12387 Cold tolerance, overwintering survival and establishment potential of the predatory mirid *Dicyphus hesperus* in the Netherlands

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Summary

Field and laboratory experiments were carried out on nymphs and diapausing and non-diapausing adults of the predatory mirid *Dicyphus hesperus* with samples derived from a culture originally collected from southern California, USA.

In laboratory assessments of cold tolerance, the supercooling points (freezing temperatures) of the three life cycle stages were similar at around -20°C. The lethal temperatures of non-diapausing nymphs, adults and diapausing adults were close to the SCP ($LTemp_{50}$ of -17.63, -17.59, and -19.2°C respectively). At 5°C, the $LTime_{50}$ was 54, 101.7 and 117.5 days for fed nymphs, adults and diapausing adults respectively. At all three exposure temperatures nymphs survived for less time than diapausing and non-diapausing adults. At -5 and 0°C there were no significant difference between fed and unfed individuals; however at 5°C, survival of fed individuals was longer in all treatments. This suggests that feeding was not apparent at the lower two temperatures, but at 5°C feeding was important in lengthening survival of *D. hesperus*.

After 5 months in the field (from November 2005 to April 2006) there was 50% survival of diapausing adults when provided with food, whereas starved diapausing adults died out after 140 days. In a similar exposure, 15% of non-diapausing adults provided with food were still alive at the end of winter (unfed adults survived 100 days). On return to the laboratory after 5 months in the field, both diapause and non-diapause adults mated and laid viable eggs and the resulting offspring developed to adult. When first instar nymphs were placed in the field in November 2005, starved samples died out after 70 days, but 5% of fed nymphs survived until the end of winter (140 days), and developed to adulthood on return to the laboratory.

Overall, the field and laboratory experiments indicate that this population of *D. hesperus* is able to enter diapause and that under temperate winter conditions survival in this life cycle stage is high. Lower levels of survival were observed in nymphs and non-diapause adults, but both of these life cycle stages survived for an entire winter,

provided that they had access to food and were capable of reproducing at the end of winter.

Contents: Page

Section 1

Introduction

1 Introduction

1.1 *Background*

Biological control is widely used in northern Europe to control pest insects and mites associated with greenhouse crops (vegetables and ornamental flowers). The emphasis on biological control reflects the fact that many glasshouse pests have become resistant to chemical control compounds and increasingly the consumer is demanding pesticide-free produce. The most successful long term glasshouse biological control schemes are the control of glasshouse whitefly *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) by the parasitoid wasp *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae) and predation of the spidermite *Tetranychus urticae* (Koch) (Acari: Tetranychidae) by the mite *Phytoseiulus persimilis* (Athias-Henriot) (Acari: Phytoseiidae). Following these successful programmes, there has been continued effort over the last 10 to 20 years to identify new or more efficient control agents.

Most of the glasshouse biocontrol agents used in northern Europe originate from tropical, semi-tropical or Mediterranean climates. For this reason, it has been widely assumed on the basis of 'climate matching', that any imported species that escape from glasshouses would be unable to survive through northern European winters. Based on introductions into the UK, this assumption is now known to be untrue. The predatory mite *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) was first released in the UK in 1991, and was found to have become established outdoors by 1999 (Jolly, 2000). Similarly, the predatory mirid *Macrolophus caliginosus* (Wagner) (Hemiptera: Miridae), first released in the UK in 1995, has been found outside of glasshouses in winter, though full establishment has not yet been confirmed (Hart *et al.*, 2002b). The impact of such exotic species on native ecosystems is unknown, but establishment of non-native species is considered to be undesirable.

In the light of the apparent problems in the UK regulatory system, a series of projects have been carried out, funded mainly by DEFRA (Department for Environment, Food and Rural Affairs), to determine the ecophysiological mechanisms that have allowed

N. californicus to establish, and by comparison, the reasons why other introduced species have failed to do so (Hart *et al.*, 2002a; Hart *et al.*, 2002b; Hatherly *et al.*, 2005a; Hatherly *et al.*, 2004; Tullett *et al.*, 2004). The data was analysed to devise a protocol by which the establishment potential of candidate biocontrol agents could be screened as part of the licensing system. The development of such a testing procedure is particularly timely against the backdrop of the desire of some European countries to introduce comprehensive 'environmental risk assessment' (ERA) for non-native biocontrol agents, and the recent funding of an EU project (REBECA) to produce 'regulatory guidelines' to be adopted (in theory) across all member states. There is an emerging consensus that regulation should be set at a level that does not inhibit the introduction of new agents (by placing excessive costs on industry), without compromising environmental safety.

1.2 Dicyphus hesperus

Dicyphus hesperus Knight (Hemiptera: Miridae) is native to North America and is an omnivorous predator that has shown potential for glasshouse biocontrol on crops such as tomato, controlling glasshouse whitefly *T. vaporariorum* and glasshouse red spider mite *T. urticae* (McGregor *et al.*, 1999). It is currently used on glasshouse tomato crops in British Columbia, Ontario and Quebec (all Canada) (Gillespie, personal communication). The developmental threshold of this species is approximately 8°C (Gillespie and Sanchez, 2004) and it is known to overwinter in reproductive diapause in North America. Strains from both California (USA) and British Columbia (Canada) have been shown to enter diapause, although with slightly different critical day length induction cues (Gillespie and Quiring, 2005).

1.3 *Experimental protocol*

As regulatory authorities now increasingly require a risk assessment dossier to be submitted as part of a license application for the release of a non-native biocontrol agent, the experiments conducted on *D. hesperus* were carried out in accordance with an established experimental protocol developed and refined during studies on five species, all of which have been previously released in biocontrol schemes in the UK

or were candidate agents (Hart *et al.*, 2002a; Hart *et al.*, 2002b; Hatherly *et al.*, 2005a; Hatherly *et al.*, 2004; Tullett *et al.*, 2004).

For this study experiments were conducted on diapausing adults of *D. hesperus* as this was believed to be the dominant overwintering stage (Gillespie, personal communication). However, as it could not be assumed that all individuals within the source population would enter diapause, experiments were also conducted on non-diapausing adults. Finally, established glasshouse populations of *D. hesperus* may escape from the glasshouse at any life stage and therefore further experiments were also conducted on first instar nymphs, even though it was thought this may not be a dominant overwintering life stage.

For logistical reasons, experiments on field survival of *D. hesperus* were conducted first. These experiments undertaken on replicate samples of non-diapausing and diapausing adults and first instar nymphs were conducted outdoors in winter under containment, and survival was assessed at regular intervals with temperatures within the microhabitat continuously monitored.

Subsequently, the cold tolerance of replicate samples of first instar nymphs and non-diapausing and diapausing adults were assessed in terms of their (i) freezing temperature (supercooling point), (ii) lethal temperature, and (iii) lethal time (assessed at -5, 0 and 5°C).

Section 2

Methods

2 Materials and Methods

2.1 *Rearing of* Dicyphus hesperus

Dicyphus hesperus were obtained from Koppert, NL from a source population that had originally been collected from California between Los Angeles and San Diego (USA). They were reared under quarantine at 23°C, 18:6LD on *Nicotiana tabacum* plants and supplemented with eggs of *Ephestia kuehniella*. Field trials were conducted with first instar nymphs and adults directly upon receipt from Koppert. For laboratory trials, in excess of 100 *D. hesperus* mated adults were placed on large *N. tabacum* plants sprinkled with *E. kuehniella* eggs. After 24 h all adults were removed. Of the emerging first instar nymphs, some were used in laboratory experiments and some were kept at 23°C, 18:6LD and used when they reached adulthood. Some of the plants that had been oviposited on by *D. hesperus* were placed at 18°C, 12:12LD and the emerging nymphs were reared to adulthood as a diapausing population. These were then used in the field and laboratory experiments as required. To determine if adults were in diapause, they were mated and left at 18°C, 12:12LD for 20 days. Females that did not lay eggs over this time period were considered to be in diapause, although they were not dissected to check for egg development within the ovaries (Gillespie and Quiring 2005). Temperatures throughout all experiments were recorded using Tinytalk® dataloggers (Gemini, UK).

2.2 *Treatments*

For laboratory and field experiments there were 6 treatments. Treatments 1 and 2 were fed and unfed first instar nymphs, treatments 3 and 4 were fed and unfed non-diapausing adults, and treatments 5 and 6 were fed and unfed adults that had been reared under a diapause-inducing regime.

2.3 *Supercooling points*

Supercooling points (SCPs) for treatments 2, 4 and 6 were measured to provide an initial indication of the cold tolerance of *D. hesperus* and also to determine whether it

was a freeze tolerant or intolerant species. For treatments 4 and 6 (adults), each individual was placed into a size 3 Beem capsule (n=23) (Agar Scientific Ltd, UK) with a temperatures probe inserted in the tube and the temperature lowered at 0.5°C min^{-1} to the SCP of the specimen (onset of the freezing exotherm). The capsules were then opened and individual *D. hesperus* placed into glass vials, with eggs of *E. kuehniella* at 20°C, 18:6 LD and mortality recorded after 24 and 48 h. The same procedure was repeated for treatment 2 (n = 26), with nymphs attached to the temperature recording probe with Vaseline grease. The supercooling points of each treatment were tested for a normal distribution, analysed using a one-way ANOVA, and differences between treatments compared using Tukey's HSD test.

2.4 *Lower lethal temperatures*

Assessing the lower lethal temperature of *D. hesperus* enabled a comparison with the SCPs to determine the extent of any pre-freeze mortality in the population. Fifty *D. hesperus* of each unfed treatment group were placed individually into Beem capsules. The mirids were exposed singly within each replicate, as preliminary control experiments had shown that *D. hesperus* adults became entangled at the higher exposure temperatures when movement was still possible, leading to higher mortality rates than expected. Ten capsules were placed in each of five boiling tubes (constituting 5 replicates of 10 insects each) and suspended in a low temperature programmable alcohol bath (Haake C50P), (Haake, Germany). Each treatment was cooled from 15°C at 0.5°C min^{-1} to a range of temperatures between 0 and -25°C. After being held at the required minimum exposure temperature for 1 min the tubes were re-warmed to 15°C at 1°C min^{-1}. Individual *D. hesperus* from each treatment were then placed in glass vials (3.5 x 2.5 cm) with *E. kuehniella* eggs at 20°C, 18:6 LD and mortality recorded after 24 and 48 h. A control treatment of 50 insects was held in the alcohol bath at 15°C for 2h 10 min, the maximum time any insects would have been in the bath during experimental exposures.

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

2.5 *Lower lethal time*

Lower lethal time experiments complement the lethal temperature work by investigating the response of *D. hesperus* to temperatures likely to be experienced in the field over a longer time period. Additionally, a relationship between laboratory survival at 5°C and field survival was observed in previous work (Hatherly *et al.*, 2005b) and these experiments served to further investigate this correlation. The same set up was used as for the field experiments, except that samples of treatments 1 to 6 were placed at -5, 0 and 5°C after being held for 1 h at 10°C to overcome possible initial mortality due to cold shock. Four replicates of 10 insects each were removed from the exposure temperature and held for 1 h at 10°C to overcome possible heat shock mortality and survival was then recorded after 24 and 48 h at 20°C, 18:6 LD. Sampling was initially 3 times a week for the first week and then weekly until 100% mortality was observed or the experiment was terminated.

The results were analysed using Probit analysis to estimate the time required to kill 10, 50 and 90% of the individuals at each temperature ($LTime_{10,50,90}$).

2.6 *Field experiments*

The field experiments were conducted to measure the effect of fluctuating temperatures on outdoor populations. Five *D. hesperus* individuals of each treatment were placed in glass vials (7 x 2.5 cm), on a 0.5 cm deep layer of agar (2%) (Oxoid Ltd technical agar, 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). Unfed treatments had no food placed in the vials, whereas fed treatments had approximately 100 *E. kuehniella* eggs added to each vial. Sufficient vials of each treatment for a 5 month field trial were placed in plastic boxes and sealed, except for four ventilation holes covered in muslin in the side of the box (3 cm in diameter), and placed in a sheltered field location at the University of Birmingham. For each treatment, 4 replicates of two vials each (10 insects per replicate) were collected at random from the field and mortality (%) recorded. Sampling was carried out on 3 occasions in the first week, weekly for the

next month, and finally every 2 weeks until the end of the experiment. Treatments 1-4 were placed in the field on the 16 November 2005 and treatments 5 and 6 on the 7 December 2005. Live adults from treatments 3-6 collected from the field on and after 13 April 2006 (experiment ended as increased daily temperatures meant that age was considered to be the limiting factor in survival and not temperature) were placed on *N. tabacum* plants sprinkled with *E. kuehniella* eggs and observed for any oviposition. Any live nymphs recovered from the field were kept in the laboratory with food and development followed through until adulthood. Throughout the field experiments, food sources had to be changed approximately every three weeks (depending on air temperature) to stop insects becoming entangled in fungal infections and to ensure that excess food was always available. To do this, small batches of vials were briefly moved into a quarantine area at 8°C and transferred to fresh vials and placed back into the field.

A control sample of 30 first instar *D. hesperus* in individual vials was maintained at 23°C, 18:6 LD and development to adulthood observed to ensure that the experimental set up and handling was not deleterious to the insects.

Section 3

Results

3 Results

3.1 *Diapause induction regime*

A total of 73.3% of *D. hesperus* reared from egg to adulthood at 18°C, 12:12 LD (n = 30) did not lay eggs and were considered to be in diapause. In a comparative population reared at 23°C, 18:6 LD, 96.6% of females laid eggs. Therefore 27% of adults reared in the diapause inducing regime could not be considered to be in diapause. It was not expected that 100% of females reared in this regime would enter diapause; however, the proportion that did was considered to be sufficiently high to continue experiments using this treatment group to determine if there were any differences in cold tolerance between non-diapausing and diapausing populations.

3.2 *Supercooling points*

No significant differences were detected in supercooling points of nymphal, non-diapausing and diapausing adult *D. hesperus* ($F_{2,70}$ = 2.4, P>0.05). The mean and range of SCPs for each treatment are shown in Table 1. All individuals were dead after freezing.

Table 1: Mean (±SE) and range of SCPs of nymphal, non-diapausing and diapausing adult *Dicyphus hesperus*.

Group	n	Mean ± SE (°C)	Range (°C)
Non-diapause nymphs	23	-20.0 ± 0.36	-16.2 to -22.6
Non-diapause adults	26	-21.1 ± 0.40	-15.8 to -24.2
Diapause adults	23	-21.0 ± 0.33	-17.2 to -23.5

3.3 *Lower lethal temperature*

Survival of *D. hesperus* nymphs and adults after the control exposure at 15°C for 2h 10 min was 100%. The lethal temperatures for 10, 50 and 90% ($LTemp_{10,50,90}$) mortality of nymphal, non-diapausing and diapausing adult *D. hesperus* are given in Figure 1. The $LTemp_{50}$ for diapausing adults was 1.5°C lower than for non-diapausing adults and nymphs, but generally, there was no significant difference between the age groups or treatments (indicated by overlapping fiducial limits). Only the $LTemp_{10}$ of diapausing adults was significantly lower than for the other two treatments (indicated by non-overlapping fiducial limits).

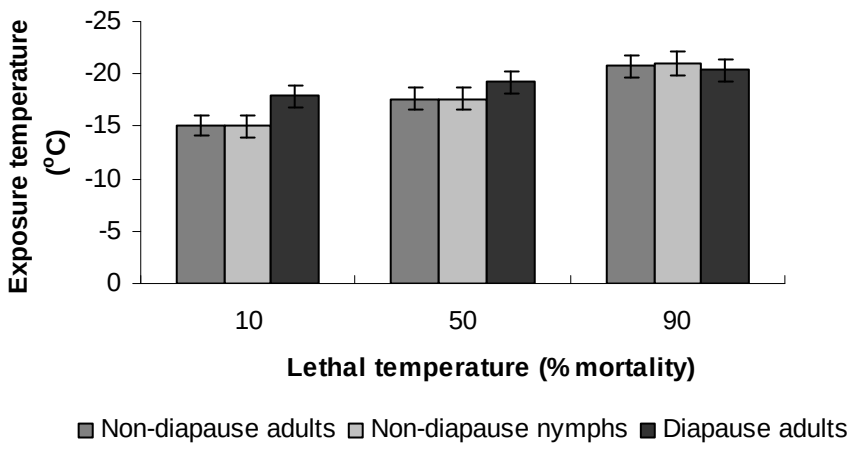

Figure 1: $LTemp_{10, 50, 90}$ (± 95% fiducial limits) of nymphal, non-diapausing and diapausing adult *D. hesperus*.

3.4 *Lower lethal time*

The lethal times for 10,50 and 90% ($LTime_{10,50,90}$) mortality at -5, 0 and 5°C of fed and unfed nymphal, non-diapausing and diapausing adult *D. hesperus* are shown in Figures 2 A, B and C .

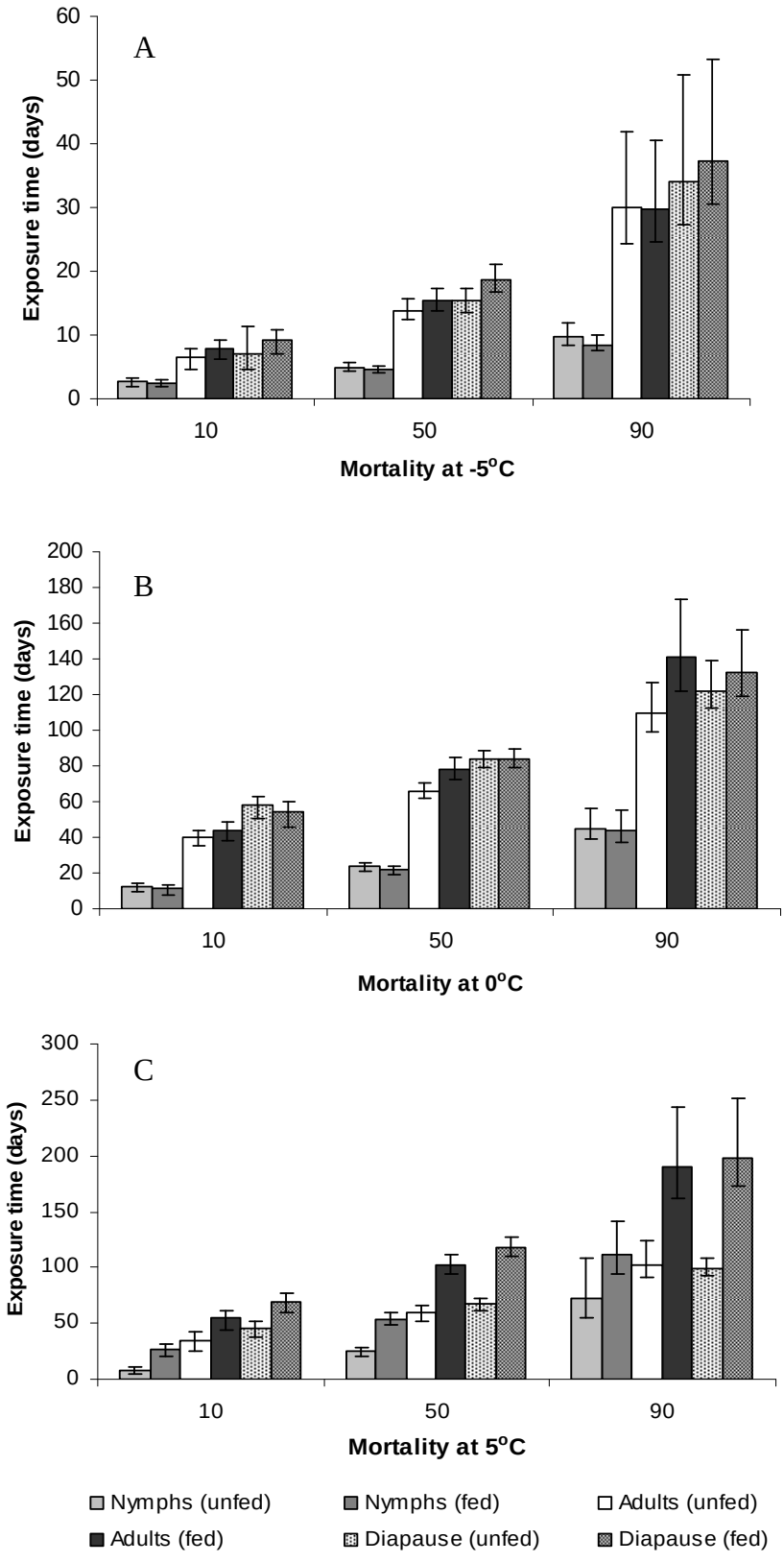

Figure 2: $LTime_{10,\ 50,\ 90}$ (± 95% fiducial limits) of fed and unfed nymphal, non-diapausing and diapausing adult *D. hesperus* at -5 (A), 0 (B) and 5°C (C).

There were no significant differences in survival between fed and unfed non-diapausing nymphs and adults or fed and unfed diapausing *D. hesperus* at -5 or 0°C. However, both fed and unfed nymphs survived significantly less time than both non-diapausing and diapausing adults at -5 and 0°C (indicated by non-overlapping fiducial limits). At 5°C, fed individuals of all treatments survived longer than unfed *D. hesperus*. Fed non-diapausing and diapausing adults survived significantly longer than fed nymphs, but there was no significant difference between the two adult treatments. At 5°C the $LTime_{50}$ was 54, 101.7 and 117.5 days for fed nymphs, non-diapausing and diapausing adults respectively.

3.5 *Field experiments*

Mortality increased in all treatments during the field exposures, but at different rates and with different final levels of survival (Figures 3-5). Trials began in mid-November 2005 for treatments 1-4 and early December 2005 for treatments 5 and 6. The mean, maximum and minimum temperatures from 16 November 2005 until 13 April 2006 were 4.3, 17 and -3.1°C respectively; mean maximum and minimum temperatures for each month are shown in Table 2. On the 29 December 2005 the temperature was never above -1°C. Between 18 November 2005 and 23 March 2006 (first and last days with temperatures below 0°C), temperatures dropped below 0°C on 34 of a possible 125 days (Figure 6). Thus, this winter was one of the coldest recorded in the UK in recent years.

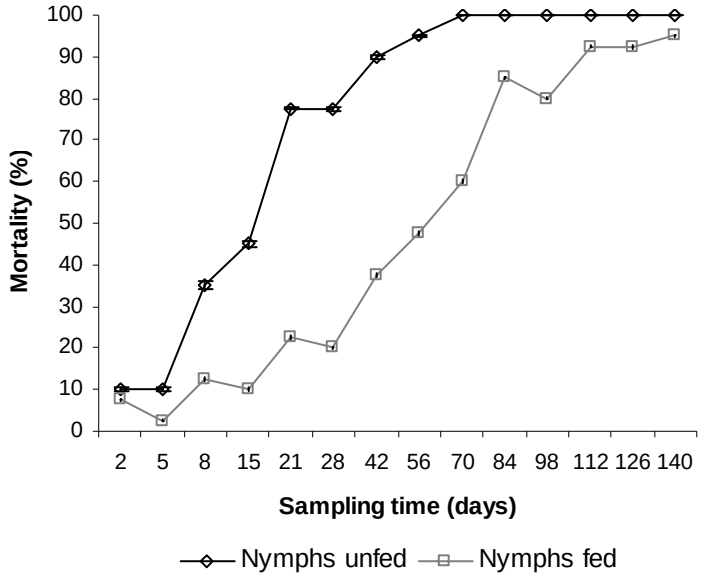

Figure 3: Mortality (%) (± SE) of fed and unfed nymphal *D. hesperus* placed in the field on 16 November 2005.

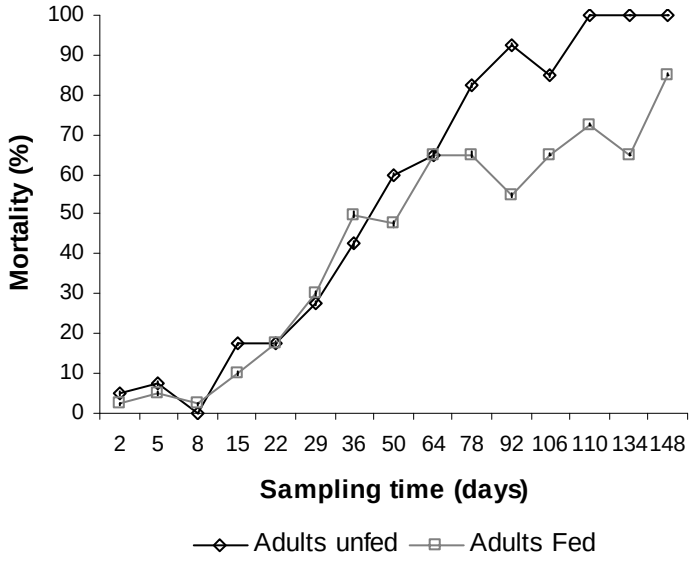

Figure 4: Mortality (%) (± SE) of fed and unfed non-diapausing adult *D. hesperus* placed in the field on 16 November 2005.

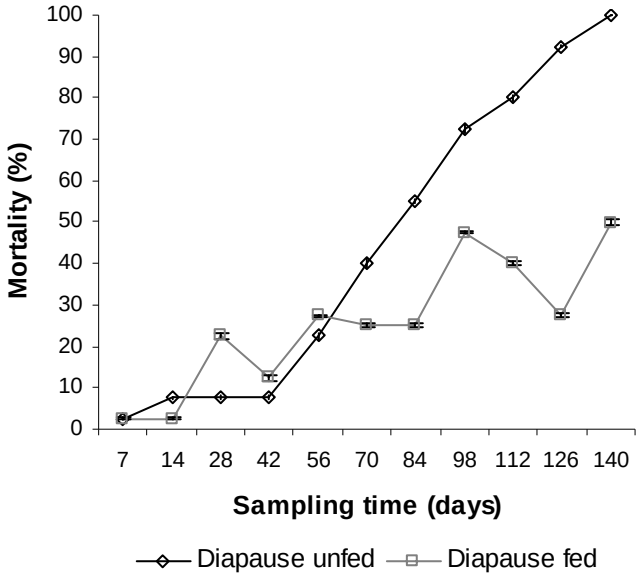

Figure 5: Mortality (%) (± SE) of fed and unfed adult *D. hesperus* reared under a diapausing inducing regime and placed in the field on 7 December 2005.

After 140 days in the field, 5% of nymphs were still alive (unfed nymphs all died after 70 days in the field), and when returned to the laboratory at 23°C after this period of time, developed to adulthood. After 148 days (5 months) in the field, 15% of fed adults were still alive (unfed adults all died after 110 days) and when returned to the laboratory laid eggs which hatched and developed into adults. They continued to lay viable eggs for 32 days. *Dicyphus hesperus* reared under the diapause inducing regime all died after 140 days when unfed, but 50% of fed individuals were still alive after this time, and when returned to the laboratory, mated and laid eggs which hatched and developed to adulthood. They survived and laid viable eggs for 23 days in the laboratory. All nymphs in the control experiments reached adulthood.

Table 2: Mean, minimum and maximum monthly temperatures at field site for entire exposure period of *D. hesperus*.

Month	Temperature (°C)		
	Mean	**Min**	**Max**
November (from 16th of the month)	1.7	-1.4	7.3
December	3.3	-2.2	8.8
January	3.2	-1.4	8.1
February	2.8	-2.7	8.1
March	5.4	-3.1	15.6
April (up to 13th of the month)	7.7	1.1	17.1

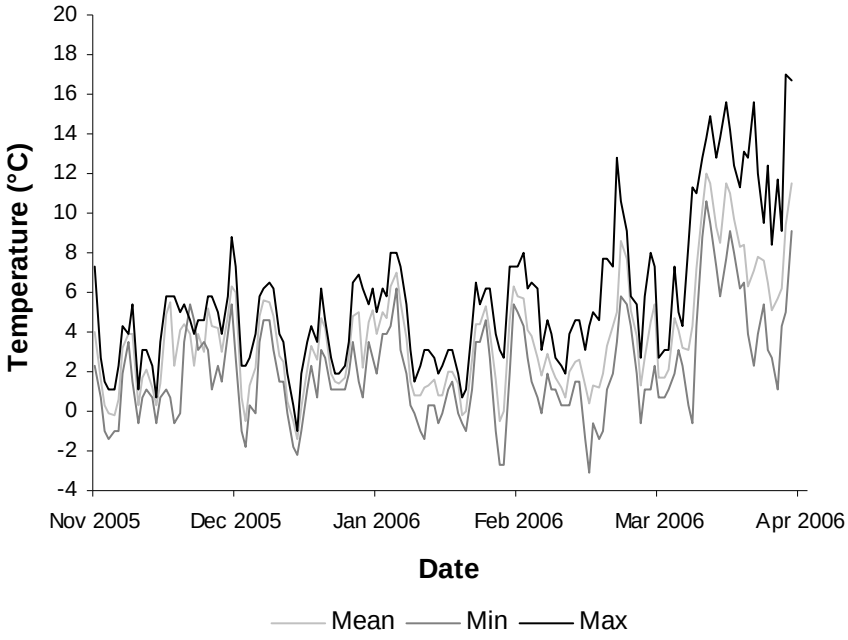

Figure 6: Mean, minimum and maximum field temperatures recorded daily during exposures of *D. hesperus*.

Survival of *D. hesperus* was higher at the end point of the winter experiment than in other species tested (Table 3). Most notably, 50% of diapausing *D. hesperus* were still alive after 140 days in the field. It is interesting to note that *N. californicus* has been

shown to overwinter in the UK and in comparison, greater numbers of *D. hesperus* survived in the field during winter.

Table 3: Comparison of survival (%) of 5 non-native biocontrol agents at the end points of field experiments during UK winters.

Species	Life stage (all fed)	Survival (%) at end point of experiment
Neoseiulus californicus	Non-diapausing adult	10% after 112 days
Macrolophus caliginosus	Non-diapausing adult	0% after 75 days
	Nymphs	3% after 200 days
Eretmocerus eremicus	Larvae	0% after 30 days
Typhlodromips montdorensis	Adults and larvae	0% after 30 days
Dicyphus hesperus	Non-diapausing adult	15% after 148 days
	Diapausing adult	50% after 140 days
	Nymphs	5% after 140 days

3.6 *Data comparison of* D. hesperus *and* M. caliginosus

A comparison of laboratory and field data obtained for adult non-diapausing *D. hesperus* and *M. caliginosus* is shown in Table 4. The latter mirid is found outside of glasshouses in the UK in winter, although all year round establishment is yet to be confirmed. From these data, it is clear that *D. hesperus* is more cold tolerant than *M. caliginosus*.

Table 4: Comparison of laboratory and field data obtained for adult unfed non-diapausing *D. hesperus* and *M. caliginosus*

Species	DT (^{o}C)	SCP (^{o}C)	LTemp$_{50}$ (^{o}C)	LTime$_{50}$ (days) at 5^{o}C	Maximum field survival (days)
D. hesperus	8.0*	-20.0	-17.6	60	110**
*M. caliginosus****	7.7	-20.2	-11.9	32.4	75

* Data from (Gillespie and Sanchez, 2004)

** For fed individuals, 15% were still alive after 148 days

*** Data on *M. caliginosus* from (Hart *et al.*, 2002b)

Section 4

Discussion

4 Discussion

The aim of this work was to determine the cold tolerance of *D. hesperus* and make recommendations on the likelihood of establishment outside the glasshouse environment in northern Europe. To put the results of *D. hesperus* into context, comparisons are made throughout this discussion with another non-native mirid *M. caliginosus* and to a lesser extent with the other species that have been evaluated using the cold tolerance screening regime used in this study.

The supercooling points recorded in the present study for *D. hesperus* were only 1°C lower than those previously recorded in the mirid *M. caliginosus*. There were no differences in lethal temperatures between *D. hesperus* nymphs, non-diapausing and diapausing adults. Comparing the lethal temperature data to the SCPs of *D. hesperus* suggests that there is a small level of pre-freeze mortality as the mean SCP for diapausing adults is -21°C but the $LTemp_{50}$ was -19.2°C. However, the $LTemp_{90}$ was similar to the recorded SCPs, suggesting that some individual *D. hesperus* were dying at or very close to their freezing temperature. The level of pre-freeze mortality was slightly lower than in *M. caliginosus* (Hart *et al.*, 2002b).

The $LTime_{50}$ at 5°C for unfed *D. hesperus* adults was 60 days in the present study. In a previous identical experiment, the $LTime_{50}$ at 5°C for unfed *M. caliginosus* adults was 32.4 days (Hart *et al.*, 2002b). At all three exposure temperatures, nymphs survived less time than non-diapausing and diapausing adults. This is partly due to the lower cold tolerance of nymphs, but may also be because adults had been feeding when experiments began whereas nymphs were used directly upon emergence from the egg and therefore may have not had accumulated any fat reserves. At 5°C the $LTime_{50}$ was 54, 101.7 and 117.5 days for fed nymphs, non-diapausing and diapausing adults respectively. Although there was no significant difference in survival between non-diapausing and diapausing adults, the latter still survived longer and this may be due to increased cold tolerance in diapausing individuals. At -5° and 0°C there were no significant differences between fed and unfed individuals; however at 5°C, survival of fed individuals was longer in all treatments. This suggests that feeding was not possible at the lower two temperatures, but at 5°C feeding was

important in lengthening survival of *D. hesperus*, which helps to explain the increased field survival of fed over unfed individuals.

All of the life cycle stages of *D. hesperus* examined in this project were able to survive throughout a colder than average UK winter, with 50% survival in fed diapausing adults after 148 days in the field (end point of experiment). Both the British Columbian and the Californian strain of *D. hesperus* can enter diapause (Gillespie and Quiring, 2005). Unfed non-diapausing and diapausing adults survived for 110 and 140 days respectively. The addition of prey increased field survival and the polyphagous nature of *D. hesperus* suggests that it is likely to be able to find food outdoors in winter should it be required. By comparison, unfed adult *M. caliginosus* survived for a maximum of 75 days in the field (Hart *et al.*, 2002b)

On return to the laboratory after nearly 5 months in the field, non-diapausing and diapausing fed *D. hesperus* adults mated successfully and laid eggs that formed viable populations. Fed nymphs returned to the laboratory after the winter in the field also developed to adulthood. It is likely that *D. hesperus* would quickly lay eggs in the field as long as a suitable prey-host plant combination was found outside the glasshouse. Even unfed adults that survived for 110 days in the field are likely to be able to either obtain food by re-entering a glasshouse or consume prey such as field populations of aphids that will begin to build up in April in northern Europe. Some individuals within the population may resort to cannibalism in times of prey shortage to prolong survival (Laycock *et al.*, 2006). Surviving nymphs that were able to develop through to adulthood when returned to the laboratory after being in the field for 140 days would also be able to return to the glasshouse throughout winter. It is likely that unfed adults survived longer than unfed nymphs in the field as they would have been feeding and thus would have accumulated fat reserves when the experiments started. First instar nymphs that were used for the experiments had just emerged from the eggs and may not have fed and therefore had less fat reserves than the adults.

Despite the diapause trial being started later than the non-diapause trials, sufficiently low temperatures were experienced during December, January and February to

suggest that the increased survival of diapausing adults when compared with non-diapausing adults was due to a biological trait and not due to differences in temperature. Nevertheless, the current study demonstrates *D. hesperus* can survive an entire winter even when not in diapause. Other mirids such as *M. caliginosus* can actively seek shelter during winter months (Hart *et al.*, 2002b) and as this is likely to be the case with *D. hesperus*, it may not require a diapause life stage to overwinter successfully in the northern Europe. The comparative table of field data obtained from previous studies shows that survival of *D. hesperus* was much higher than the 5 other species at the end points of the experiments. Overall, the laboratory and field data show that *D. hesperus* is sufficiently cold tolerant to survive a 'typical' winter in temperate climates. Previous work has shown that development of *D. hesperus* from egg hatch to adult is approximately 48 and 22 days at 14 and 22°C respectively, and development to adulthood was still possible at 35°C; an overall developmental threshold of 8°C was calculated (Gillespie and Sanchez, 2004). This supports the fact that *D. hesperus* is likely to be able to comfortably complete its development within a northern European climate. The developmental threshold of *M. caliginosus* was estimated to be between 7.7 and 8.4°C and the theoretical number of generations possible in the UK was 2 (Hart *et al.*, 2002b). It is likely that *D. hesperus* will also be able to complete a minimum of two generations a year in the UK and countries with a similar climte, as developmental times from egg to adult of 34.9 days at 22°C have previously been recorded for this species (Gillespie and Sanchez, 2004), and these are very similar to developmental times recorded for *M. caliginosus* (35.9 days at 23°C) (Hart *et al.*, 2002b).

A previous risk classification system used to compare $LTime_{50}$ at 5°C in the laboratory with maximum field survival (Hatherly *et al.*, 2005b) would place *D. hesperus* in the 'high risk' group, as it is able to survive a UK winter as a non-diapause population and in addition, some strains are able to enter diapause. Under this system, *M. caliginosus* has been classed as an 'intermediate risk', as survival for an entire winter in the field was marginal.

If a species such *D. hesperus* is ecophysiologically able to survive outdoors through northern European winters, then for long term outdoor establishment, it must also be

able to utilise wild prey or other food resources. No work on prey consumption was conducted in the present study; however, *D. hesperus* is known to be polyphagous and has previously been shown to feed on *T. vaporariorum*, *T. urticae* (McGregor *et al.*, 1999) and *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (Shipp and Wang, 2006). It is likely that *D. hesperus* will also be able to feed on common aphids such as *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) and outdoor whitefly species such as cabbage whitefly, *Aleyrodes proletella* (Linnaeus) (Hemiptera: Aleyrodidae), but this assumption would need to be tested.

Dicyphus hesperus is the sixth species to be subjected to a screening protocol to predict establishment potential outside the glasshouse. In the current investigation the $LTime_{50}$ at 5°C of unfed adult *D. hesperus* was 60 days and the corresponding field survival was 110 days. This further strengthens the relationship between laboratory and field survival (Hatherly *et al.*, 2005b) as shown in Figure 7 in which *D. hesperus* is the most cold hardy species so far investigated.

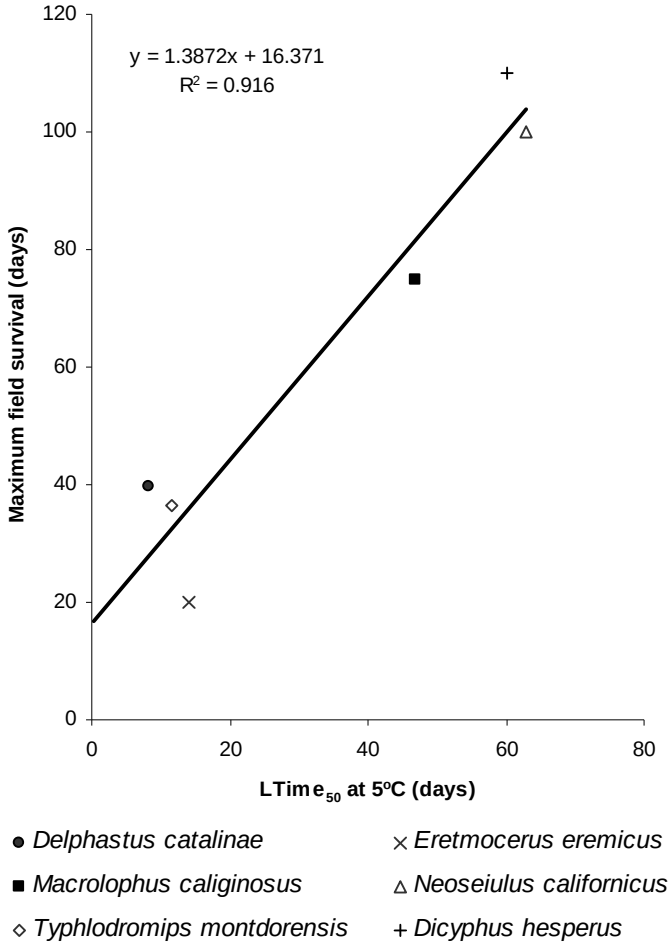

Figure 7: Relationship between maximum field survival (days) and $LTime_{50}$ at 5°C (days) for 6 non-native biocontrol agents. Data refer to unfed adults of all species except *E. eremicus* that were exposed as unfed larvae (adapted from Hatherly *et al.*, 2005b).

This relationship may benefit biocontrol companies with limited research and development budgets, as the expensive and time consuming field trials could at least initially be avoided. The $LTime_{50}$ should be viewed as an initial rapid screen ('quick scan') for establishment potential, identifying the need for further experimentation. Only with species where the risk of establishment was unclear from the laboratory data would field studies need to be conducted. It is likely that a candidate species with a short $LTime_{50}$ at 5°C will also have a short winter field survival and therefore the risk of establishment may be low, for example, in the predatory mite *Typhlodromips montdorensis*. Conversely, the reverse would be true where a long $LTime_{50}$ would

indicate that substantial long term field survival of the species is likely, as with *D. hesperus*.

The current work has shown that *D. hesperus* nymphs and non-diapausing and diapausing adults are able to survive an entire UK winter outdoors, and the supplementation of food significantly increases survival further. After nearly 5 months in the field, nymphs returned to the laboratory developed to adulthood and non-diapausing and diapausing adults were able to mate and form viable populations after return to the laboratory. The comparison with other species subjected to the current screening protocol shows that *D. hesperus* is the most cold tolerant of the 6 species and that establishment of this species outdoors is likely in northern Europe.

Section 5

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5 References

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