Within the three control treatments no *M. caliginosus* first instar nymphs reached adulthood. On agar, 80% of nymphs were dead by day 2 and the remaining 20% by day 5. None moulted to second instar. On uninfested *B. oleracea* leaves, 90% were dead by day 2 and the remaining 10% by day 4. None moulted to second instar. On uninfested *N. tabacum* 67% died by day 3 and 33% moulted to second instar on day 4 and then died by day 5.

The total developmental times and the time spent at each life stage for the parental generation of *M. caliginosus* reared on different prey sources at 24°C are shown in Table 10. The corresponding P values are shown in Table 11. The developmental times for *M. caliginosus* fed on *T. vaporariorum* and *M. persicae* at 12°C are shown in Table 12 and the corresponding P values in Table 13. Only 2 of 40 individual *M. caliginosus* reached adulthood at 12°C when reared on *A. proletella* and were therefore not included in the analyses. The total developmental time of *M. caliginosus* 24°C (days ± SE) on *T. vaporariorum* (28.8 ± 0.53) was similar to those reared on *M. persicae* (31.2 ± 0.56) ($P > 0.05$), but significantly shorter than when reared on *A. proletella* (34.7 ± 1.18) ($P < 0.01$) at 24°C.

Table 10: Time spent (days ± SE) at each life stage of *Macrolophus caliginosus* (F1 generation) at 24°C on three different prey sources and sex of adults.

Food source	n	Egg	1st instar	2nd instar	3rd instar	4th instar	5th instar	Egg-adult	Female (%)	Male (%)
T. vaporaiorum	96	11.0 ± 0.20	3.8 ± 0.17	3.4 ± 0.15	3.3 ± 0.15	3.4 ± 0.16	3.9 ± 0.18	28.8 ± 0.53	58	42
M. persicae	79	11.5 ± 0.28	4.9 ± 0.30	4.1 ± 0.21	3.6 ± 0.18	3.4 ± 0.20	3.7 ± 0.26	31.2 ± 0.56	52	48
A. proletella	41	10.7 ± 0.26	4.5 ± 0.36	5.0 ± 0.49	4.5 ± 0.42	5.1 ± 0.52	4.9 ± 0.46	34.7 ± 1.18	55	45

Table 11: Developmental time and P values for each life stage of *Macrolophus caliginosus* (F1 generation) at 24°C fed on *Trialeurodes vaporariorum* (T.v), *Myzus persicae* (M.p) or *Aleyrodes proletella* (A.p).

Life stage	Developmental time	P value
Egg	T.v = M.p = A.p	> 0.05
1st instar	T.v < M.p	< 0.01
	T.v < A.p	< 0.05
	M.p = A.p	> 0.05
2nd instar	T.v < M.p & A.p	< 0.01
	M.p < A.p	< 0.01
3rd, 4th & 5th instar	T.v = M.p	>0.05
	T.v < A.p	<0.01
	M.p < A.p	<0.01
Total time egg-adult	T.v = M.p	> 0.05
	T.v < A.p	< 0.01
	M.p = A.p	> 0.05

Table 12: Time spent (days ± SE) at each life stage of *Macrolophus caliginosus* (F1 generation) at 12°C on three different prey sources and sex of adults. NB *Aleyrodes proletella* only two replicates.

Food source	n	Egg	1st instar	2nd instar	3rd instar	4th instar	5th instar	Egg-adult	Female (%)	Male (%)
T. vaporaiorum	30	24.0 ± 0.59	10.5 ± 0.59	9.8 ± 0.52	9.9 ± 0.60	11.5 ± 0.44	15.5 ± 0.54	81.2 ± 1.90	60	40
M. persicae	36	23.9 ± 0.39	13.8 ± 0.29	13.6 ± 0.27	13.9 ± 0.27	14.1 ± 0.30	15.3 ± 0.29	94.6 ± 1.06	47	53
A.proletella	2	26.5 ± 1.5	9.0 ± 1	12 ± 1	10.5 ± 0.5	9.0 ± 1	11.5 ± 0.5	78.5 ± 3.5	0	100

As only two replicates of *M. caliginosus* fed on *A. proletella* reached adulthood and both of these were male, no further work could be conducted on this population.

Table 13: Developmental time and P values for each life stage of *Macrolophus caliginosus* (F1 generation) at 12°C on two different prey sources (*Aleyrodes proletella* omitted from analyses as only two replicates).

Life stage	Developmental time	P value
Egg	T.v = M.p	> 0.05
1st, 2nd, 3rd, 4th instar	T.v < M.p	< 0.01
5th instar	T.v = M.p	> 0.05
Total time egg-adult	T.v < M.p	< 0.05

The total developmental time of the F2 generation of *M. caliginosus* reared on *T. vaporariorum* or *M. persicae* 24°C is shown in Table 14 and the corresponding P values in Table 15. Total developmental time was similar for the F2 generation of *M. caliginosus* reared on *T. vaporariorum* and *M. persicae*, but slightly longer for *M. caliginosus* fed on *A. proletella*.

Table 14: Time spent (days ± SE) at each life stage of *Macrolophus caliginosus* (F2 generation) at 24°C on three different prey sources and sex of adults.

Food source	n	Egg	1st instar	2nd instar	3rd instar	4th instar	5th instar	Egg-adult	Female (%)	Male (%)
T. vaporaiorum	30	11.9 ± 0.15	4.4 ± 0.14	3.9 ± 0.14	3.7 ± 0.15	3.6 ± 0.16	4.0 ± 0.13	31.5 ± 0.39	43	57
M. persicae	30	12.4 ± 0.17	4.7 ± 0.18	3.7 ± 0.11	3.8 ± 0.14	3.4 ± 0.14	3.1 ± 0.08	31.1 ± 0.40	40	60
A. proletella	10	11.1 ± 0.45	4.6 ± 0.39	5.1 ± 0.47	4.5 ± 0.44	5.3 ± 0.54	4.8 ± 0.41	35.4 ± 1.18	50	50

Table 15: Developmental time and P values for each life stage of *Macrolophus caliginosus* (F2 generation) at 24°C on two different prey sources.

Life stage	Developmental time	P value
Egg , 1st, 2nd, 3rd 4th instar	T.v = M.p	> 0.05
5th instar	T.v > M.p	< 0.01
Total time egg-adult	T.v = M.p	> 0.05

3.5.2 Fecundity

The mean total number of eggs (± SE) produced by the F1 generation is 56.7 ± 6.6, 53.1 ± 6.3 and 34.6 ± 5.2 for *M. caliginosus* fed on *T. vaporariorum*, *M. persicae* or *A. proletella* respectively (Figure 18). There was no difference between the mean number of eggs laid by *M. caliginosus* when fed on *T. vaporariorum* or *M. persicae* at 24°C ($F_{1,53}$ = 0.02, P > 0.05). *Macrolophus caliginosus* fed on *A. proletella* was not included in the analyses as the sample size (n = 10) was only a third of that of *M. caliginosus* fed on the other two prey sources and problems with premature leaf death may have been contributing to lower recorded oviposition numbers. The number of eggs laid per day (± SE) peaked on day 6 for both *M. caliginosus* fed on *T. vaporariorum* (3.7 ± 0.57) and on *M. persicae* (3.7 ± 0.55) and on day 5 when fed on *A. proletella* (2.4 ± 0.7). The mean number of eggs laid per day fell to 0 on days 41, 48 and 52 for *M. caliginosus* fed on *T. vaporariorum*, *M. persicae* or *A. proletella* respectively.

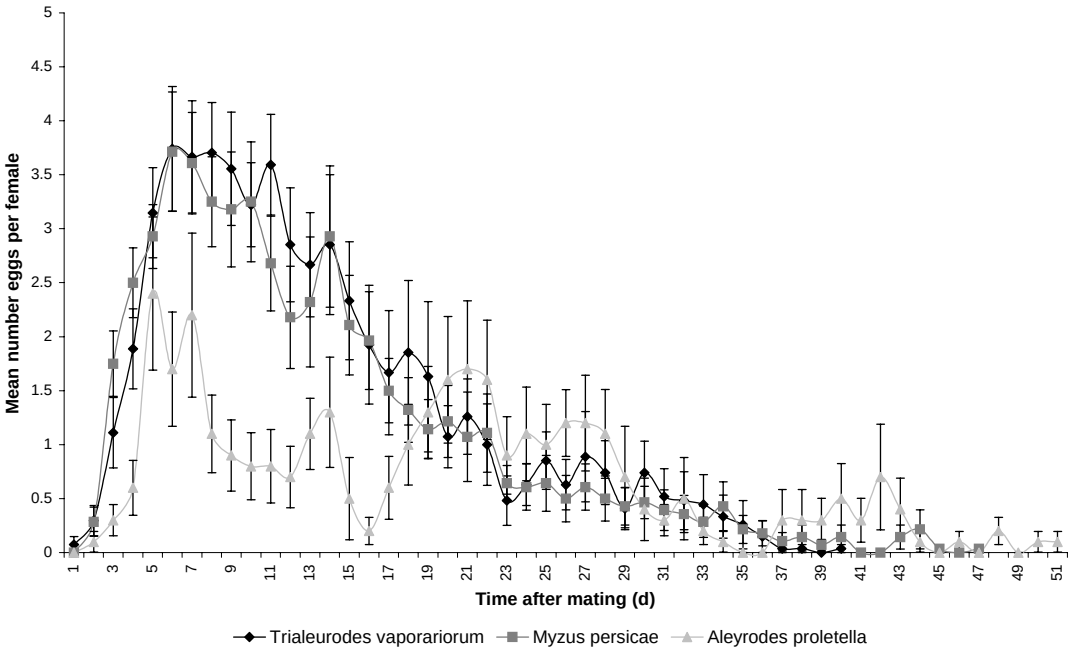


Figure 18: Mean number of eggs (± SE) laid per *Macrolophus caliginosus* (F1) per day at 24°C when fed on three different prey sources.

The mean (± SE) number of eggs laid per F1 generation *M. caliginosus* per day when fed *T. vaporariorum* or *M. persicae* at 12°C is shown in Figure 19. The mean total number of eggs (± SE) laid was 21.6 ± 9.8 and 14.2 ± 4.1 for *M. caliginosus* fed on *T. vaporariorum*, and on *M. persicae*. Only two individual *M. caliginosus* fed on *A. proletella* reached adulthood at 12°C and no egg production was recorded.

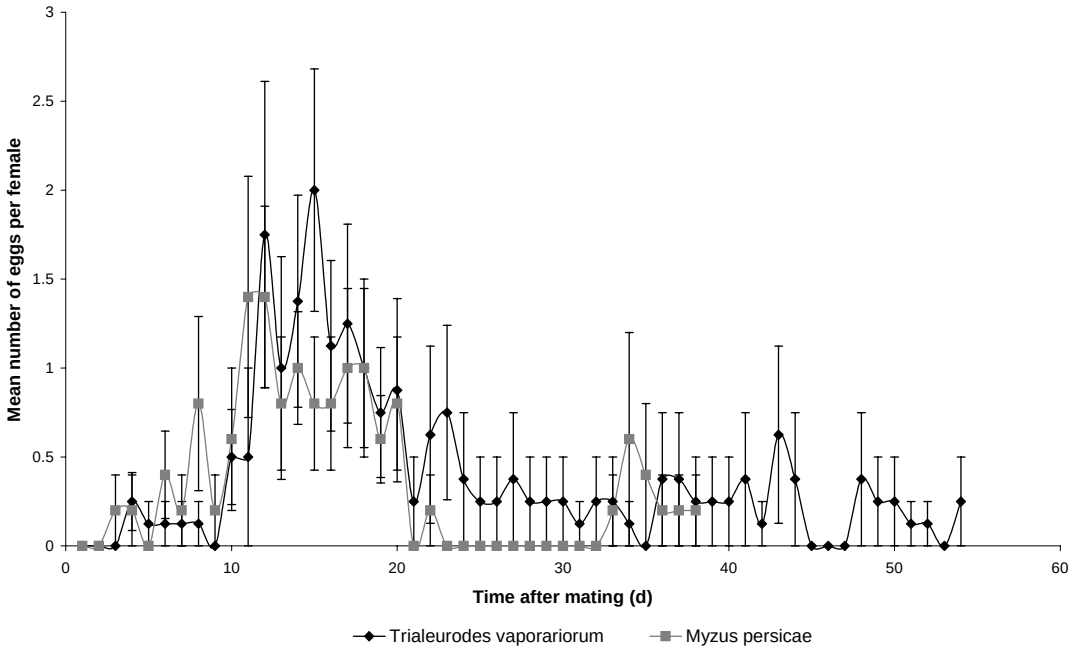


Figure 19: Mean number of eggs (± SE) laid per *Macrolophus caliginosus* (F1) per day at 12°C when fed on two different prey sources.

3.5.3 Longevity, time spent reproducing and total survival time after mating

There was no difference in the total longevity (± SE) of F1 generation *M. caliginosus* fed on *T. vaporariorum* (58.1 days ± 1.6) and on *M. persicae* (61.1 days ± 2.3) at 24°C ($F_{1,53}$ = 0.75, P > 0.05). The total longevity of the F1 generation of *M. caliginosus* fed on *A. proletella* was 69.5 ± 3.5 and significantly longer than *M. caliginosus* fed on the other two prey sources ($F_{1,33}$ = 0.6, P < 0.05). However, this final statistical comparison should be treated with caution as as the sample size of *M. caliginosus* fed on *A. proletella* was much smaller than for *M. caliginosus* fed on the other two prey sources. The number of eggs laid per day peaked on day 14 for *M. caliginosus* fed on *T. vaporariorum* (1.4 ± 0.59) and day 12 when fed on *M. persicae* (1.4 ± 0.67). The mean number of eggs laid per day fell to 0 on days 55 and 39 for *M. caliginosus* fed on *T. vaporariorum* and on *M. persicae* respectively.

There was no difference in the time spent reproducing by the F1 generation *M. caliginosus* fed on *T. vaporariorum* (23.7 days ± 1.6) and on *M. persicae* (24.8 days ± 2.3) at 24°C ($F_{1,53}$ = 0.07, P > 0.05). Also, there was no difference in total survival

time after mating of F1 generation *M. caliginosus* fed on *T. vaporariorum* (30.4 days ± 1.7) and on *M. persicae* (30.4 days ± 2.4) at 24°C ($F_{1,53} = 0.19$, $P > 0.05$).

Table 16 shows the mean total number of eggs laid and the mean total longevity per *M. caliginosus* fed on *A. proletella*, *T. vaporariorum* or *M. persicae*. As only two *M. caliginosus* fed on *A. proletella* reached adulthood at 12°C, no fecundity or longevity experiments were conducted.

Table 16: Mean total eggs laid (± SE) and total longevity (days ± SE) per *Macrolophus caliginosus* on different prey sources at 12 and 24°C.

Treatment	n	Mean total eggs laid (± SE)	Total longevity (days ± SE)
F1 *M. caliginosus* fed on *A. proletella* at 12°C	0	n/a	n/a
F2 *M. caliginosus* fed on *A. proletella* at 24°C	4	12.5 ± 5.9	46.5 ± 4.1
F1 *M. caliginosus* fed on *T. vaporariorum* at 12°C	8	21.6 ± 9.8	122 ± 6.9
F2 *M. caliginosus* fed on *T. vaporariorum* at 24°C	16	35.5 ± 6.6	55.7 ± 2.5
F1 *M. caliginosus* fed on *M. persicae* at 12°C	6	14.2 ± 4.1	130 ± 6.5
F2 *M. caliginosus* fed on *M. persicae* at 24°C	14	36.2 ± 5	54.3 ± 1.5

The time spent reproducing and the total survival after mating per *M. caliginosus* fed on *A. proletella*, *T. vaporariorum* and *M. persicae* is shown in Table 17.

Table 17: Time spent reproducing (days ± SE) and total survival time (days ± SE) after mating for *Macrolophus caliginosus* on different prey sources at 12 and 24°C where applicable.

Treatment	n	Time spent reproducing (days ± SE)	Total survival after mating (days ± SE)
F1 *M. caliginosus* fed on *A. proletella* at 24°C	10	31.7 ± 4.7	38.7 ± 3.7
F2 *M. caliginosus* fed on *A. proletella* at 24°C	4	11.5 ± 3.2	14.5 ± 4.0
F2 *M. caliginosus* fed on *T. vaporariorum* at 24°C	16	14.8 ± 1.1	24.3 ± 2.5
F1 *M. caliginosus* fed on *T. vaporariorum* at 12°C	8	23.3 ± 5.8	45.2 ± 7.2
F2 *M. caliginosus* fed on *M. persicae* at 24°C	14	18.4 ± 1.6	23.1 ± 1.8
F1 *M. caliginosus* fed on *M. persicae* at 12°C	5	20.1 ± 4.7	34.7 ± 7.7

3.6 Discussion

If outdoor climatic regimes favour *M. caliginosus*, it will also have to be able to utilise a wild prey source to sustain long term populations. Its primary glasshouse prey *T. vaporariorum*, will often escape from the glasshouse, but is unlikely to be available as a food source throughout an entire year as it lacks cold tolerance. Therefore the initial aim of this work was to determine whether *M. caliginosus* could feed on the two alternate prey sources, *M. persicae* and *A. proletella*, that would be available outdoors.

The two temperatures used to investigate the development of *M. caliginosus* on different prey sources were 12 and 24°C. The former was above the previously estimated developmental threshold of 8.4°C (Hart *et al.*, 2002b) and 24°C was slightly lower than the optimum temperature for development of 25-26°C (Hansen *et al.*,

1999), but regarded as a suitable temperature for both predator and prey development. During the current investigation with the control treatments there was no development beyond the first instar of any *M. caliginosus* reared on agar or on unifested *B. oleracea* leaves. On unifested *N. tabacum* leaves 33% of *M. caliginosus* moulted to second instar, but died before moulting again. *Macrolophus caliginosus* has been classified as a plant feeder (Albajes and Alomar, 1999) and can survive by feeding on the sap of tomato plants when its prey is absent (Sampson and Jacobson, 1999). Sap of the ivy geranium, *Pelargonium peltatum* (Linnaeus) (Geraniaceae) is an important supplement of *M. caliginosus* nymphs fed on *Ephestia kuehniella* (Keller) (Phycitini: Pyralidae) eggs (Grenier *et al.*, 1989) and *M. caliginosus* females can survive for two weeks on tomato sap alone (van Schelt *et al.*, 1995). In the present study, even though some *M. caliginosus* reached second instar when kept on *N. tabacum* leaves it appears that the sap from the leaves alone may not be an adequate food source to complete development. However, it is likely *M. caliginosus* could overcome a short period of prey scarcity by feeding on plant sap. If more time had been available, experiments using adult *M. caliginosus* on the control treatments might have revealed the maximum time period it could survive on a plant diet as the sole food source. In a recent comparison of three plant predators, *M. caliginosus* consumed less *T. vaporariorum* than *Dicyphus tamaninii* (Wagner) (Heteroptera: Miridae) and *Orius majusculus* (Reuter) (Heteroptera: Anthocoridae), but remained on the plant at low prey densities, whereas *D. tamaninii* and *O. majusculus* did not (Montserrat *et al.*, 2004). This suggests that *M. caliginosus* may be supplementing its diet with plant sap at low prey densities, whereas *D. tamaninii* and *O. majusculus* are more likely to disperse in search of live prey.

As development of *M. caliginosus* on plants alone was unsuccessful in the present study it was important to determine development on live prey. At 24°C *M. caliginosus* took 28.8, 31.2 and 34.7 days to develop from egg to adult when fed on *T. vaporariorum*, *M. persicae* and *A. proletella* respectively. Therefore both the alternative prey sources *M. persicae* and *A. proletella* were adequate food sources for *M. caliginosus* to complete its development. Even though development of *M. caliginosus* was significantly slower when fed on *A. proletella* than on *T. vaporariorum*, in terms of establishment success, this is unlikely to hold much significance. Hart *et al*., (2002b) found that development from egg to adult of *M.*

caliginosus reared on *T. vaporariorum* on *N. tabacum* was 25 days at 26°C. This was slightly faster than previous observations, where development with the same prey on tomato was 30 days at 25°C (Fauvel *et al.*, 1987). In the current work there were minor differences in developmental times at 24°C between life stages of the three treatments. However, from an establishment perspective, only total developmental time from egg to adult is of high importance. Development of the F2 generation from egg to adult was 31.6, 31.3 and 33.9 days for *M. caliginosus* fed on *T. vaporariorum*, *M. persicae* and *A. proletella* respectively and therefore very similar to the F1 generation. However, the sample size for the F2 generation of *M. caliginosus* fed on *A. proletella* was only ten and therefore this result needs to be viewed with caution.

Malausa and Trottin-Caudal (1996) found that *M. caliginosus* could feed on *M. persicae*, *Aphis gossypii* (Glover) (Hemiptera: Aphididae) and *T. urticae*, although the latter two were not as good as food sources as *M. persicae*. When fed *T. urticae* on tomato at 22°C development of *M. caliginosus* nymphs was 27 days (Hansen *et al.*, 1999) and 28.2 days when fed on *Tetranychus turkestani* (Ugarov and Nikolski) (Acari: Tetranychidae) (Fauvel *et al.*, 1987). The related *Macrolophus pygmaeus* (Rambur) (Heteroptera: Miridae) marginally prefers *T. vaporariorum* over *M. persicae* as a food source (Perdikis and Lykouressis, 2000), but like *M. caliginosus*, can utilise both prey types readily. This may explain why there were no large differences in life history traits between *M. caliginosus* fed on *T. vaporariorum* or *M. persicae* in the current investigation. Also, *M. caliginosus* will preferably feed on aphids over mites when presented simultaneously (Foglar *et al.*, 1990). In the present work, development was possible on both *M. persicae* and *A. proletella* so the commonly held view that *M. caliginosus* is a highly polyphagous predator (Montserrat *et al.*, 2000; Riudavets and Castane, 1998) is supported.

The next stage of the current investigation was to determine the effect of varying prey sources on fitness of *M. caliginosus*. Due to the small sample size (n = 10) of F1 generation of *M. caliginosus* fed on *A. proletella*, the fecundity data needs to be viewed with caution. Also, during the fecundity studies, some leaves died before the experiments had been completed and therefore, as *M. caliginosus* lays its eggs in stems and veins of leaves (Constant *et al.*, 1996; Fauvel *et al.*, 1987; Ferran *et al.*,

1996), it is likely that not all the eggs had hatched. This contributed to large variation between replicates and oviposition rates should therefore be viewed only as an indication of fecundity of *M. caliginosus* on *T. vaporariorum*, *M. persicae* and *A. proletella*. Mean fecundity per *M. caliginosus* female fed on *T. vaporariorum* and *M. persicae* was 56.7 and 53.1 respectively, and on *A. proletella* it was 34.6. Riudavets and Castane (1998) found *M. caliginosus* could lay 83 eggs per female and van Schelt *et al.,* (1995) observed an oviposition rate of two to seven eggs per day for first 15 days of a study when fed on *T. vaporariorum*. Therefore, although some variation in fecundity is evident between studies, *M. caliginosus* is highly fecund when fed on *T. vaporariorum*. *Macrolophus caliginosus* fecundity is also high (125 eggs/female) when fed on eggs of *E. kuehniella* (Keller), but low when fed on *T. turkestani* (20 eggs/female) (Fauvel *et al.*, 1987) and *T. urticae* (20.2 eggs/female, with maximum of 53 eggs) (Hansen *et al.*, 1999). In addition to the small sample size of *M. caliginosus* fed on *A. proletella* and the large variation between replicates, a further reason for the lower fecundity may have been that due to culturing problems, less *A. proletella* were available as food during the experiments than during the exposures with *T. vaporariorum* and *M. persicae*. Also, *A. proletella* was reared on *B. oleracea* leaves and although *M. caliginosus* was provided with a *N. tabacum* leaf for oviposition it is possible that *M. caliginosus* prefers to oviposit on the same substrate as that occupied by its prey. With *N. tabacum* as an oviposition substrate the number of eggs laid per female per day was 3.7 and 1.2 eggs per day on an artificial substrate (Constant *et al.*, 1996). Nevertheless, in the present study oviposition of *M. caliginosus* fed on *A. proletella* was possible, suggesting that *A. proletella* may provide an adequate, even if not optimal food source for *M. caliginosus* outside the glasshouse.

For establishment outside the glasshouse, initially development, and ultimately fecundity, will have a major influence on the success of *M. caliginosus*. However, the longevity of *M. caliginosus* on varying prey sources is also of interest as it may help determine the severity of any possible non-target effects on the environment outside the glasshouse. Total longevity of the F1 generation of *M. caliginosus* was 58.1, 61.1 and 69.5 days for *M. caliginosus* fed on *T. vaporariorum*, *M. persicae* and *A. proletella* respectively. Other life history traits recorded were total time spent reproducing and total survival after mating. There were no differences between the F1 generations of *M. caliginosus* fed on *T. vaporariorum* or *M. persicae* for both traits.

This further strengthens the view that *M. persicae* may be just as adequate a food source as *T. vaporariorum*. There are some differences between the time spent reproducing and total survival after mating for each treatment in the remaining data, but as sample sizes varied from 16 to as low as four, they should only be regarded as an indication of the response to these treatments.

Work in this investigation conducted at 24°C has indicated that development and fecundity of *M. caliginosus* is possible on *T. vaporariorum*, *M. persicae* and *A. proletella*. *Macrolophus caliginosus* fed on *M. persicae* is as successful as *M. caliginosus* fed on *T. vaporariorum*. *Aleyrodes proletella* seems to be slightly less suited as a prey source for *M. caliginosus*, but it is still able to sustain a population. Any differences in life history of *M. caliginosus* that were apparent between different prey sources may become even more marked at sub-optimal temperatures, such as 12°C, that are likely to be experienced frequently during a UK year outside the glasshouse. In the present study *M. caliginosus* took 81.1 and 94.7 days to develop from egg to adult when fed on *T. vaporariorum* and *M. persicae* at 12°C respectively. Only two (4%) *M. caliginosus* fed on *A. proletella* reached adulthood at 12°C (both male, therefore no fecundity work conducted). This indicates that *A. proletella* at 12°C may not be an adequate food source for *M. caliginosus*. Development of *M. caliginosus* on *T. vaporariorum* has been found to be 130 days from egg to adult at 11°C (Hart *et al.*, 2002b). In the current work at 12°C the mean total number of eggs laid was 21.6 and 14.2 respectively for *M. caliginosus* fed on *T. vaporariorum* and *M. persicae*. This was lower than at 24°C, suggesting that temperature has a negative effect on fecundity. However, a higher percentage of leaves died at 12 than at 24°C before all the eggs were able to hatch and therefore the results recorded are unlikely to be a maximum indication of fecundity at 12°C.

Glasshouse crops such as tomatoes are now often grown for 11 months a year and therefore *M. caliginosus* has prey available almost continually throughout the year in the glasshouse except at the beginning of the season when crops are first colonised by pest species. As *M. caliginosus* has been observed outside UK glasshouses in the winter it is likely to be able to feed in winter. Fed populations have been shown to survive for 50-75 days in mid-winter in the UK and a small proportion of nymphs survived for an entire UK winter (Hart *et al.*, 2002b). *Macrolophus caliginosus* is

likely to be able to feed on species such as *T. urticae* (Enkegaard *et al.*, 2001; Hansen *et al.*, 1999), *A. gossypii* (Malausa and Trottin-Caudal, 1996), *F. occidentalis* (Montserrat *et al.*, 2000; Riudavets and Castane, 1998) at varying times of the year in glasshouses. Although some of the above species such as *F. occidentalis* are unlikely to be available outside UK glasshouses during the winter (McDonald *et al.*, 1997), it seems probable that some prey source will always be accessible outside the glasshouse during part of the winter.

Macrolophus caliginosus has a high capacity to move in and out of the glasshouse in the Mediterranean climate (Gabarra *et al.*, 2004). Part of this chapter aimed to predict how well *M. caliginosus* would survive outside the glasshouse. Previous thermal biology studies concluded that long term exposure to low temperature in winter was most likely to kill any *M. caliginosus* populations outside the glasshouse (Hart *et al.*, 2002b). However, even if *M. caliginosus* is not 'established' outside the glasshouse environment it will be able to move easily between the glasshouse and the 'open field'. Some individuals may therefore survive their entire life cycle outside the glasshouse during milder winters and may eventually become locally established in areas where the temperature regimes are less harsh and where abundant alternate food sources are available.

If local establishment does ultimately occur it may not be too serious in terms of affects on non-target organisms. In the UK it is estimated that *M. caliginosus* could complete two generations a year outside the glasshouse (Hart *et al.*, 2002b) and this may be lower if no adequate food source can be found. As *M. caliginosus* can feed on mites, thrips, whitefly and aphids it may not encounter or feed on rarer 'non-pest' species that are native to the UK. However, *M. caliginosus* can feed on *E. formosa* pupae whenever available (Castane *et al.*, 2004) and can also feed on other natural enemies such as the leaf miner parasitoid *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) (Nedstam and Johansson-Kron, 1999) and *P. persimilis* (Koskula *et al.*, 1999). Therefore, even though *M. caliginosus* will feed on a large number of pest species, it can also feed on some natural enemies when available. In most years the most abundant prey source is likely to be a pest species. Therefore, if *M. caliginosus* did establish locally outside the glasshouse in the UK, in most years it would be likely to feed primarily on pest insects and mites. However, in some years it is possible that

some natural enemies and possibly UK native insects may get predated. The only way to determine these responses is to conduct regular surveys to determine the occurrence of *M. caliginosus* outside UK glasshouses.

This section of work has outlined an initial sequence of experiments that can be used to determine the efficacy of *M. caliginosus* fed on alternative prey sources, as possibly encountered outside the glasshouse in the UK. To date the suggested protocol would be:

Step:

1. Determine whether a glasshouse biological control agent can sustain a population on non glasshouse prey.
2. If a population can be sustained then compare developmental parameters of the glasshouse biological control agent on its glasshouse prey to at least two non glasshouse prey sources at an optimal and a sub-optimal temperature.
3. Conduct choice tests to determine which prey and host plant would preferably be chosen by the biological control agent.

With more research these steps may form the basis of a screening process to detect aternative prey sources available to *M. caliginosus* and in turn, other non-native glasshouse biological control agents.

Chapter 4: Interactions between *Neoseiulus californicus, Typhlodromips montdorensis* and *Typhlodromus pyri*

4.1 Mites in UK orchards

Neoseiulus californicus has established outside the glasshouse environment in several areas in the south east and south west of England since it was first released under licence as a biological control agent in the UK in 1991 (Jolly, 2000). It has generally been found on strawberries and hops, but also been sighted on apples (Fitzgerald, pers comm). The 'wild' UK strain of *N. californicus* is able to enter diapause outside the glasshouse (Hart *et al.*, 2002a; Jolly, 2000) and studies on its thermal biology suggest that it is able to survive a 'typical' UK winter (Hart *et al.*, 2002a). It is possible therefore that *N. californicus* may become more common in UK orchards and other fruit growing areas.

The most abundant predatory mite in UK apple orchards is the native *Typhlodromus pyri* (Fitzgerald and Solomon, 2002). It successfully controls the European red mite, *Panonychus ulmi* in the UK where organophosphorous resistant strains of the predator are used in biological control (Solomon *et al.*, 2000; Solomon *et al.*, 1993). During sampling from 1997 to 2000, *N. californicus* was found in Claston, East Malling, Ledbury, Stroud, Ticehurst and Chartham. In most of these cases, *T. pyri* was found at the same sites (Jolly, 2001). It is of interest to investigate how *T. pyri* and *N. californicus* may interact.

When fed *Tetranychus urticae* or *P. ulmi*, *N. californicus* develops quicker from egg to adult than *T. pyri* (Jolly, 2000). In the UK three to four generations of *T. pyri* are possible a year (Solomon *et al.*, 2000), compared with *N. californicus* where up to six generations a year are theoretically possible (Hart *et al.*, 2002a). Therefore if *N. californicus* spreads within the UK it is possible that it will compete with *T. pyri* for the same prey and may over time displace *T. pyri* from UK fruit growing regions.

There is evidence that competition between phytoseiid mites can lead to the displacement of a species from an orchard (MacRae and Croft, 1997; Schausberger, 1997; Zhang and Croft, 1995). For example, it is thought that the ability of the generalist *T. pyri* to feed on immatures of *Metaseiulus occidentalis* (Nesbitt) (Acari: Phytoseiidae) during spring and autumn, when *T. urticae* populations are low, contributed to the displacement of the specialist *M. occidentalis* in orchard test plots (MacRae and Croft, 1997). Although all three species are generalists, the ability of *Euseius finlandicus* to outcompete *T. pyri* and *Kampimodromus aberrans* (Oudemans) (Acari: Phytoseiidae) in laboratory tests is attributed to it moving faster than the other two species (Schausberger, 1997). *Amblyseius andersoni* outcompetes *Amblyseius fallacis* (Garman) (Acari: Phytoseiidae), *Typhlodromus occidentalis* (Nesbitt) (Acari: Phytoseiidae) and *T. pyri* when all four species are fed on *T. urticae*. *Typhlodromus occidentalis*, the smallest and least polyphagous of the four species was always outcompeted by the other three species (Zhang and Croft, 1995). This study also demonstrated that a small polyphagous species such as *T. pyri* could compete with a larger oligophagous species such as *A. fallacis*. Therefore, the size and degree of polyphagy of a predator can affect the competitiveness of immature phytoseiids (Zhang and Croft, 1995).

Although *N. californicus* is cannibalistic at low prey densities, *T. pyri* is more cannibalistic (Croft and Croft, 1993; Croft and Croft, 1996; Croft *et al.*, 1996) and this may also affect their interaction. It will therefore be interesting to consider the implications for phytoseiid population compositions in UK fruit growing regions of the interactions of the two generalists *T. pyri* and *N. californicus*.

The present study has demonstrated that *T. montdorensis* is unlikely to be able to establish outside the glasshouse environment in the UK. If DEFRA grant a licence for its release, growers may introduce the species into their glasshouses at the same time as the commercially widely used *N. californicus*. It was therefore considered of interest to examine the interactions between *T. montdorensis* and *N. californicus* when released together and to determine whether the interaction was detrimental or beneficial to a glasshouse biological control programme.

4.2 Species

4.2.1 Neoseiulus californicus

Neoseiulus californicus (Photographs 4 and 6) is a generalist predator originating in California. It has been used extensively for biological control programmes against *T. urticae* in different parts of the world, in a wide range of management and climatic conditions (McMurtry and Croft, 1997). It is used widely in the Mediterranean region where it is reported to occur naturally (Castagnoli and Simoni, 1991). It was introduced into the UK for glasshouse biological control in 1991 and has since established locally in southern Britain (Jolly, 2000). Subsequent work on its thermal biology has revealed that it can complete a generation and reproduce in the field during a UK winter (Hart *et al.*, 2002a).

4.2.2 Typhlodromus pyri

Typhlodromus pyri (Photographs 5 and 7) is a generalist predator (Wei and Walde, 1997). It will feed primarily on *P. ulmi* (Dicke, 1988; Dicke and Dejong, 1988), but will also feed and reproduce equally successfully on *T. urticae* (Herbert, 1956). It is also known to feed on eriophyiids mites, mildew (Chant, 1959) and pollen (Dosse, 1961). *Typhlodromus pyri* was the species most commonly found in sprayed UK apple orchards in the late 1950s (Chant, 1959) and at the end of the last century (Fitzgerald and Solomon, 2002). It is now used as a biological control agent in the majority of apple orchards in the Netherlands, Switzerland and parts of Italy (Blommers, 1994).

4.2.3 Typhlodromips montdorensis

Typhlodromips montdorensis is a predatory mite originating in New Caledonia currently being tested as a glasshouse biological control agent against thrips and mites in the UK (more detail in Chapters 1 and 2).

4.3 Aims

The aims of this chapter were to:

1. Determine whether *N. californicus* can feed on *T. montdorensis* and *T. pyri*.
2. Determine whether *T. montdorensis* and *T. pyri* can feed on *N. californicus*.
3. Compare total prey consumption, longevity and fecundity of each mite species on its prey and investigate interactions that may occur in the field and the glasshouse.

4.4 Methods

4.4.1 Rearing mite species

Stock cultures of *N. californicus*, *T. pyri* and *T. montdorensis* were fed with *T. urticae* and cattail pollen (*Typha* sp) and held at 25°C, 18:6 LD on black tiles (same method for rearing as in Chapter 2). Approximately 100 eggs of each species were taken and placed on separate rearing stages (as in Chapter 2) at 25°C, 18:6 LD. The eggs of each species were left to hatch and juvenile stages were fed with eggs and juvenile stages of *T. urticae* that were brushed off field bean leaves daily. Pollen was also added daily.

4.4.2 Experimental set-up

To determine whether *N. californicus* would feed on *T. montdorensis* and *T. pyri* and whether *T. montdorensis* and *T. pyri* would feed on *N. californicus* the initial sequence of experiments involved a no-choice design, i.e. an adult mite was provided with only the larval stages of its phytoseiid prey. As a comparison/control, adults of each species were fed solely on *T. urticae* larvae. The final scenario was to present adult mites of each species with 50% *T. urticae* and 50% of the target larval phytoseiid as a food source (choice test). Larvae were selected as the food source as studies have demonstrated the importance of immature stages, particularly larvae, as food sources for phytoseiids when their primary prey is unavailable in the field (Croft

and MacRae, 1992; Croft *et al.*, 1992). Additionally, larvae were easy to handle and could be quickly selected once they had hatched.

One 7 day old adult male and one 7 day old adult female of each species (22 pairs in total for each species) that had developed from the original 100 eggs were taken and placed on individual rearing stages with excess food and left to mate. After 24 h the male was removed and the female was transferred to an individual rearing arena (Chapter 2), not supplied with any food and left for a further 24 h to make sure that all individuals had been starved for an equal period of time. A 1 cm long piece of black cotton thread frayed at one end was placed in each arena for oviposition. After 24 h, 11 treatments were set up, with each treatment consisting of 22 mated females on individual rearing stages supplied with a specific prey species (all *T. urticae* used as prey were mixed first and second instar). The treatments (1-11) were as follows:

1. *N. californicus* female with 16 *T. urticae*
2. *N. californicus* female with 16 *T. pyri* larvae
3. *N. californicus* female with 8 *T. pyri* and 8 *T. urticae*
4. *N. californicus* female with 16 *T. montdorensis* larvae
5. *N. californicus* female with 8 *T. montdorensis* larvae and 8 *T. urticae*
6. *T. montdorensis* female with 16 *T. urticae*
7. *T. montdorensis* female with 8 *N. californicus* larvae and 8 *T. urticae*
8. *T. montdorensis* female with 16 *N. californicus* larvae
9. *T. pyri* female with 16 *T. urticae*
10. *T. pyri* female with 8 *N. californicus* larvae and 8 *T. urticae*
11. *T. pyri* female with 16 *N. californicus* larvae

Every 24 h the number of eggs laid, the number of each food source consumed (determined by larval carcasses), and whether the female was alive or dead, was recorded. All excess food and carcasses on the stages were removed and replaced with an identical amount of food as previously supplied. This procedure was repeated until the female had died. Oviposition rate, longevity and prey consumption was recorded for each female of each species.

Total egg production, longevity, total prey consumption and the number of prey consumed during the first 5 days of the experiments were analysed by a One-way ANOVA for each species kept on each of its prey sources. Differences between treatments were compared by Tukey's HSD. The number of prey consumed over the first 5 days was analysed as no individuals died during the first 5 days of each experiment. Treatments 1, 6 and 9 were also compared by One-way ANOVA. These were the only treatments that allowed direct comparison between the 3 mite species, as the sole prey source in each case was *T. urticae*. In addition, the number of eggs laid per female per day for the first 5 days of the experiment was calculated for treatments 1, 6 and 9. For treatments 3, 5, 7, 10 consisting of two prey sources, for example *N. californicus* fed on 8 *T. urticae* and 8 *T. montdorensis*, the ratio of the number of *T. urticae* and *T. montdorensis* consumed was compared.

4.5 Results

The mean total egg production, longevity, total prey consumed and prey consumed over the first 5 days of the experiment are shown in Table 18, for *N. californicus*, *T. montdorensis* and *T. pyri* fed solely on *T. urticae*. The mean number of *T. urticae* consumed per female per day during the first 5 days of the experiment was 7, 7.9 and 8.5 for *N. californicus*, *T. montdorensis* and *T. pyri* respectively.

Table 18: Mean number of eggs (± SE) laid, number of eggs laid per female per day for the first 5 days of the experiment, longevity, total prey consumed and prey consumed over first 5 days for *Neoseiulus californicus* (N.c), *Typhlodromips montdorensis* (T.m) and *Typhlodromus pyri* (T.p) fed on *Tetranychus urticae* (T.u).

Treatment	Total number of eggs laid (± SE)	Eggs per female per day for 1st 5 days	Longevity (days ± SE)	Total prey consumed (± SE)	Prey consumed in 1st 5 days (± SE)
N.c fed 16 T.u	31.5 ± 2.3	3.8	27.9 ± 2.8	144 ± 9	154 ± 5.9
T.m fed 16 T.u	22.4 ± 2.1	2.8	15.0 ± 1.6	114 ± 11.2	175 ± 4.9
T.p fed 16 T.u	14.8 ± 1.4	3.8	21.5 ± 2.3	125 ± 9.1	187 ± 5.8

Neoseiulus californicus and *T. montdorensis* both laid significantly more eggs than *T. pyri* ($P < 0.01$). *Neoseiulus californicus* laid more eggs than *T. montdorensis*, although

this was not significant ($P > 0.05$). *Neoseiulus californicus* and *T. pyri* survived significantly longer than *T. montdorensis* ($P < 0.01$ and $P < 0.05$ respectively). There was no difference in longevity between *N. californicus* and *T. pyri* ($P > 0.05$). Total prey consumption of *N. californicus* was significantly greater than *T. montdorensis* ($P < 0.01$), but there were no differences in total prey consumed between *N. californicus* and *T. pyri* and *T. montdorensis* and *T. pyri* ($P > 0.05$). Over the first 5 days of the experiment *T. montdorensis* and *T. pyri* consumed significantly more prey than *N. californicus* ($P < 0.05$).

The mean total egg production, longevity, total prey consumed, prey consumed over the first 5 days of the experiment and the ratio of *T. urticae* consumed compared with *T. montdorensis* are summarised in Table 19, for *N. californicus* fed on 16 *T. urticae*, 8 *T. urticae* and 8 *T. montdorensis*, or 16 *T. montdorensis*.

Table 19: Mean number of eggs (± SE) laid, longevity, total prey consumed, prey consumed over the first 5 days of the experiment and the ratio of *Tetranychus urticae* consumed compared with *Typhlodromips montdorensis* for *Neoseiulus californicus* (N.c), fed on *Typhlodromips montdorensis* (T.m) and/or *Tetranychus urticae* (T.u).

N. californicus fed on:	Total number of eggs laid (± SE)	Longevity (days ± SE)	Total prey consumed (± SE)	Prey consumed in 1st 5 days (± SE)	Ratio of T.u:T.m consumed
16 T.u	31.5 ± 2.3	27.9 ± 2.8	144 ± 9	154 ± 5.9	n/a
8 T.u & 8 T.m	24.5 ± 2.1	21.2 ± 2.0	138 ± 11	157 ± 2.4	2.6:1
16 T.m	21.7 ± 2.0	16.0 ± 1.9	70.5 ± 6.7	124 ± 5.0	n/a

Neoseiulus californicus laid significantly more eggs ($P < 0.01$) and survived significantly longer ($P < 0.01$) when fed on *T. urticae* alone than when fed only on *T. montdorensis*. Over the entire experiment and during the first 5 days of the experiment *N. californicus* fed solely on *T. urticae* or fed both *T. urticae* and *T. montdorensis* consumed significantly more prey than when fed only on *T. montdorensis* ($P < 0.01$, for both treatments). *Neoseiulus californicus* consumed 2.6 *T. urticae* for every 1 *T. montdorensis* when fed on both prey sources.

The mean total egg production, longevity, total prey consumed, prey consumed over the first 5 days of the experiment and the ratio of *T. urticae* consumed compared with *T. pyri* are given in Table 20, for *N. californicus* fed on 16 *T. urticae*, 8 *T. urticae* and 8 *T. pyri*, or 16 *T. pyri*.

Table 20: Mean number of eggs (± SE) laid, longevity, total prey consumed, prey consumed over the first 5 days of the experiment and the ratio of *Tetranychus urticae* consumed compared with *Typhlodromus pyri* for *Neoseiulus californicus* (N.c), fed on *Typhlodromus pyri* (T.p) and/or *Tetranychus urticae* (T.u).

N. californicus fed on:	Total number of eggs laid (± SE)	Longevity (days ± SE)	Total prey consumed (± SE)	Prey consumed in 1st 5 days (± SE)	Ratio of T.u:T.p consumed
16 T.u	31.5 ± 2.3	27.9 ± 2.8	144 ± 9	154 ± 5.9	n/a
8 T.u & 8 T.p	20.6 ± 2.2	11.1 ± 0.9	72.5 ± 6.6	140 ± 2.9	2.2:1
16 T.p	12.6 ± 1.4	10.3 ± 1.0	44.8 ± 3.7	112 ± 6.9	n/a

Neoseiulus californicus laid significantly more eggs when fed on *T. urticae* alone than when fed solely on *T. pyri* ($P < 0.01$). When fed on both *T. urticae* and *T. pyri*, *N. californicus* laid significantly more eggs than when fed on *T. pyri* alone ($P < 0.01$). It survived significantly longer when fed on *T. urticae* alone than when fed either on *T. urticae* and *T. pyri*, or solely on *T. pyri* ($P < 0.01$). *Neoseiulus californicus* consumed significantly more prey over the whole experiment when fed exclusively on *T. urticae* than when fed just on *T. pyri* ($P < 0.01$). When fed on both *T. urticae* and *T. pyri*, *N. californicus* consumed significantly more prey than when fed on *T. pyri* alone ($P < 0.01$). During the first 5 days of the experiment *N. californicus*, fed only on *T. urticae* and when fed both *T. urticae* and *T. pyri*, consumed significantly more prey than when fed only with *T. pyri* ($P < 0.01$, for both treatments). *Neoseiulus californicus* consumed 2.2 *T. urticae* for every 1 *T. pyri* when fed on both prey sources.

The mean total egg production, longevity, total prey consumed, prey consumed over the first 5 days of the experiment and the ratio of *T. urticae* consumed compared with *N. californicus* are shown in Table 21, for *T. montdorensis* fed on 16 *T. urticae*, 8 *T. urticae* and 8 *N. californicus*, or 16 *N. californicus*.

Table 21: Mean number of eggs (± SE) laid, longevity, total prey consumed, prey consumed over the first 5 days of the experiment and the ratio of *Tetranychus urticae* consumed compared with *Neoseiulus californicus* for *Typhlodromips montdorensis* (T.m), fed on *Neoseiulus californicus* (N.c) and/or *Tetranychus urticae* (T.u).

T. montdorensis fed on:	Total number of eggs laid (± SE)	Longevity (days ± SE)	Total prey consumed (± SE)	Prey consumed in 1st 5 days (± SE)	Ratio of T.u:N.c consumed
16 T.u	22.4 ± 2.1	15.0 ± 1.6	114 ± 11.2	175 ± 4.9	n/a
8 T.u & 8 N.c	12.4 ± 1.3	8.81 ± 0.6	69.9 ± 4.9	186 ± 2.2	1.04:1
16 N.c	16.7 ± 1.3	10.2 ± 0.7	82.6 ± 6.8	172 ± 6.5	n/a

Typhlodromips montdorensis laid significantly more eggs when fed on *T. urticae* alone than when fed on both *T. urticae* and *N. californicus* ($P < 0.01$). This species also survived significantly longer and consumed significantly more prey when fed exclusively on *T. urticae* than when fed both *T. urticae* and *N. californicus* or when fed only on *N. californicus* ($P < 0.01$, for both treatments). Over the first 5 days of the experiment there were no differences in prey consumption between *T. montdorensis* on each of the prey regimes ($P < 0.05$). *Typhlodromips montdorensis* consumed 1.04 *T. urticae* for every 1 *N. californicus* when fed on both prey sources.

The mean total egg production, longevity, total prey consumed, prey consumed over the first 5 days of the experiment and the ratio of *T. urticae* consumed compared with *N. californicus* are given in Table 22, for *T. pyri* fed on 16 *T. urticae*, 8 *T. urticae* and 8 *N. californicus*, or 16 *N. californicus*.

Table 22: Mean number of eggs (± SE) laid, longevity, total prey consumed, prey consumed over the first 5 days of the experiment and the ratio of *Tetranychus urticae* consumed compared with *Neoseiulus californicus* for *Typhlodromus pyri* (T.p), fed on *Neoseiulus californicus* (N.c) and/or *Tetranychus urticae* (T.u).

T. pyri fed on:	Total number of eggs laid (± SE)	Longevity (days ± SE)	Total prey consumed (± SE)	Prey consumed in 1st 5 days (± SE)	Ratio of T.u:N.c consumed
16 T.u	14.8 ± 1.4	21.5 ± 2.3	125 ± 9.1	187 ± 5.8	n/a
8 T.u & 8 N.c	13.3 ± 1.3	18.4 ± 1.3	87.7 ± 6.5	130 ± 2.4	2.9:1
16 N.c	12.6 ± 1.9	21.3 ± 2.8	57.2 ± 7.2	77 ± 2.6	n/a

There was no difference between the total number of eggs laid and the longevity for *T. pyri* fed on the three prey regimes. Over the entire experiment and during the first 5 days of the experiment, *T. pyri* fed only on *T. urticae* consumed significantly more prey than when feeding on both *T. urticae* and *N. californicus* or on *N. californicus* alone ($P < 0.01$). Similarly, *T. pyri* fed on both *T. urticae* and *N. californicus* consumed significantly more prey than when fed on *N. californicus* alone ($P < 0.01$). *Typhlodromus pyri* consumed 2.9 *T. urticae* for every 1 *N. californicus* when fed on both prey sources.

4.6 Discussion

During the present study the only direct comparisons made between *N. californicus*, *T. montdorensis* and *T. pyri* were when each species was fed solely on *T. urticae*. Direct comparisons between each species during choice tests were deemed unnecessary as the prey source during each choice test was different for each mite species. In a number of experiments the variation between different replicates was higher than could be expected. This was primarily caused by some individuals surviving up to 40 days longer than others. This generally had little effect on prey consumption as individuals surviving for long periods fed very little after about 25 days.

When fed solely on *T. urticae*, *N. californicus* survived longer than *T. montdorensis* and *T. pyri*. Over its lifetime *N. californicus* consumed more prey than *T. montdorensis* or *T. pyri*. However, this result is slightly skewed in favour of *N. californicus* because it survived longer than the other two species. Over the first 5 days of each experiment the highest number of prey consumed was by *T. pyri* and the least by *N. californicus*. *Neoseiulus californicus* consumed approximately 7 *T. urticae* larvae per female per day during this time compared with 8.5 by *T. pyri*. In terms of possible interactions between the two species in the field this small difference is unlikely to have a significant effect. The number of eggs laid per female per day during the first 5 days of the experiment for *N. californicus* and *T. pyri* fed on *T. urticae* was 3.8 and this is comparable to other studies where *N. californicus* laid between 2.37 and 3.81 eggs when preying on tetranychid mites including *T. urticae* (Castagnoli and Simoni, 1991; Friese and Gilstrap, 1982; Ma and Laing, 1973). *Typhlodromips montdorensis* laid 2.8 eggs per day in the present study; Steiner and Goodwin (2002a) found that *T. montdorensis* laid 3.25 eggs per day at 30°C when fed first instar thrips. In the current investigation, if the results are viewed as a population of mites, *N. californicus* was the most and *T. pyri* the least fecund of the three species.

When considering *T. urticae* as the sole prey source the results indicate that, due to its higher fecundity, longevity and prey consumption, *N. californicus* would be more successful as its predator than *T. montdorensis*. However, if these two species were released in tandem, interactions must be considered. The present study shows that *N. californicus* can feed on larval stages of *T. montdorensis*; however, fecundity and longevity is greatly reduced than when fed exclusively on *T. urticae* and prey consumption is lower when *T. montdorensis* is the sole food source. This may be due to *T. montdorensis* being more nutritious than *T. urticae*, but when presented with a mixed diet, *N. californicus* showed a marked preference for *T. urticae*, suggesting that it is its preferred food source. Also, over the first 5 days of the experiment it consumed less prey when fed solely on *T. montdorensis* than when fed a mixed diet or only on *T. urticae*. It is likely that *N. californicus* can develop and reproduce when fed only on *T. montdorensis* for a limited period of time, but if no other prey becomes available, it may lose some of its effectiveness as a biological control agent in the glasshouse. Similarly, *T. montdorensis* is most successful when fed exclusively on *T. urticae*, but in the mixed diet choice experiments, it showed no preference for *T.*

urticae over *N. californicus*. Interestingly, overall performance of *T. montdorensis* was lowest during the choice experiments. This confirms that *T. montdorensis* did not show a strong preference for any particular prey during the experiments.

If both *N. californicus* and *T. montdorensis* were released in the same glasshouse it is likely that they would co-exist. As *N. californicus* shows a preference for *T. urticae* it is likely to feed primarily on this source, but at the beginning and the end of the cropping season, when *T. urticae* is in short supply, it may be able to feed on *T. montdorensis* to maintain its population for a short time. In contrast, *T. montdorensis* shows no preference for *T. urticae* and therefore may feed on whatever it encounters. If this scenario occurs it is likely to be counter-productive for a grower to release both species, as they are more likely to interfere with each other in the control of *T. urticae* rather than enhance control. It would be more beneficial to release a generalist predator such as *N. californicus* and a more specialised species for example *P. persimilis*. The specialist is likely to provide effective short term pest suppression and the generalist will have a greater ability to persist at low prey levels, i.e. at the beginning and the end of a cropping season. However, when releasing both a specialist and a generalist care must be taken, as for example, *N. californicus* preys more on *P. persimilis* than *vice versa* (Schausberger and Walzer, 2001). Also when provided with phytoseiid prey, *P. persimilis* has higher mortality than *N. californicus* (Walzer and Schausberger, 1999) and this may also influence the efficacy of biological control in the glasshouse.

How successfully *N. californicus* and *T. pyri* feed, survive and reproduce on each other in the laboratory may give some indication as to what may happen in the field. In the current work *N. californicus* laid more eggs, lived longer and consumed more prey when fed solely on *T. urticae* than when fed on a mixed diet of *T. urticae* and *T. pyri* or on *T. pyri*. In contrast, *T. pyri* consumed more prey when fed only on *T. urticae*, but there was no difference in fecundity and longevity when fed on a mixed diet of *T. urticae* and *N. californicus* or just on *N. californicus*. When fed both *T. urticae* and *T. pyri*, *N. californicus* showed a preference for feeding on *T. urticae* and *T. pyri* showed an even greater preference to feed on *T. urticae* compared with *N. californicus* when given a mixed diet. Both *N. californicus* and *T. pyri* are able to feed on larval stages of each other. Overall, *N. californicus* is more fecund; however, both

species consume approximately equal numbers of their prey. Therefore if *N. californicus* and *T. pyri* are in close proximity in the field, a large number of other factors will determine whether these species co-exist or one outcompetes the other.

In a study between *T. pyri*, *A. andersoni*, *M. occidentalis* and *N. fallacis*, *T. pyri* had the greatest ability to avoid predation through rapid movement, hiding in confined areas and being able to avoid sustained contact with other species (Croft and Croft, 1996). It is possible that *T. pyri* may be well equipped to avoid *N. californicus*, if the latter is in low numbers. When prey is in short supply, for example late in the season, displacement of *M. occidentalis* by *T. pyri* has been observed, probably due to the greater ability of *T. pyri* to feed on other phytoseiids (Croft and MacRae, 1992). *Neoseiulus californicus* is also able to feed on other phytoseiids and therefore it and *T. pyri* may not feed solely on each other when their primary prey source is in short supply. *Typhlodromus pyri* is more successful when it is cool at the start and end of the season than in warm weather (Croft *et al.*, 1992). *Neoseiulus californicus* originated in California and is also thought to be native to the Mediterranean region and it may therefore be more successful during the summer in the UK. Therefore *N. californicus* and *T. pyri* may 'avoid' each other throughout much of the year in UK orchards, only coming into close contact during cool periods in the summer. However, in France, *N. californicus* searched for prey on trees until November and then again from February and the first generation developed in the field during March and April (Raworth *et al.*, 1994). This is likely to be later in the UK, but *N. californicus* may be able to build up large numbers before *T. pyri*. In contrast, *T. pyri* has been observed to appear on leaves much earlier in orchards in Canada than other phytoseiids (Marshall *et al.*, 2001), although *N. californicus* was not mentioned in the study. There is therefore a need to conduct a study to determine at what time of the year both species will be predominant in UK orchards. Although work in France and Canada may suggest these species will essentially 'avoid' each other, it is more likely that the UK climate will favour both species at similar times of the year. In the UK, three to four generations of *T. pyri* are possible each year (Solomon *et al.*, 2000), however, for *N. californicus* six generations a year are theoretically possible (Hart *et al.*, 2002a). Therefore both species are able to reproduce well in the UK climate. When fed *T. urticae* or *P. ulmi*, *N. californicus* develops faster from egg to adult than

T. pyri (Jolly, 2000), and this, in combination with higher voltinism, may give *N. californicus* a slight advantage over *T. pyri*.

Early and late in the season when prey is not as widely available as during the summer months, the ability of *T. pyri* and *N. californicus* to exploit other prey sources may have an influence on how they interact. *Typhlodromus pyri* can show a clear preference for certain food sources; for example, it prefers *P. ulmi* to apple rust mite, *Aculus schlechtendali* (Nalepa) (Acarina: Eriophyidae) (Dicke, 1988; Dicke and Dejong, 1988), despite development being quicker and reproductive capacity higher on the latter (Dicke *et al.*, 1990). It has been well documented that *N. californicus* (Castagnoli *et al.*, 1999; Castagnoli *et al.*, 2001; Raworth *et al.*, 1994) and *T. pyri* (Croft *et al.*, 1992; Dosse, 1961; Walde *et al.*, 1992) can feed on pollen and during the present study both species fed on cattail pollen in culture. Both species are likely to be able to exploit alternative food sources in the field.

Movement throughout an orchard will also have an influence on how *N. californicus* and *T. pyri* interact. When *T. pyri* was released in an orchard it became the dominant predator and fed on *P. ulmi* and *T. urticae*, but moved slowly through the orchard (4 years to move 84 m) (Marshall *et al.*, 2001). It is unknown how quickly *N. californicus* will spread throughout orchards, but it has been suggested that in France it is able to migrate to trees from release sites equally well over bare ground as from shrubs to adjacent tree rows (Raworth *et al.*, 1994). It is likely *N. californicus* and *T. pyri* will be able to exist in tandem in a large scale environment, for example, in an orchard. On a smaller scale, such as in individual trees, competitive exclusion may occur (Zhang and Croft, 1995). If one of the species has a strong competitive advantage then, over a long time scale it may begin to outcompete the other predator on a large scale and at more regular intervals.

There are a large number of predatory mite species present in UK orchards. A recent five year survey revealed *T. pyri* was the most abundant, but *Phytoseiulus macropilis* (Banks) (Acari: Phytoseiidae) and *E. finlandicus* were also common and *A. andersoni*, *Paraseiulus soleiger* (Ribaga) (Acari: Phytoseiidae) and *K. aberrans* were also locally abundant (Fitzgerald and Solomon, 2002). It is possible that the next long term survey will reveal *N. californicus* as an abundant species; however, whether it will report

significantly reduced numbers of *T. pyri* remains to be seen. Additionally, pesticide usage will continue to have an impact on mite populations in orchards. *Typhlodromus pyri* is known to be resistant to commonly used organophosphorus insecticides in orchards (Kapetanakis and Cranham, 1983) and accordingly has been used extensively in IPM (Solomon *et al.*, 1993). *Neoseiulus californicus* may not be resistant to pesticides and this in the short term may prevent *N. californicus* spreading beyond orchards that do not use pesticides. It remains hard to predict what will happen between *N. californicus* and *T. pyri* in the field until extensive studies have been conducted. It is more likely, that due to the abundance of phytoseiid mites in UK orchards, all species may be able to co-exist to a certain extent and that no species will become extinct from competition.

In conclusion, the present study has demonstrated that *N. californicus* can feed on *T. montdorensis* and *T. pyri* and both *T. montdorensis* and *T. pyri* can feed on *N. californicus*. Oviposition is possible for each mite species when fed solely on their respective phytoseiid prey, but it seems that both *N. californicus* and *T. pyri* can only sustain a population on their respective phytoseiid prey for a short time. *Typhlodromips montdorensis* does not have a preference for *T. urticae* over *N. californicus*, but a balanced diet is likely to optimise its performance in the glasshouse. *Neoseiulus californicus* is established in parts of the UK and will inevitably encounter native *T. pyri* in UK orchards. Regular field surveys will prove vital to monitoring the spread of *N. californicus* and to determine whether *T. pyri* remains the most common species of phytoseiid mite in UK orchards.

Chapter 5: General Discussion

This general discussion will be divided into four sections discussing, respectively, the establishment potential of *T. montdorensis*, the value of thermal data as a screen for establishment, wider issues concerning risk assessments, and practicalities of risk assessment for the biological control industry.

5.1 Establishment potential of *Typhlodromips montdorensis* in the UK

Since the establishment of *N. californicus* (Jolly, 2000) in the south-east and west of England and the possible establishment of *M. caliginosus* outside glasshouses (Hart *et al.*, 2002b), DEFRA has taken a more precautionary approach before granting licences for the release of non-native biological control agents in the UK. In the light of interest in the use of *T. montdorensis* as a biological control agent in the UK it was decided to investigate the outdoor establishment potential of this non-native predatory mite.

In the current study, estimates of developmental threshold and voltinism indicate that from 1986-2000 an average of six generations would be possible per year in the Midlands region of the UK. Development of *T. montdorensis* is likely to be confined to periods between April and September of each year. Five or six generations may be possible during this period. Between October and March, no complete generation is thought possible and therefore, for *T. montdorensis* to be able to survive the winter, it must remain in a sheltered location or cold harden, and survive until it can develop and complete a generation. From 1986-2000 temperatures above the developmental threshold of *T. montdorensis* were reached only between mid April and the end of September. Therefore, *T. montdorensis* would have to survive six months of the year at temperatures below its developmental threshold and some exposures to temperatures in the range of -5 to -10°C. In order to test the likelihood of such survival, studies on its thermal biology were conducted.

Studies on SCPs concluded that *T. montdorensis* was a freeze intolerant species and was susceptible to pre-freeze mortality with no survival at temperatures above the

SCP. When *T. montdorensis* was exposed to low temperatures for a short period of time high mortality was recorded at sub-zero temperatures. However, these temperatures were relatively low and would not be experienced on a regular basis during a UK winter. Therefore *T. montdorensis* was held at -5, 0 and 5°C as these temperatures, particularly 0 and 5°C, were considered to be more common during a UK winter. Mortality was high over relatively short periods of time, with maximum survival of 90% for an acclimated larval population of only 18 days at 5°C. This result was encouraging with regard to the hypothesis that winter survival of *T. montdorensis* would be limited by its lack of cold tolerance. To further test this, winter field survival and reproduction trials were conducted and the ability to enter diapause was investigated.

Work on laboratory cultures of *T. montdorensis* showed that no individuals of the current strain entered diapause under the regimes tested. The experiments on laboratory development, cold hardiness and diapause suggested that *T. montdorensis* might not be able to overwinter outside the glasshouse in the UK.

The two long-term field experiments showed that the maximum survival time of *T. montdorensis* was 35 days with or without the provision of prey. During mid-winter 100% mortality was reached after 14 days and egg mortality was 100% after 7 days. If mean temperatures were below 3°C with some sub-zero exposure, 100% of *T. montdorensis* died within 7 days, which could be expected as survival at 5°C in the laboratory rarely exceeded 10 days. Therefore, although some individuals survived for 35 days in early winter, low temperatures in mid-winter would cause 100% mortality. Survival was not increased by acclimation and no viable eggs were laid in the field throughout the winter. The limited amount of time *T. montdorensis* survived in the field also suggested that re-colonisation into the glasshouse after primary escape during the winter would be unlikely.

Comparisons between the pre-release studies conducted on *T. montdorensis* and data on *N. californicus* obtained post-release in the UK suggest that *N. californicus* is more cold tolerant than *T. montdorensis*. Additionally, *N. californicus* could theoretically complete a generation during a UK winter; some field populations survived for up to

three months and the UK strain could enter diapause (Hart *et al.*, 2002a). Based on the data collected in the current investigation it is concluded that *T. montdorensis* is a 'safe' candidate for release into UK glasshouses as it does not seem to be able to overwinter.

5.2 Value of thermal data as a screen for establishment

The protocol for screening of non-native biological control agents has now been used on five species. Of these, work on *N. californicus* (Hart *et al.*, 2002a), *M. caliginosus* (Hart *et al.*, 2002b), *D. catalinae* (Tullett, unpublished data) and *E. eremicus* (Tullett *et al.*, 2004) was done post-release and work on *T. montdorensis* (Hatherly *et al.*, 2004) was done prior to the licensing of this species. *Neoseiulus californicus* and *M. caliginosus* were commonly used for glasshouse biological control, whereas *D. catalinae* and *E. eremicus* were less widely used, although the latter has recently been used more frequently. The data obtained suggests that *N. californicus* and *M. caliginosus* may be able to overwinter outside the glasshouse in the UK whereas the other three species will die out early in winter. The various laboratory indices of cold tolerance were related to survival times in the field to identify correlative relationships. The $LTime_{50}$ at 5ºC was found to be strongly correlated with field survival when fitted with a simple linear regression (Figure 20).

Another interesting technique that could be used to predict whether the climate in a given area may favour the establishment of non-native biological control agents is the use of climate envelopes. Emprical models can be used to identify important climatic variables that describe the range of an insect species. These variables are often described as the 'climate envelope' in which the insect can live (Lindsey and Bayoh, 2004). They have been successfully used to map distributions of butterflies (Hill *et al.*, 2002) and mosquitoes (Bayoh *et al.*, 2001; Lindsay and Bayoh 2004) By using climatic data of known distribution ranges of non-native biological control agents it may be possible to map their distribution if they were introduced into the UK. Although this meathod may prove difficult for species with a large number of different strains used for biological control it may be possible to investigate its use for some non-native biological control agents and use this system in tandem with the

protocol described in this study to predict establishement potential of non-native biological control agents.

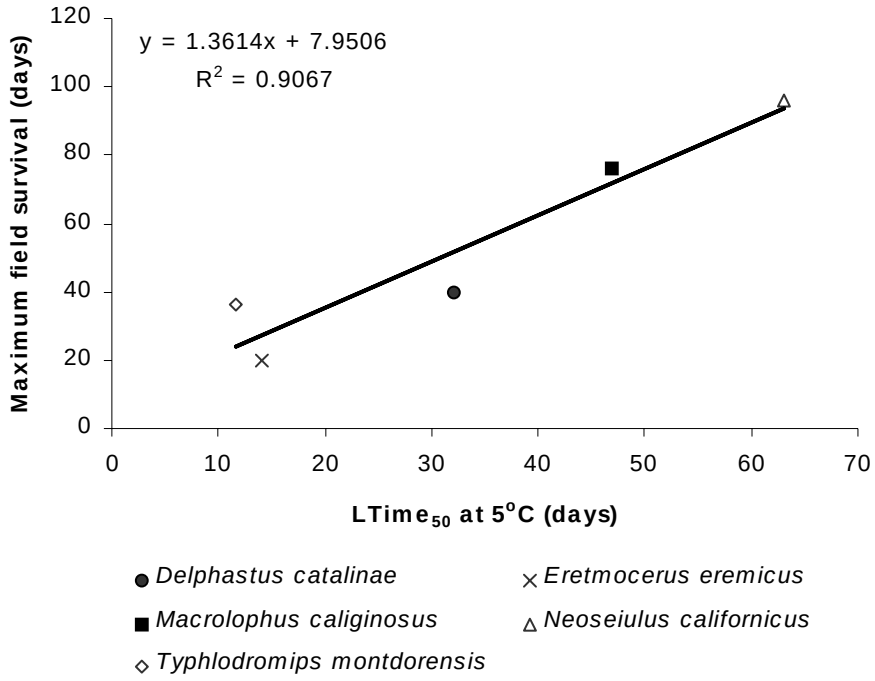


Figure 20: Relationship between maximum field survival (days) and $LTime_{50}$ at 5ºC (days) with a simple ($R^2 = 0.9067$) linear regression, y = 1.3614x + 7.9506 for five non-native biological control agents.

As $LTime_{50}$ at 5ºC increases, field survival also increases. *Neoseiulus californicus* had the longest $LTime_{50}$ with populations surviving for up to three months in the field. Additionally, the mites reproduced before dying and diapause strains are able to survive throughout the winter (Jolly, 2000). In contrast, *T. montdorensis* have low $LTime_{50}$ and field survival is correspondingly short. The survival time of *M. caliginosus* lies between that of *D. catalinae* and *N. californicus*. The analysis suggests that laboratory assessment of the $LTime_{50}$ at 5ºC may be a reliable predictor of field survival. If this proves to be a consistent relationship, it would benefit biological control companies that have limited research and development (R and D) budgets, as conducting expensive and time consuming field experiments could be avoided. Consequently, the costs of preparing an environmental risk assessment dossier as part of a licence application would be reduced. A similar relationship between laboratory assessment of cold tolerance and field survival has also been found for a number of crop pest species (Bale and Walters, 2001), suggesting that this

may be a robust system of predicting establishment potential. However, care should be taken when dealing with insect species that are known to enter diapause as the relationship outlined in Figure 20 may not hold true in species that can increase their cold tolerance by entering a diapause state. It is likely that for such species, field experiments would still have to be conducted by biological control companies and the laboratory prediction of field survival could only be used for species thought not to diapause for example *T. montdorensis*.

In summary, an analysis across five species of non-native biological control agents has identified a correlation between the duration of survival in the laboratory at 5ºC and survival in the field in winter. This relationship could be routinely incorporated into the risk assessment protocol conducted by biological control companies as part of the licensing system, and has the advantage of focusing limited R and D budget expenditure on the critical factors that determine establishment success.

5.2.1 Screening process for alternate prey

The establishment of non-native species outside glasshouses can be viewed as a two-stage process. If abiotic factors such as temperature do not allow a species to survive the winter then biotic factors that may be involved in the establishment do not need to be investigated. This would not apply if it were believed that non-native biological control agents that could not overwinter, but could temporarily establish during the summer could still feed and would cause substantial damage during a single cropping season. As a non-native species that can not overwinter will only cause a maximum of one season's damage, it is more feasible to invest resources in studying non-native species that can overwinter and determine whether they can exploit alternate food sources. Therefore, if abiotic factors do not prevent survival through winter, a screening process to determine alternative prey sources for the biological control agent would be of value.

Preliminary studies were conducted during the current investigation on the use of alternate prey by *M. caliginosus*. It is reported to be found outside glasshouses in the UK (Hart *et al.*, 2002b) and therefore abiotic factors may not be causing complete extinction of escaped populations. For this reason, its performance on its primary

glasshouse prey *T. vaporariorum* was compared with that on two alternative prey sources, *M. persicae* and *A. proletella*. It was found that *M. caliginosus* could sustain a population on *M. persicae* and could complete a generation on *A. proletella*, although performance on *A. proletella* was significantly lower than on *T. vaporariorum* and *M. persicae* for reasons discussed in Chapter 3. The present study concluded that *M. caliginosus* was likely to be able to feed on a number of alternative prey sources outside the glasshouse in the UK. Previous studies concluded that *M. caliginosus* was a highly polyphagous species (Enkegaard *et al.*, 2001; Fauvel *et al.*, 1987; Malausa and Trottin-Caudal, 1996) and this supports the view of the current work that *M. caliginosus* is likely to find a food source all year round outside the glasshouse. This work will be continued in more detail as part of a project funded by DEFRA on the use of alternate prey by non-native biological control agents due to start in October 2004 at the University of Birmingham.

Once it has been determined whether a species can use alternate prey outside the glasshouse, it is important to assess the status (i.e. absent, only present during the summer or present all year round) of the non-native species outside the glasshouse. This has been done with *N. californicus*. Sampling between 1997 and 2000 revealed a number of sites where *N. californicus* was present outside the glasshouse and the native *T. pyri* was also found at most of these sites (Jolly, 2000). As *T. pyri* is the most common species in UK orchards and successfully controls *P. ulmi* (Fitzgerald and Solomon, 2002) it was considered important to see how *N. californicus* and *T. pyri* may interact when they come into close proximity in the field. The present study revealed that both *N. californicus* and *T. pyri* could feed on larval stages of the other. However, if presented with a mixed diet, both species showed a preference for *T. urticae* over their phytoseiid prey. *Neoseiulus californicus* was more fecund and lived longer than *T. pyri*, giving the former a possible advantage in the field. Nevertheless, the large number of mites present in UK orchards and the generalist nature of *N. californicus* and *T. pyri* will hopefully mean that the two species will not feed specifically on each other. However, to draw any firm conclusions on interactions between the two species, field surveys need to be conducted and areas where both species occur need to be monitored closely.

In addition to the work on *N. californicus* and *T. pyri* together, a similar study was conducted with *N. californicus* and *T. montdorensis*. If a licence is granted for the release of *T. montdorensis*, growers may decide to use it and *N. californicus* at the same time in their glasshouses. The present study revealed that both species could feed on larvae of the other. *Neoseiulus californicus* had a preference for *T. urticae* over *T. montdorensis*, but *T. montdorensis* showed no such preference. As both are generalist species, it is unlikely to be beneficial to release them both at the same time. In the long term, as this study showed that *N. californicus* is more fecund than *T. montdorensis*, growers are more likely to release the former in the hope that it will quickly produce a sustainable population to control *T. urticae* infestations.

5.3 Wider issues concerning risk assessments

There are current moves to harmonise the legislation that surrounds the release of non-native biological control agents in the EU. In 1998 an EU funded research project on 'Evaluating Environmental Risks of Biological Control Introductions into Europe' (ERBIC) was undertaken. It aimed to review and examine the environmental impact of past biological control introductions and to produce guidelines for further introductions in Europe (Lynch and Thomas, 2000; van Lenteren *et al.*, 2003). More recently there has been a workshop on 'Harmonisation of the regulation of invertebrate biological agents (IBCAs) in Europe' in Zurich, Switzerland. The meeting aimed to combine guidance documents produced by both the Economic Cooperation and Development (OECD) and the European and Mediterranean Plant Protection Organisation (EPPO) into one set of guidelines that would be easy to apply in all European countries. Countries such as the UK and Norway have extensive regulation for the introduction of non-native species whereas Greece for example, has no such regulation in place. In the north of Europe, for example in Norway, Sweden and the UK, most biological control occurs within glasshouses and the outdoor establishment of a non-native species is considered undesirable. In Mediterranean countries, such as Spain and Italy, biological control is conducted both outdoors and within glasshouses, but establishment of glasshouse agents outdoors is still considered to be undesirable. There are obviously different climatic and regulatory attitudes between different countries, but it is hoped that a set of guidelines can be produced that will satisfy all countries concerned. Clearly, there must still be some country-

specific regulation. For example, as *T. montdorensis* is unlikely to establish outside UK glasshouses, it is feasible that countries such as Sweden and Norway with colder winters than the UK will be prepared to release *T. montdorensis*. However, central and southern European countries may be reluctant to allow the release of species such as *T. montdorensis* until they have conducted their own research.

Worldwide, there are even more complicated issues. For example, the data on *T. montdorensis* may suggest that it would be a safe species to release in Canada due to the cold Canadian winters preventing establishment outside the glasshouse. However, Canada exports 80% of its glasshouse crops, mainly to the USA (Gillespie, pers comm), hence *T. montdorensis* would then be likely to be shipped to other countries on contaminated plant products. In contrast, exports of glasshouse crops from the UK are much lower thus there would be less chance of *T. montdorensis* being 'exported'. Globalisation and pest invasion, for example an increase in ship and flight containers, are likely to increase such problems (Loope and Howarth, 2002). When taking issues such as exports into account, care must be taken as there is a risk that the use of biological control agents will be reduced because of concern over possible spread to other countries. This might lead to an increase in the use of other pest control methods such as chemical pesticides. Overall, it may be more beneficial to risk the establishment of some non-native species and in turn reduce the use of pesticides. If a non-native species can potentially establish in a country, it is important to conduct cost benefit analyses, as this may reveal that the benefit to the horticulture industry and the country as a whole outweighs the environmental costs and risks, and therefore a licence for its release may still be granted. Ultimately, decisions on licensing are largely dependent on the individual governmental organisations responsible for the regulation of non-native species in each country.

5.4 Practicalities of risk assessment

A number of further issues should briefly be mentioned when considering the practicalities of risk assessment. For example, if DEFRA grants a licence to a biological control company, who is responsible if an introduction subsequently causes environmental problems? If the environmental risk assessment protocol is approved and the biological control company does all that is required, then it is unlikely they

will accept responsibility for any post-release problems. The most probable scenario is that a research institute or university would carry out the original research and would then report to DEFRA. If a licence were then granted, it would be important that DEFRA ensures that biological control companies submit regular reports on the numbers of each agent that are released and conduct field surveys to determine whether a species was establishing and spreading away from the glasshouse release sites.

A further issue to consider is the cost of the R and D required to prepare an environmental risk assessment dossier as part of a licence application. Small biological control companies argue that they have limited R and D budgets that are inadequate to conduct comprehensive research on every potential biological control agent under consideration for release. Ultimately, they may feel that stringent guidelines on the introduction of non-native species into the UK may render the biological control industry financially unviable. A case can be made for a government agency to fund research on 'environmentally friendly' pest control. However, in this case, all of the information would need to be shared with all producers of biological control agents. However, if a company funds the research necessary to submit a licence application, there may need to be a system to protect the initial investment of the company, perhaps by a period of exclusive use of the biological control agent, for example for five years.

Much time and resources are currently being invested in producing guidelines for the release of non-native species across Europe. Governments, research scientists and industry are all involved in this harmonisation process and it is hoped that in the next few years a solution will be found that suits all parties concerned.

6 References

ACRE. (2001). Applications from Syngenta Bioline to release *Amblyseius montdorensis* and *Eretmocerus eremicus* for use as glasshouse biocontrol agents. Department for Environment, Food & Rural Affairs, London.

Albajes, R., and Alomar, O. (1999). Current and potential use of polyphagous predators. *In* "Integrated pest and disease management in greenhouse crops" (R. Albajes, M. L. Gullino, J. C. van Lenteren, and Y. Elad, Eds.), pp. 265-275. Kluwer Academic Publishers, Dordrecht.

Bale, J. S. (1987). Insect cold hardiness - freezing and supercooling - an ecophysiological perspective. *Journal of Insect Physiology*, **33,** 899-908.

Bale, J. S. (1989). Cold hardiness and overwintering of Insects. *Agricultural Zoology Reviews*, **3,** 157-192.

Bale, J. S. (1991). Insects at low-temperature - a predictable relationship. *Functional Ecology*, **5,** 291-298.

Bale, J. S. (1993). Classes of insect cold-hardiness. *Functional Ecology*, **7,** 751-753.

Bale, J. S. (1996). Insect cold hardiness: A matter of life and death. *European Journal of Entomology*, **93,** 369-382.

Bale, J. S. (2002). Insects and low temperatures: from molecular biology to distributions and abundance. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, **357,** 849-861.

Bale, J. S., Harrington, R., and Clough, M. S. (1988). Low temperature mortality of the peach-potato aphid *Myzus persicae*. *Ecological Entomology*, **13,** 121-129.

Bale, J. S., Masters, G. J., Hodkinson, I. D., Awmack, C., Bezemer, T. M., Brown, V. K., Butterfield, J., Buse, A., Coulson, J. C., Farrar, J., Good, J. E. G., Harrington, R., Hartley, S., Jones, T. H., Lindroth, R. L., Press, M. C., Symrnioudis, I., Watt, A. D., and Whittaker, J. B. (2002). Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology*, **8,** 1-16.

Bale, J. S., Odoherty, R., Atkinson, H. J., and Stevenson, R. A. (1984). An automatic thermoelectric cooling method and computer-based recording-system for supercooling point studies on small invertebrates. *Cryobiology*, **21,** 340-347.

Bale, J. S., and Walters, K. F. A. (1997). Testing procedures for determining the overwintering potential of alien pests, pp. 55.

Bale, J. S., and Walters, K. F. A. (2001). Overwintering biology as a guide to the establishment potential of non-native arthropods in the UK (Chap 18, 343-354). *In* "Environment and Animal Development. Genes, Life Histories and Plasticity" (D. Atkinson and M. Thorndyke, Eds.), pp. 358. BIOS Scientific Publishers Ltd, Oxford, UK.

Barratt, B. I. P., Evans, A. A., Ferguson, C. M., Barker, G. M., McNeill, M. R., and Phillips, C. B. (1997). Laboratory nontarget host range of the introduced parasitoids *Microctonus aethiopoides* and *M. hyperodae* (Hymenoptera: Braconidae) compared with field parasitism in New Zealand. *Environmental Entomology*, **26,** 694-702.

Barratt, B. I. P., Ferguson, C. M., Goldson, S. L., Phillips, C. M., and Hannah, D. J. (1999). Predicting the risk from biological control agents introduction: a New Zealand approach. *In* "Nontarget effects of insect biological control" (J. J. Duan, Ed.), pp. 59-75. Kluwer Academic Publishers, London.

Barrett, J. (2001). Thermal hysteresis proteins. *International Journal of Biochemistry & Cell Biology*, **33,** 105-117.

Baust, J. G. (1973). Mechanisms of cryoprotection in freezing tolerant animal systems. *Cryobiology*, **10,** 197-205.

Baust, J. G. (1982). Environmental triggers to cold hardening. *Comparative Biochemistry and Physiology*, **73A,** 563-570.

Baust, J. G., and Rojas, R. R. (1985). Insect Cold Hardiness - Facts and Fancy. *Journal of Insect Physiology*, **31,** 755-759.

Bayoh, M. N., Thomas C.J., and Lindsay S.W. (2001). Mapping distributions of chromosomal forms of *Anopheles gambiae* in West Africa using climate data. *Medical and Veterinary Entomology*, **15**(3): 267-274.

Beard, J. J. (2001). A review of Australian *Neoseiulus* Hughes and *Typhlodromips* de Leon (Acari : Phytoseiidae : Amblyseiinae). *Invertebrate Taxonomy*, **15,** 73-158.

Beirne, B. P. (1985). Avoidable obstacles to colonization in classical biological - control of insects. *Canadian Journal of Zoology - Revue Canadienne De Zoologie*, **63,** 743-747.

Bennett, F. D. (1993). Do introduced parasitoids displace native ones. *Florida Entomologist*, **76,** 54-63.

Blommers, L. H. M. (1994). Integrated pest management in European apple orchards. *Annual Review of Entomology*, **39,** 213-241.

Blum, B., Ehlers, R., Haukeland-Salinas, S., Hokkanen, H., Jung, K., Kuhlmann, U., Menzler-Hokkanen, I., Ravensberg, W., Strasser, H., Warrior, P., and Wilson, M. (2003). Biological control agents: Safety and regulatory policy. *Biocontrol*, **48,** 477-484.

Bonde, J. (1989). Biological studies including population-growth parameters of the predatory mite *Amblyseius barkeri* Acarina, Phytoseiidae at 25-degrees-C in the Laboratory. *Entomophaga*, **34,** 275-287.

Broufas, G. D., and Koveos, D. S. (2000). Effect of different pollens on development, survivorship and reproduction of *Euseius finlandicus* (Acari : Phytoseiidae). *Environmental Entomology*, **29,** 743-749.

Broufas, G. D., Koveos, D. S., and Georgatsis, D. I. (2002). Overwintering sites and winter mortality of *Euseius finlandicus* (Acari : Phytoseiidae) in a peach orchard in northern Greece. *Experimental and Applied Acarology*, **26,** 1-12.

Caltagirone, L. E. (1981). Landmark examples in classical biological control. *Annual Review of Entomology*, **26,** 213-232.

Campbell, A., Frazer, B. D., Gilbert, N., Gutierrez, A. P., and Mackauer, M. (1974). Temperature requirements of some aphids and their parasites. *Journal of Applied Ecology*, **11,** 431-438.

Cannon, R. J. C. (1987). Effects of low-temperature acclimation on the survival and cold tolerance of an Antarctic mite. *Journal of Insect Physiology*, **33,** 509-521.

Carruthers, R. I., and Onsager, J. A. (1993). Perspective on the use of exotic natural enemies for biological-control of pest grasshoppers (Orthoptera, Acrididae). *Environmental Entomology*, **22,** 885-903.

Castagnoli, M., and Simoni, S. (1991). Influence of temperature on population increase of *Amblyseius californicus* (McGregor) (Acarina: Phytoseiidae). *Redia*, **74,** 621-640.

Castagnoli, M., and Simoni, S. (1999). Effect of long-term feeding history on functional and numerical response of *Neoseiulus californicus* (Acari : Phytoseiidae). *Experimental and Applied Acarology*, **23,** 217-234.

Castagnoli, M., Simoni, S., and Biliotti, N. (1999). Mass rearing of *Amblyseius californicus* (Acari: Phytoseiisae) on two alternative food sources. *In* "Ecology and Evolution of the Acari" (J. Bruin, L. P. S. van der Geest, and M. W. Sabelis, Eds.), pp. 425-431. Kluwer Academic Publishers, Netherlands.

Castagnoli, M., Simoni, S., and Nachman, G. (2001). Short-term changes in consumption and oviposition rates of *Neoseiulus californicus* strains (Acari : Phytoseiidae) after a diet shift. *Experimental and Applied Acarology*, **25,** 969-983.

Castane, C., Alomar, O., Goula, M., and Gabarra, R. (2004). Colonization of tomato greenhouses by the predatory mirid bugs *Macrolophus caliginosus* and *Dicyphus tamaninii*. *Biological Control*, **30,** 591-597.

Caughley, G., and Gunn, A. (1996). "Conservation biology in theory and practice." Blackwell Science, Oxford.

Chant, D. A. (1959). Phytoseiid mites (Acarina: Phytoseiidae). Part I. Bionomics of seven species in southeastern England. *Canadian Entomologist*, **12 (Suppl.),** 1-166.

Chant, D. A. (1985). Systematics and Taxonomy. *In* "World crop pests: Spider mites: their biology, natural enemies and control." (M. W. Sabelis, Ed.), Vol. 1B, pp. 3-32. Elsevier Science Publishers.

Chen, C. P., Denlinger, D. L., and Lee, R. E. (1991). Seasonal-variation in generation time, diapause and cold hardiness in a central Ohio population of the flesh fly, *Sarcophaga bullata*. *Ecological Entomology*, **16,** 155-162.

Chen, C. P., Lee, R. E., and Denlinger, D. L. (1990). A comparison of the responses of tropical and temperate flies (Diptera, Sarcophagidae) to cold and heat-stress. *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology*, **160,** 543-547.

Chen, C. P., and Walker, V. K. (1994). Cold-shock and chilling tolerance in drosophila. *Journal of Insect Physiology*, **40,** 661-669.

Clarke, B., Murray, J., and Johnson, M. S. (1984). The extinction of endemic species by a program of biological - control. *Pacific Science*, **38,** 97-104.

Constant, B., Grenier, S., and Bonnot, G. (1996). Artificial substrate for egg laying and embryonic development by the predatory bug *Macrolophus caliginosus* (Heteroptera: Miridae). *Biological Control*, **7,** 140-147.

Cory, J. S., and Myers, J. H. (2000). Direct and indirect ecological effects of biological control. *Trends in Ecology & Evolution*, **15,** 137-139.

Coulson, S. J., and Bale, J. S. (1990). Characterization and limitations of the rapid cold-hardening response in the housefly *Musca domestica* (Diptera, Muscidae). *Journal of Insect Physiology*, **36,** 207-211.

Coulson, S. J., Leinaas, H. P., Ims, R. A., and Sovik, G. (2000). Experimental manipulation of the winter surface ice layer: the effects on a high Arctic soil microarthropod community. *Ecography*, **23,** 299-306.

Croft, B. A., and Croft, M. B. (1993). Larval survival and feeding by immature *Metaseiulus occidentalis, Neoseiulus fallacis, Amblyseius andersoni* and *Typhlodromus pyri* on life stage groups of *Tetranychus urticae* Koch and phytoseiid larvae. *Experimental & Applied Acarology*, **17,** 685-693.

Croft, B. A., and Croft, M. B. (1996). Intra- and interspecific predation among adult female phytoseiid mites (Acari: Phytoseiidae): Effects on survival and reproduction. *Environmental Entomology*, **25,** 853-858.

Croft, B. A., Kim, S. S., and Kim, D. I. (1996). Intra- and interspecific predation on four life stage groups by the adult females of *Metaseiulus occidentalis*, *Typhlodromus pyri*, *Neoseiulus fallacis* and *Amblyseius andersoni*. *Experimental & Applied Acarology*, **20,** 435-444.

Croft, B. A., and MacRae, I. V. (1992). Biological control of apple mites by mixed populations of *Metaseiulus occidentalis* (Nesbitt) and *Typhlodromus pyri* (Scheuten) (Acari, Phytoseiidae). *Environmental Entomology*, **21,** 202-209.

Croft, B. A., MacRae, I. V., and Currans, K. G. (1992). Factors affecting biological control of apple mites by mixed populations of *Metaseiulus occidentalis* and *Typhlodromus pyri*. *Experimental & Applied Acarology*, **14,** 343-355.

Davey, M. C., Pickup, J., and Block, W. (1992). Temperature-variation and its biological significance in fellfield habitats on a maritime Antarctic island. *Antarctic Science*, **4,** 383-388.

Delfosse, E. S. (1988). *Echium* appeal won by CSIRO. *News Bulletin of the Australian Entomological Society*, **24,** 149-152.

Denlinger, D. L. (1986). Dormancy in tropical insects. *Annual Review of Entomology*, **31,** 239-264.

Denlinger, D. L. (1991). Relationships between cold hardiness and diapause. *In* "Insects at low temperature" (R. E. Lee and D. L. Denlinger, Eds.), pp. 174-199 (Chapter 8). Chapman and Hall.

Denlinger, D. L. (2002). Regulation of diapause. *Annual Review of Entomology*, **47,** 93-122.

Dent, D. (2000). "Integrated Pest Management." Chapman & Hall, UK,

DETR. (1997). The regulation and control of the release of non-native animals and plants into the wild in Great Britain., pp. 36. DETR, London.

DeVries, A. L. (1971). Glycoproteins as biological antifreeze agents in antarctic fishes. *Science*, **172,** 1152-55.

DeVries, A. L. (1986). Antifreeze glycopeptides and peptides: Interactions with ice and water. *Methods Enzymology*, **127,** 293-303.

Dicke, M. (1988). Prey preference of the Phytoseiid mite *Typhlodromus pyri* .1. Response to volatile kairomones. *Experimental & Applied Acarology*, **4,** 1-13.

Dicke, M., and Dejong, M. (1988). Prey preference of the Phytoseiid mite *Typhlodromus pyri* .2. electrophoretic diet analysis. *Experimental & Applied Acarology*, **4,** 15-25.

Dicke, M., Sabelis, M. W., Dejong, M., and Alers, M. P. T. (1990). Do phytoseiid mites select the best prey species in terms of reproductive success. *Experimental & Applied Acarology*, **8,** 161-173.

Dosse, G. (1959). Ueber einige neue Raubmilbenarten (Phytoseiidae). *Planzenschutzberichte*, **21,** 44-61.

Dosse, G. (1961). Ueber die bedeutung der pollenahrung fuer *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae). *Entomologia Experimentalis et Applicata*, **4,** 191-195.

Draper, N. R., and Smith, H. (1981). "Applied regression analysis." John Wiley & Sons, Inc.,

Duman, J. G. (1982). Insect antifreezes and ice nucleating agents. *Cryobiology*, **19,** 613-627.

Duman, J. G. (1984). Change in overwintering mechanism of the Cucujid beetle, *Cucujus clavipes*. *Journal of Insect Physiology*, **30,** 235-239.

Duman, J. G. (2001). Antifreeze and ice nucleator proteins in terrestrial arthropods. *Annual Review of Physiology*, **63,** 327-357.

Duman, J. G., and Horwath, K. L. (1983). The role of haemolymph proteins in the cold tolerance of insects. *Annual Review of Physiology*, **45,** 261-270.

Duso, C., and Camporese, P. (1991). Developmental times and oviposition rates of predatory mites *Typhlodromus pyri* and *Amblyseius andersoni* (Acari, Phytoseiidae) reared on different foods. *Experimental & Applied Acarology*, **13,** 117-128.

Easton, C. M., and Horwath, K. L. (1994). Characterization of primary-cell cultures derived from fat-body of the beetle, *Tenebrio molitor*, and the immunolocalization of a thermal hysteresis protein in-vitro. *Journal of Insect Physiology*, **40,** 537-547.

Ehler, L. E. (1998). Invasion biology and biological control. *Biological Control*, **13,** 127-133.

Ehler, L. E. (1999). Critical issues related to nontarget effects of classical control of insects. *In* "Nontarget effects of biological control" (J. J. Duan, Ed.), pp. 3-13. Kluwer Academic Publishers, London.

Elliott, N., Kieckhefer, R., and Kauffman, W. (1996). Effects of an invading coccinellid on native coccinellids in an agricultural landscape. *Oecologia*, **105,** 537-544.

Enkegaard, A., Brodsgaard, H. F., and Hansen, D. L. (2001). *Macrolophus caliginosus*: functional response to whiteflies and preference and switching capacity between whiteflies and spider mites. *Entomologia Experimentalis et Applicata*, **101,** 81-88.

Fauvel, G., Malausa, J. C., and Kaspar, B. (1987). Laboratory studies on the main biological characteristics of *Macrolophus caliginosus* (Heteroptera, Miridae). *Entomophaga*, **32,** 529-543.

Ferran, A., Rortais, A., Malausa, J. C., Gambier, J., and Lambin, M. (1996). Ovipositional behaviour of *Macrolophus caliginosus* (Heteroptera: Miridae) on tobacco leaves. *Bulletin of Entomological Research*, **86,** 123-128.

Fields, P. G., Fleurat-Lessard, F., Lavenseau, L., Febvay, G., Peypelut, L., and Bonnot, G. (1998). The effect of cold acclimation and deacclimation on cold tolerance, trehalose and free amino acid levels in *Sitophilus granarius* and *Cryptolestes ferrugineus* (Coleoptera). *Journal of Insect Physiology*, **44,** 955-965.

Finney, D. J. (1971). "Probit analysis." University Press, Cambridge.

Fitzgerald, J. D., and Solomon, M. G. (1991). Diapause induction and duration in the Phytoseiid mite *Typhlodromus pyri*. *Experimental & Applied Acarology*, **12,** 135-145.

Fitzgerald, J. D., and Solomon, M. G. (2002). Distribution of predatory phytoseiid mites in commercial cider apple orchards and unsprayed apple trees in the UK: Implications for biocontrol of phytophagous mites. *International Journal of Acarology*, **28,** 181-186.

Foglar, H., Malausa, J. C., and Wajnberg, E. (1990). The functional-response and preference of *Macrolophus caliginosus* Heteroptera, Miridae for 2 of its prey - *Myzus persicae* and *Tetranychus urticae*. *Entomophaga*, **35,** 465-474.

Follett, P. A., Duan, J., Messing, R. H., and Jones, V. P. (2000). Parasitoid drift after biological control introductions: re-examining pandora's box. *American Entomologist*, **46,** 82-94.

Friese, D. D., and Gilstrap, F. E. (1982). Influence of prey availability on reproduction and prey consumption of *Phytoseiulus persimilis*, *Amblyseius californicus* and *Metaseiulus occidentalis* (Acarina: Phytoseiidae). *International Journal of Acarology*, **8,** 85-89.

Friese, D. D., and Gilstrap, F. E. (1985). Prey requirements and developmental times of 3 Phytoseiid species predaceous on spider mites (Acari, Phytoseiidae). *Southwestern Entomologist*, **10,** 83-88.

Fry, J. C. (1993). "Biological data analysis: a practical approach." University Press, Oxford.

Funasaki, G. Y., Lai, P. Y., Nakahara, L. M., Beardsley, J. W., and Ota, A. K. (1988). A review of biological control introductions in Hawaii: 1890-1985. *Proceedings of the Hawaiian Entomological Society*, **28,** 105-160.

Gabarra, R., Alomar, O., Castane, C., Goula, M., and Albajes, R. (2004). Movement of greenhouse whitefly and its predators between in- and outside of Mediterranean greenhouses. *Agriculture Ecosystems & Environment*, **102,** 341-348.

Goldson, S. L., McNeill, M. R., Phillips, C. B., and Proffitt, J. R. (1992). Host specificity testing and suitability of the parasitoid *Microctonus hyperodae* (Hym, Braconidae, Euphorinae) as a biological-control agent of *Listronotus bonariensis* (Col, Curculionidae) in New Zealand. *Entomophaga*, **37,** 483-498.

Goodwin, S., and Steiner, M. Y. (2002). Developments in IPM for protected cropping in Australia. *Bulletin IOBC/WPRS*, **25,** 81-84.

Gotoh, T., Yamaguchi, K., and Mori, K. (2004). Effect of temperature on life history of the predatory mite *Amblyseius* (*Neoseiulus*) *californicus* (Acari : Phytoseiidae). *Experimental and Applied Acarology*, **32,** 15-30.

Grenier, S., Guillaud, J., Delobel, B., and Bonnot, G. (1989). Nutrition and rearing of the predacious bug *Macrolophus caliginosus* Heteroptera, Miridae with artificial media. *Entomophaga*, **34,** 77-86.

Hamilton, R. L., Mullins, D. E., and Orcutt, D. M. (1985). Freezing tolerance in the woodroach *Cryptocercus punctulatus* (Scudder). *Experientia*, **41,** 1535-1537.

Hansen, D. L., Brodsgaard, H. F., and Enkegaard, A. (1999). Life table characteristics of *Macrolophus caliginosus* preying upon *Tetranychus urticae*. *Entomologia Experimentalis et Applicata*, **93,** 269-275.

Harrington, R., Fleming, R. A., and Woiwod, I. P. (2001). Climate change impacts on insect management and conservation in temperate regions: can they be predicted? *Agricultural and Forest Entomology*, **3,** 233-240.

Harris, P. (1988). Environmental-impact of weed-control insects - weed stands have been replaced by diverse plant-communities. *Bioscience*, **38,** 542-548.

Hart, A. J., and Bale, J. S. (1997). Evidence for the first strongly freeze-tolerant insect found in the UK. *Ecological Entomology*, **22,** 242-245.

Hart, A. J., Bale, J. S., and Fenlon, J. S. (1997). Developmental threshold, day-degree requirements and voltinism of the aphid predator *Episyrphus balteatus* (Diptera: Syrphidae). *Annals of Applied Biology*, **130,** 427-437.

Hart, A. J., Bale, J. S., Tullett, A. G., Worland, M. R., and Walters, K. F. A. (2002a). Effects of temperature on the establishment potential of the predatory mite *Amblyseius californicus* McGregor (Acari: Phytoseiidae) in the UK. *Journal of Insect Physiology*, **48,** 593-599.

Hart, A. J., Tullett, A. G., Bale, J. S., and Walters, K. F. A. (2002b). Effects of temperature on the establishment potential in the UK of the non - native glasshouse biocontrol agent *Macrolophus caliginosus*. *Physiological Entomology*, **27,** 112-123.

Hatherly, I. S., Bale, J. S., Walters, K. F. A., and Worland, M. R. (2004). Thermal biology of *Typhlodromips montdorensis*: implications for its introduction as a glasshouse biological control agent in the UK. *Entomologia Experimentalis et Applicata*, **111,** 97-109.

Hayes, A. J., and McArdle, B. H. (1987). A laboratory study on the predatory mite *Typhlodromus pyri* (Acarina, Phytoseiidae) .1. The effect of temperature and food - consumption on the rate of development of the eggs and immature stages. *Researches on Population Ecology*, **29,** 73-83.

Herbert, H. J. (1956). Laboratory studies on some factors in the life history of the predacious mite *Typhlodromus tiliae* Oudemans (Acarina: Phytoseiidae). *Canadian Entomologist*, **88,** 701-704.

Higley, L. G., Pedigo, L. P., and Ostlie, K. R. (1986). Degday - a program for calculating degree-days, and assumptions behind the degree-day approach. *Environmental Entomology*, **15,** 999-1016.

Hill, J. K., Thomas, C.D., Fox, R., Telfer, M. G., Willis, S. G., Asher, J., and Huntley, B. (2002). Responses of butterflies to twentieth century climate warming: implications for future ranges. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **269**(1505): 2163-2171.

Hokkanen, H. M. T., and Pimentel, D. (1989). New associations in biological-control - theory and practice. *Canadian Entomologist*, **121,** 829-840.

Hopper, K. R. (1998). Is classical biological control safe - or much ado about nothing? *In* "Sixth Australasian Applied Entomological Research Conference", Vol. 1, pp. 501-510, University of Queensland, Brisbane, Australia.

Horwath, K. L., and Duman, J. G. (1984). Yearly variations in the overwintering mechanisms of the cold - hardy beetle *Dendroides canadensis*. *Physiological Zoology*, **57,** 40-45.

Houghton, J., Meirafilho, L., Callender, B., Harris, N., Kattenberg, A., and Maskell, K. (1996). "Climate change 1995 - The science of climate change. Contribution of working group I to the second assessment of the Intergovernmental Panel on climate change." Cambridge: University Press.

Howarth, F. G. (1991). Environmental impacts of classical biological-control. *Annual Review of Entomology*, **36,** 485-509.

Howling, G. G., Harrington, R., Clark, S. J., and Bale, J. S. (1993). The use of multiple-regression via principal components in forecasting early-season aphid (Homoptera, Aphididae) flight. *Bulletin of Entomological Research*, **83,** 377-381.

Hoy, M. A., and Flaherty, D. L. (1975). Photoperiodic induction of diapause in a predaceous mite, *Metaseiulus occidentalis*. *Annals of the Entomological Society of America*, **63,** 960-963.

Huffaker, C. B., Messenger, P. S., and DeBach, P. (1971). The natural enemy component in natural control and the theory of biological control. *In* "Biological Control" (C. B. Huffaker, Ed.), pp. 16-62. Plenum, New York.

Hughes, L. (2000). Biological consequences of global warming: is the signal already apparent? *Trends in Ecology & Evolution*, **15,** 56-61.

Hussey, N. W., and Bravenboer, L. (1971). Control of pests in glasshouse culture by the introduction of natural enemies. *In* "Biological Control" (C. B. Huffaker, Ed.), pp. 195-216. Plenum, New York.

James, D. G. (1988). Reproductive diapause in *Typhlodromus occidentalis* Nesbitt (Acarina, Phytoseiidae) from Southern New-South-Wales. *Journal of the Australian Entomological Society*, **27,** 55-59.

Jolly, R. J. (2000). The predatory mite *Neoseiulus californicus*: its potential as a biocontrol agent for the fruit tree red spider mite *Panonychus ulmi* in the UK. *Proceedings of the 2000 Brighton Conference - Pests & Diseases 2000*, **1,** 487-490.

Jolly, R. L. (2001). The status of the predatory mite *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) in the UK, and its potential as a biocontrol agent of *Panonychus ulmi* (Koch) (Acari: Tetranychidae). Unpublished PhD Thesis, pp. 173. University of Birmingham.

Kapetanakis, E. G., and Cranham, J. E. (1983). Laboratory evaluation of resistance to pesticides in the phytoseiid predator *Typhlodromus pyri* from English apple orchards. *Annals of Applied Biology*, **103,** 389-400.

Karl, T. R., Nicholls, N., and Gregory, J. (1997). The coming climate. *Scientific American*, **276,** 78-83.

Kelty, J. D., and Lee, R. E. (1999). Induction of rapid cold hardening by cooling at ecologically relevant rates in *Drosophila melanogaster*. *Journal of Insect Physiology*, **45,** 719-726.

Klok, C. J., and Chown, S. L. (1997). Critical thermal limits, temperature tolerance and water balance of a sub-Antarctic caterpillar, *Pringleophaga marioni* (Lepidoptera: Tineidae). *Journal of Insect Physiology*, **43,** 685-694.

Knight, C. A., and Duman, J. G. (1986). Inhibition of recrystallization of ice by insect thermal hysteresis proteins - a possible cryoprotective role. *Cryobiology*, **23,** 256-262.

Koskula, H., Vanninen, I., and Lindquist, I. (1999). The role of *Macrolophus caliginosus* (Heteroptera: Miridae) in controlling the two-spotted spider mite in greenhouse tomato under North European conditions. *IOBC/WPRS Bulletin*, **22,** 129-132.

Kostal, V. (1993). Cold-hardiness and supercooling capacity in diapausing and nondiapausing stages of the cabbage root fly *Delia radicum*. *Cryobiology*, **30,** 524-531.

Kostiainen, T., and Hoy, M. A. (1994). Egg-harvesting allows large-scale rearing of *Amblyseius finlandicus* (Acari, Phytoseiidae) in the laboratory. *Experimental & Applied Acarology*, **18,** 155-165.

Kristiansen, E., Pedersen, S., Ramlov, H., and Zachariassen, K. E. (1999). Antifreeze activity in the cerambycid beetle *Rhagium inquisitor*. *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology*, **169,** 55-60.

Lamb, R. J. (1992). Developmental rate of *Acyrthosiphon pisum* (Homoptera, Aphididae) at low temperatures - implications for estimating rate parameters for insects. *Environmental Entomology*, **21,** 10-19.

Lawton, J. H. (1995). The response of insects to environmental change. *In* "Insects in a Changing Environment" (R. Harrington and N. E. Stork, Eds.), pp. 3-26. Academic Press, London, London.

Leather, S. (1983). Forecasting aphid outbreaks using winter egg counts: an assessment of its feasibility and an example of its applications in Finland. *Zeitschrift fuer angewandte Entomologie*, **96,** 282-287.

Leather, S., Walters, K. F. A., and Bale, J. S. (1993). "The ecology of insect overwintering." Cambridge University Press.

Lindsay, S. W. and Bayoh, M. N. (2004). Mapping members of the *Anopheles gambiae* complex using climate data. *Physiological Entomology*, **29**(3): 204-209.

Lockwood, J. A. (1993). Environmental-issues involved in biological-control of rangeland grasshoppers (Orthoptera, Acrididae) with exotic agents. *Environmental Entomology*, **22,** 503-518.

Lockwood, J. A. (1999). Nontarget effects of Biological control: what are we trying to miss. *In* "Nontarget effects of biological control" (J. J. Duan, Ed.), pp. 15-31. Kluwer Academic Publishers, Boston.

Loomans, A. J. M., van Lenteren, J. C., Bigler, F., Burgio, G., Hokkanen, H. M. T., and Thomas, M. B. (2002). Evaluating environmental risks of biological control introductions: how to select safe natural enemies? *Bulletin IOBC/WPRS*, **25,** 147-150.

Loope, L. L., and Howarth, F. G. (2002). Globalization and pest invasion: where will we be in five years? *In* "1st International symposium on biological control of arthropods" (R. Reardon, Ed.), Vol. 1, pp. 573. USDA, Honolulu, Hawaii, USA.

Lynch, L. D., and Thomas, M. B. (2000). Nontarget effects in the biocontrol of insects with insects, nematodes and microbial agents: the evidence. *BiocontrolNews and Information*, **21,** 117N130N.

Ma, W. L., and Laing, J. E. (1973). Biology, potential for increase and prey consumption of *Amblyseius chilenensis* (Dosse) (Acarina: Phytoseiidae). *Entomophaga*, **18,** 47-60.

Mach, R. N. e. a. (2000). Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications*, **10,** 689-710.

MacRae, I. V., and Croft, B. A. (1997). Intra- and interspecific predation by adult female *Metaseiulus occidentalis* and *Typhlodromus pyri* (Acari: Phytoseiidae) when provisioned with varying densities and ratios of *Tetranychus urticae* (Acari: Tetranychidae) and phytoseiid larvae. *Experimental & Applied Acarology*, **21,** 235-245.

Malausa, J. C., and Trottin-Caudal, Y. (1996). Advances in the strategy of use of the predaceous bug *Macrolophus caliginosus* (Heteroptera: Miridae) in glasshouse crops. *In* "Zoophytophagous Heteroptera: Implications for life history and integrated pest management. Proceedings: Thomas Say Publications in Entomology" (O. Alomar and R. N. Wiedenmann, Eds.), pp. 178-189. Entomological Society of America.

Mansingh, A. (1971). Physiological classification of dormancies in insects. *Canadian Entomologist*, **103,** 983-1009.

Marshall, D. B., Thistlewood, H. M. A., and Lester, P. J. (2001). Release, establishment, and movement of the predator *Typhlodromus pyri* (Acari : Phytoseiidae) on apple. *Canadian Entomologist*, **133,** 279-292.

Masaki, S. (1980). Summer diapause. *Annual Review of Entomology*, **25,** 1-25.

McDonald, J. R., Bale, J. S., and Walters, K. F. A. (1997). Low temperature mortality and overwintering of the western flower thrips *Frankliniella occidentalis* (Thysanoptera: Thripidae). *Bulletin of Entomological Research*, **87,** 497-505.

McDonald, J. R., Head, J., Bale, J. S., and Walters, K. F. A. (2000). Cold tolerance, overwintering and establishment potential of *Thrips palmi*. *Physiological Entomology*, **25,** 159-166.

McMurtry, J. A., and Croft, B. A. (1997). Life-styles of phytoseiid mites and their role in biological control. *Annual Review of Entomology*, **42,** 291-321.

Memmott, J. (1999). Food webs as a tool for studying nontarget effects in biological control. *In* "Nontarget effects of biological control" (P. A. Follett and J. J. Duan, Eds.), pp. 147-163. Kluwer Academic Publishers, Boston.

Messing, R. H. (1999). The impact of nontarget concerns on the practice of biological control. *In* "Nontarget effects of biological control" (P. A. Follett and J. J. Duan, Eds.), pp. 45-55. Kluwer Academic Publishers, London.

Met Office (2004). Historic station data. Available: http://www.metoffice.gov.uk/climate/uk/stationdata/index.html [Accessed 02/12/2004]

Montserrat, M., Albajes, R., and Castane, C. (2000). Functional response of four Heteropteran predators preying on greenhouse whitefly (Homoptera : Aleyrodidae) and western flower thrips (Thysanoptera : Thripidae). *Environmental Entomology*, **29,** 1075-1082.

Montserrat, M., Albajes, R., and Castane, C. (2004). Behavioral responses of three plant-inhabiting predators to different prey densities. *Biological Control*, **30,** 256-264.

Moore, N. W. (1989). "The bird of time." University Press, Cambridge.

Morewood, W. D. (1992). Cold hardiness of *Phytoseiulus persimilis* Athias-Henriot and *Amblyseius cucumeris* (Oudemans) (Acarina, Phytoseiidae). *Canadian Entomologist*, **124,** 1015-1025.

Morewood, W. D. (1993). Diapause and cold hardiness of phytoseiid mites (Acarina, Phytoseiidae). *European Journal of Entomology*, **90,** 3-10.

Morewood, W. D., and Gilkeson, L. A. (1991). Diapause induction in the thrips predator *Amblyseius cucumeris* Acarina, Phytoseiidae under greenhouse conditions. *Entomophaga*, **36,** 253-263.

Morrisey, R. E., and Baust, J. G. (1976). The ontogeny of cold tolerance in the gall fly *Eurosta solidaginis*. *Journal of Insect Physiology*, **22,** 431-437.

Murdoch, W. W., Chesson, J., and Chesson, P. L. (1985). Biological-control in theory and practice. *American Naturalist*, **125,** 344-366.

Murray, J., Murray, E., Johnson, M. S., and Clarke, S. (1988). The extinction of *Partula* on Moorea. *Pacific Science*, **42,** 150-153.

Nedstam, B., and Johansson-Kron, M. (1999). *Diglyphus isaea* (Walker) and *Macrolophus caliginosus* (Wagner) for biological control of *Liromyza bryoniae* (Kaltenbach) in tomato. *IOBC/WPRS Bulletin*, **22,** 261-263.

Nedved, O. (1993). Comparison of cold hardiness in two ladybird beetles (Coleoptera: Coccinellidae) with contrasting hibernation behaviour. *European Journal of Entomology*, **90,** 465-470.

Nedved, O. (1998). Modelling the relationship between cold injury and accumulated degree days in terrestrial arthropods. *Cryo-Letters*, **19,** 267-274.

Nedved, O. (2000). Snow white and the seven dwarfs: A multivariate approach to classification of cold tolerance. *Cryo-Letters*, **21,** 339-348.

Nilssen, A., and Tenow, O. (1990). Diapause, embryo growth and supercooling capacity of *Epirrita autumnata* eggs from Northern Fennoscandia. *Entomologia Experimentalis et Applicata*, **57,** 39-55.

Orr, Garcia-Salazar, and Landis. (1999). Trichogramma non-target impacts: a model for biological control risk assessment. *In* "Nontarget effects of biological control" (J. J. Duan, Ed.). Kluwer Academic Publishers, London.

Overmeer, W. P. J. (1985a). Diapause. *In* "World crop pests: spider mites: their biology, natural enemies and control" (M. W. Sabelis, Ed.), Vol. 1B, pp. 95-101. Elsevier Science Publishers.

Overmeer, W. P. J. (1985b). Techniques: rearing and handling. *In* "*World Crop Pests: Spider Mites: their Biology, Natural Enemies and Control*" (W. Helle and M. W. Sabelis, Eds.), Vol. 1B, pp. 161-170. Elsevier Science Publishers.

Overmeer, W. P. J., Nelis, H. J. C. F., Deleenheer, A. P., Calis, J. N. M., and Veerman, A. (1989). Effect of diet on the photoperiodic induction of diapause in 3 species of predatory mite, *Amblyseius potentillae, A. cucumeris* and *Typhlodromus pyri*. *Experimental & Applied Acarology*, **7,** 281-287.

Perdikis, D., and Lykouressis, D. (2000). Effects of various items, host plants, and temperatures on the development and survival of *Macrolophus pygmaeus* Rambur (Hemiptera : Miridae). *Biological Control*, **17,** 55-60.

Pimentel, D., Glenister, C., Fast, S., and Gallahan, D. (1984). Environmental risks of biological pest controls. *Oikos*, **42,** 283-290.

Pratt, P. D., Schausberger, P., and Croft, B. A. (1999). Prey-food types of *Neoseiulus fallacis* (Acari : Phytoseiidae) and literature versus experimentally derived prey-food estimates for five phytoseiid species. *Experimental and Applied Acarology*, **23,** 551-565.

Pullin, A. S. (1996). Physiological relationships between insect diapause and cold tolerance: Coevolution or coincidence? *European Journal of Entomology*, **93,** 121-129.

Pullin, A. S., and Bale, J. S. (1989). Influence of diapause and temperature on cryoprotectant synthesis and cold hardiness in pupae of *Pieris brassicae*. *Comparative Biochemistry and Physiology a-Physiology*, **94,** 499-503.

Ramlov, H. (1998). Untitled. *Cryo-Letters*, **19,** 4-4.

Ratcliffe, D. (1979). The end of the large blue butterfly. *New Scientist*, **8,** 457-458.

Raworth, D. A., Fauvel, G., and Auger, P. (1994). Location, reproduction and movement of *Neoseiulus californicus* (Acari, Phytoseiidae) during the autumn, winter and spring in orchards in the South of France. *Experimental & Applied Acarology*, **18,** 593-602.

Rickards, J., Kelleher, M. J., and Storey, K. B. (1987). Strategies of freeze avoidance in larvae of the Goldenrod Gall Moth, *Epiblema scudderiana* - winter profiles of a natural - population. *Journal of Insect Physiology*, **33,** 443-450.

Ring, R. A. (1982). Freezing-tolerant insects with low supercooling points. *Comparative Biochemistry and Physiology a-Physiology*, **73,** 605-612.

Riudavets, J., and Castane, C. (1998). Identification and evaluation of native predators of *Frankliniella occidentalis* (Thysanoptera : Thripidae) in the Mediterranean. *Environmental Entomology*, **27,** 86-93.

Roberts, L. I. N. (1986). The practice of biological control - implications for conservation, science and the community. *Weta News Bulletin of the Entomological Society of New Zealand*, **9,** 76-84.

Rock, G. C., Yeargan, D. R., and Rabb, R. L. (1971). Diapause in the phytoseiid mite, *Neoseiulus fallacis*. *Journal of Insect Physiology*, **17,** 1651-1659.

Russel, D. A. (1986). The role of the entomological society in New Zealand. *Weta*, **9,** 44-54.

Salt, R. W. (1936). Studies on the freezing process in insects. *Techincal Bulletin Minisota Agricultural Experimental Station*, **116,** 1-41.

Salt, R. W. (1961). Principles of insect cold hardiness. *Annual Review of Entomology*, **6,** 55-74.

Sampson, A. C., and Jacobson, R. J. (1999). *Macrolophus caliginosus* Wagner (Heteroptera: Miridae): A predator causing damage to UK tomatoes. *IOBC Bulletin*, **22,** 213-216.

Samways, M. J. (1988). Classical biological control and insect conservation: are they compatible? *Environmental Conservation*, **15,** 349-354.

Sands, D. P. A. (1997). The 'safety' of biological control agents: assessing their impact on beneficial and other non-target hosts. *Memoirs of the Museum of Victoria*, **56,** 611-615.

Sands, D. P. A. (1998). Guidelines for testing host specificity of agents for biological control of arthropods. *In* "Sixth Australasian Applied Entomological Research Conference", Vol. 1, pp. 556-560, University of Queensland, Brisbane, Australia.

Saunders, D. S., and Hayward, S. A. L. (1998). Geographical and diapause-related cold tolerance in the blow fly, *Calliphora vicina*. *Journal of Insect Physiology*, **44,** 541-551.

Schausberger, P. (1992). Comparative investigations on the effect of different foods on development and reproduction of *Amblyseius aberrans* Oud and *Amblyseius finlandicus* Oud (Acarina, Phytoseiidae). *Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie*, **113,** 476-486.

Schausberger, P. (1997). Inter- and intraspecific predation on immatures by adult females in *Euseius finlandicus, Typhlodromus pyri* and *Kampimodromus aberrans* (Acari: Phytoseiidae). *Experimental & Applied Acarology*, **21,** 131-150.

Schausberger, P., and Walzer, A. (2001). Combined versus single species release of predaceous mites: Predator-predator interactions and pest suppression. *Biological Control*, **20,** 269-278.

Schicha, E. (1979). Three new species of *Amblyseius* Berlese from New Caledonia and Australia (Acari: Phytoseiidae). *Australian Entomology Magazine*, **6,** 41-48.

Schicha, E. (1987). "Phytoseiidae of Australia and Neighboring Areas." Indira Publishing,

Secord, D., and Kareiva, P. (1996). Perils and pitfalls in the host specificity paradigm. *Bioscience*, **46,** 448-453.

Simberloff, D., and Stiling, P. (1996a). How risky is biological control? *Ecology*, **77,** 1965-1974.

Simberloff, D., and Stiling, P. (1996b). Risks of species introduced for biological control. *Biological Conservation*, **78,** 185-192.

Sinclair, B. J. (1999). Insect cold tolerance: How many kinds of frozen? *European Journal of Entomology*, **96,** 157-164.

Sinclair, B. J., Vernon, P., Klok, C. J., and Chown, S. L. (2003). Insects at low temperature: an ecological perspective. *Trends in Ecology & Evolution*, **18,** 257-262.

Sinclair, B. J., and Wharton, D. A. (1997). Avoidance of intracellular freezing by the freezing-tolerant New Zealand Alpine Weta *Hemideina maori* (Orthoptera: Stenopelmatidae). *Journal of Insect Physiology*, **43,** 621-625.

Slachta, M., Vambera, J., Zahradnickova, H., and Kostal, V. (2002). Entering diapause is a prerequisite for successful cold- acclimation in adult *Graphosoma lineatum* (Heteroptera : Pentatomidae). *Journal of Insect Physiology*, **48,** 1031-1039.

Solomon, M. G., Cross, J. V., Fitzgerald, J. D., Campbell, C. A. M., Jolly, R. L., Olszak, R. W., Niemczyk, E., and Vogt, H. (2000). Biocontrol of pests of apples and pears in northern and central Europe - 3. Predators. *Biocontrol Science and Technology*, **10,** 91-128.

Solomon, M. G., Easterbrook, M. A., and Fitzgerald, J. D. (1993). Mite management programs based on organophosphate resistant *Typhlodromus pyri* in UK apple orchards. *Crop Protection*, **12,** 249-254.

Somme, L. (1961). On the overwintering of houseflies (*Musca domestica* L.) and stable flies (*Stomoxys calcitrinas* (L.) in Norway. *Norsk Entomologisk Tidsskrift*, **11,** 191-223.

Somme, L. (1964). Effects of glycerol on cold hardiness in insects. *Canadian Journal of Zoology*, **42,** 89-101.

Somme, L. (1982). Supercooling and winter survival in terrestrial arthropods. *Comparative Biochemistry and Physiology a-Physiology*, **73,** 519-543.

Somme, L. (1996). The effect of prolonged exposures at low temperatures in insects. *Cryo-Letters*, **17,** 341-346.

Speyer, E. R. (1927). An important parasite of the greenhouse white-fly (*Trialeurodes vaporariorum* Westwood). *Bulletin Entomological Research*, **17,** 301-308.

Stanley, J. N., and Julien, M. H. (1998). The need for post-release studies to improve risk assessments and decision making in classical biological control. *In* "Sixth Australasian Applied Entomological Research Conference", Vol. 1, pp. 561-564, The University of Queensland, Brisbane, Australia.

Steiner, M. Y. (2002). Progress towards integrated pest management for thrips (Thysanoptera: Thripidae) in strawberries in Australia. *Bulletin IOBC/WPRS*, **25,** 253-256.

Steiner, M. Y., and Goodwin, S. (2002a). Development of a new thrips predator, *Typhlodromips montdorensis* (Schicha) (Acari: Phytoseiidae) indigenous to Australia. *Bulletin IOBC/WPRS*, **25,** 245-247.

Steiner, M. Y., and Goodwin, S. (2002b). Management of thrips on cucumber with *Typhlodromips montdorensis* (Schicha) (Acari: Phytoseiidae). *Bulletin IOBC/WPRS*, **25,** 249-252.

Steiner, M. Y., Goodwin, S., Wellham, T. M., Barchia, I. M., and Spohr, L. J. (2003a). Biological studies of the Australian predatory mite *Typhlodromalus lailae* (Schicha) (Acari : Phytoseiidae). *Australian Journal of Entomology*, **42,** 131-137.

Steiner, M. Y., Goodwin, S., Wellham, T. M., Barchia, I. M., and Spohr, L. J. (2003b). Biological studies of the Australian predatory mite *Typhlodromips montdorensis* (Schicha) (Acari : Phytoseiidae), a potential biocontrol agent for western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera : Thripidae). *Australian Journal of Entomology*, **42,** 124-130.

Storey, K. B., and Storey, J. M. (1996). Natural freezing survival in animals. *Annual Review of Ecology and Systematics*, **27,** 365-386.

Tauber, M. J., and Tauber, C. A. (1976). Insect seasonality: diapause maintenance, termination, and postdiapause development. *Annual Review of Entomology*, **21,** 81-107.

Tauber, M. J., Tauber, C. A., and Masaki, S. (1986). "Seasonal adaptations of Insects." University Press, Oxford.

Thomas, H., and Stoddart, J. L. (1980). Leaf senescence. *Annual Review of Plant Physiology*, **31,** 83-111.

Thomas, M. B., and Willis, A. J. (1998). Biocontrol-risky but necessary? *Trends in Ecology & Evolution*, **13,** 325-329.

Todd, C. M., and Block, W. (1995). A comparison of the cold-hardiness attributes in larvae of 4 species of Diptera. *Cryo-Letters*, **16,** 137-146.

Tullett, A. G., Hart, A. J., Worland, M. R., and Bale, J. S. (2004). Assessing the effects of low temperature on the establishment potential in Britain of the non-native biological control agent *Eretmocerus eremicus*. *Physiological Entomology*, **29,** 363-371.

van der Geest, L. P. S., Overmeer, W. P. J., and Vanzon, A. Q. (1991). Cold-hardiness in the predatory mite *Amblyseius potentillae* (Acari, Phytoseiidae). *Experimental & Applied Acarology*, **11,** 167-176.

van der Laak, S. (1982). Physiological adaptations to low temperature in freezing tolerant *Phyllodecta laticollis* beetles. *Comparative Biochemistry and Physiology*, **73A,** 613-620.

van Driesche, R. G., and Bellows, T. S. (1996). "Biological Control." Chapman and Hall, London, UK,

van Driesche, R. G., and Hoddle, M. (1997). Should arthropod parasitoids and predators be subject to host range testing when used as biological control agents? *Agriculture and Human Values*, **14,** 211-226.

van Houten, Y. M. (1989). Photoperiodic control of adult diapause in the predacious mite, *Amblyseius potentillae*: repeated diapause induction and termination. *Physiological Entomology*, **14,** 341-348.

van Houten, Y. M., Overmeer, W. P. J., van Zon, A. Q., and Veerman, A. (1988). Thermoperiodic induction of diapause in the predacious mite, *Amblyseius potentillae*. *Journal of Insect Physiology*, **34,** 285-290.

van Houten, Y. M., van Stratum, P., Bruin, J., and Veerman, A. (1995). Selection for non-diapause in *Amblyseius cucumeris* and *A. barkeri* and exploration of the effectiveness of selected strains for thrips control. *Entomologia Experimentalis et Applicata*, **77,** 289-295.

van Houten, Y. M., and Veenendaal, R. L. (1990). Effects of photoperiod, temperature, food and relative-humidity on the induction of diapause in the predatory mite *Amblyseius potentillae*. *Experimental & Applied Acarology*, **10,** 111-128.

van Lenteren, J. C. (1995). Frequency and consequences of insect invasions. *In* "Biological control: Benefits and risks" (J. M. Lynch, Ed.), pp. 30-43. University Press, Cambridge.

van Lenteren, J. C. (2000). A greenhouse without pesticides: fact or fantasy? *Crop Protection*, **19,** 375-384.

van Lenteren, J. C., Babendreier, D., Bigler, F., Burgio, G., Hokkanen, H. M. T., Kuske, S., Loomans, A. J. M., Menzler-Hokkanen, I., van Rijn, P. C. J., Thomas, M. B., Tommasini, M. G., and Zeng, Q. Q. (2003). Environmental risk assessment of exotic natural enemies used in inundative biological control. *Biocontrol*, **48,** 3-38.

van Lenteren, J. C., Bigler, F., Burgio, G., Hokkanen, H., and Thomas, M. B. (2002). Risks of importation and release of exotic biological control agents: how to determine host specificity. *Bulletin IOBC/WPRS*, **25,** 281-284.

van Lenteren, J. C., and Loomans, A. J. M. (2000). Biological control of insects: always safe? Risks of introduction and release of exotic natural enemies. *In* "Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society, N.E.V., Amsterdam," (F. J. A. J. Meeuwsen, Ed.), Vol. 11, pp. 3-22.

van Lenteren, J. C., and Manzaroli, G. (1999). Evaluation and use of predators and parasitoids for biological control of pests in greenhouses. *In* "Integrated pest and disease management in greenhouse crops" (Y. Elad, Ed.). Kluwer Academic Press, Boston.

van Lenteren, J. C., and Woets, J. (1988). Biological and integrated pest-control in greenhouses. *Annual Review of Entomology*, **33,** 239-269.

van Schelt, J., Klapwijk, J., Letard, M., and Aucouturier, C. (1995). The use of *Macrolophus caliginosus* as a whitefly predator in protected crops. *In* "Bemisia: 1995. Taxonomy, biology, damage, control and management." (D. Gerling and R. T. Mayer, Eds.), pp. 515-521. Intercept, Andover, UK.

Veerman, A. (1992). Diapause in Phytoseiid mites - a review. *Experimental & Applied Acarology*, **14,** 1-60.

Veerman, A. (1994). Photoperiodic and thermoperiodic control of diapause in plant-inhabiting mites - a review. *Netherlands Journal of Zoology*, **44,** 139-155.

Waage, J. K., and Greathead, D. J. (1988). Biological control - challenges and opportunities. *Philosophical Transactions of the Royal Society of London Series B - Biological Sciences*, **318,** 111-128.

Wagner, T. L., Wu, H. I., Sharpe, P. J. H., Schoolfield, R. M., and Coulson, R. N. (1984). Modelling insect development rates - a literature-review and application of a biophysical model. *Annals of the Entomological Society of America*, **77,** 208-225.

Walde, S. J., Nyrop, J. P., and Hardman, J. M. (1992). Dynamics of *Panonychus ulmi* and *Typhlodromus pyri* - factors contributing to persistence. *Experimental & Applied Acarology*, **14,** 261-291.

Walzer, A., and Schausberger, P. (1999). Cannibalism and interspecific predation in the phytoseiid mites *Phytoseiulus persimilis* and *Neoseiulus californicus*: predation rates and effects on reproduction and juvenile development. *Biocontrol*, **43,** 457-468.

Way, M. J., Cammell, M. E., Taylor, L. R., and Woiwod, I. P. (1981). The use of egg counts and suction trap samples to forecast the infestation of Spring-sown field beans,

Vicia faba, by the black bean aphid, *Aphis fabae*. *Annals of Applied Biology*, **98,** 21-34.

Wei, Q. C., and Walde, S. J. (1997). The functional response of *Typhlodromus pyri* to its prey, *Panonychus ulmi*: the effect of pollen. *Experimental & Applied Acarology*, **21,** 677-684.

Wittwer, S. H., and Castilla, N. (1995). Protected cultivation of horticultural crops worldwide. *Horticultural Technology*, **5,** 6-23.

Wu, D. W., and Duman, J. G. (1991). Activation of antifreeze proteins from larvae of the beetle *Dendroides canadensis*. *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology*, **161,** 279-283.

Yamamura, K., and Kiritani, K. (1998). A simple method to estimate the potential increase in the number of generations under global warming in temperate zones. *Applied Entomology and Zoology*, **33,** 289-298.

Yi, S. X., and Lee, R. E. (2003). Detecting freeze injury and seasonal cold-hardening of cells and tissues in the gall fly larvae, *Eurosta solidaginis* (Diptera : Tephritidae) using fluorescent vital dyes. *Journal of Insect Physiology*, **49,** 999-1004.

Young, S. R., and Block, W. (1980). Experimental studies on the cold tolerance of *Alaskozetes antarcticus*. *Journal of Insect Physiology*, **26,** 227-234.

Zachariassen, K. E. (1982). Nucleating-agents in cold-hardy insects. *Comparative Biochemistry and Physiology a-Physiology*, **73,** 557-562.

Zachariassen, K. E. (1985). Physiology of cold tolerance in insects. *Physiological Reviews*, **65,** 799-832.

Zachariassen, K. E., and Hammel, H. T. (1976). Nucleating agents in the haemolymph of insects tolerant to freezing. *Nature*, **262,** 285-287.

Zavaleta, E. S., Hobbs, R. J., and Mooney, H. A. (2001). Viewing invasive species removal in a whole-ecosystem context. *Trends in Ecology & Evolution*, **16,** 454-459.

Zhang, Z. Q., and Croft, B. A. (1994). A comparative life history study of immature *Amblyseius fallacis, A. andersoni*, *Typhlodromus occidentalis* and *T. pyri* (Acari: Phytoseiidae) with a review of larval feeding patterns in the family. *Experimental and Applied Acarology*, **18,** 635-657.

Zhang, Z. Q., and Croft, B. A. (1995). Interspecific competition and predation between immature *Amblyseius fallacis*, *Amblyseius andersoni*, *Typhlodromus occidentalis* and *Typhlodromus pyri* (Acari, Phytoseiidae). *Experimental & Applied Acarology*, **19,** 247-257.