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Use of thermal data as a screen for the establishment potential of non-native biological control agents in the UK

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Abstract. A series of recent studies have investigated the development, cold tolerance and winter field survival of glasshouse biological control agents that are non-native to the UK: *Neoseiulus californicus*, *Macrolophus caliginosus*, *Delphastus catalinae*, *Eretmocerus eremicus* and *Typhlodromips montdorensis*. The combined data sets for these species have been analysed to identify laboratory indices of cold tolerance that correlate with the duration of survival under winter field conditions. A strong correlation was found between the laboratory LTime$_{50}$ at 5 °C and maximum field survival time. Both *N. californicus* and *M. caliginosus* survived substantially longer in the laboratory and in the field than the other species. It is suggested that the LTime$_{50}$ at 5 °C may provide a reliable 'first stage' screen of establishment potential for candidate non-native biological control agents in the UK.

Key words: biocontrol, cold tolerance, diapause, environmental risk assessment, establishment potential, non-native species, overwintering

Introduction

Temperature is the most important factor affecting establishment of non-native ectotherms in the UK (Bale and Walters, 2001). To survive, an alien arthropod must have an adequate thermal budget to complete its life cycle, reproduce and prepare to survive the winter, either in diapause or as a cold tolerant non-diapausing life stage. Long term establishment will also depend upon the ability of the species to locate and utilise wild prey or host sources.

Licences for the release of non-native biological control agents into the UK are granted by the Department for Environment, Food and Rural Affairs (DEFRA). Information supplied by biological control companies as part of the licensing system constitutes a form of risk

assessment, covering areas such as similarities and differences in climate between the native and introduced countries, host range and overwintering. Applications are usually compiled on the basis of 'available information'. However, this approach is problematic, because the information necessary to compile a comprehensive risk assessment dossier is often unavailable, as the relevant experimental studies have never been done. In areas where key information is lacking, other forms of data have been used as a 'proxy' in the risk assessment. One of the best examples of this situation is the absence of direct assessments of cold tolerance and the use of 'climate matching' between the native and introduced ranges, as a means of predicting likely survival in the introduced environment (Cameron et al., 1993; Goolsby et al., 2005). Most non-native glasshouse biological control agents introduced into the UK are of Mediterranean, subtropical or tropical origin (van Lenteren, 1997) and in consequence, it has often been assumed that they lack the necessary cold tolerance to establish outside the glasshouse environment in temperate climates such as the UK. This assumption has been drawn into doubt following observations of the mirid bug, *Macrolophus caliginosus* Wagner (Heteroptera: Miridae) outside glasshouses during winter after it was first introduced into the UK in 1995 (Hart et al., 2002b). Additionally, the predatory mite *Neoseiulus californicus* McGregor (Acari: Phytoseiidae) was introduced in 1991 and has since established locally in the UK (Jolly, 2000). The establishment of non-native species outside the glasshouse may have undesirable effects on native populations, either by competition or predation (Howarth, 1991; Simberloff and Stiling, 1996a, b; Ehler, 1999; van Lenteren and Manzaroli, 1999).

In designing a risk assessment protocol for establishment potential of non-native biological control agents, a balance has to be reached between the range of information that is crucial in making informed decisions, and the ability of biological control companies to fund the necessary research, especially in areas where essential data are not available. Against this background, studies have been undertaken on the thermal biology of a number of non-native biological control agents with the aim of identifying laboratory indices of development and survival that predict establishment potential in the field (Hart et al., 2002a, b; Hatherly et al., 2004; Tullett et al., 2004). The attraction of such a system is that it would streamline the range of experiments necessary for the licensing of future candidate species.

An experimental protocol covering a wide range of tests to reflect the variety of ways that low temperatures may affect insect populations has now been applied to five biological control agents that are

not native to the UK (Hart et al., 2002a, b; Hatherly et al., 2004; Tullett et al., 2004). Of these, work on *N. californicus* (Hart et al., 2002a), *M. caliginosus* (Hart et al., 2002b), *Delphastus catalinae* Gordon (Coleoptera: Coccinellidae) (Tullett, unpublished data) and *Eretmocerus eremicus* Rose & Zolnerowich (Aphelinidae: Hymenoptera) (Tullett et al., 2004) was conducted post-release, and studies on *Typhlodromips montdorensis* Schicha (Acari: Phytoseiidae) (Hatherly et al., 2004) were undertaken prior to any licence application for this species. This paper summarises the results of these studies and identifies a common relationship between a laboratory index of cold tolerance and winter survival in the field that has the potential to be used as a pre-release screen for candidate biological control agents.

Results and discussion

The summary data for the five species is presented in Table 1. Where possible, results are of comparable life stages and physiological states between species. Developmental thresholds (DT), thermal budgets (in day-degrees) and voltinism (number of theoretical generations a year) were determined using weighted linear regression as calculated from the egg to adult developmental times of each species at varying temperatures. Supercooling points (SCPs), lethal temperature required to kill 50% of the population (LTemp$_{50}$), lethal time required to kill 50% of the population at 5 °C (LTime$_{50}$), and maximum field survival are for non-acclimated unfed adults. The exception was *E. eremicus*, where LTime$_{50}$ at 5 °C and maximum field survival are for unfed non-acclimated larvae. *Eretmocerus eremicus* were exposed as larvae within parasitised hosts as this is the form in which this species is shipped by biocontrol companies and is also the usual overwintering stage for parasitoids in the UK. Full details of all methods are given in Hart et al. (2002a, b), Hatherly et al. (2004) and Tullett et al. (2004).

Developmental threshold, thermal budget and voltinism

Weighted linear regression was used to estimate the developmental threshold of each species (Draper and Smith, 1981). This method proved to be consistently more accurate than the use of simple linear regression (Campbell et al., 1974), as the estimated developmental thresholds were closer to the observed limits to development in the laboratory experiments. *Macrolophus caliginosus* has the highest thermal budget above its developmental threshold and *T. montdorensis* the lowest. However, the higher developmental threshold of

Table 1. Summary of laboratory and field data obtained for five non-native biological control agents

Species	DT (°C)	Thermal budget	Voltinism	SCP (°C)	$LTemp_{50}$ (°C)	$LTime_{50}$ (days) at 5 °C	Acclimation effect in: laboratory/field (Y/N), x = not conducted	Maximum field survival (days)
D. catalinae	9.8	321.5	2	−21.5	−11.7	8.3	yes/x	28
E. eremicus	9.2	310.1	3	−25	−20.5	14	yes/yes	30
M. caliginosus	7.7	495	2	−20.2	−11.9	32.4	no/x	75
N. californicus	8.6	142.9	7	−21.6	−13.9	38.6	yes/x	100
T. montdorensis	10.3	108.7	6	−24.1	−6.7	9.5	yes/no	35

T. montdorensis means that development through its life cycle is likely to start later in the favourable season for growth and development than for *M. caliginosus*. The low developmental thresholds of *M. caliginosus* and *N. californicus* increase their potential to mature to adult and begin reproduction soon after winter. The number of generations theoretically possible each year in the West Midlands region of the UK calculated from temperature data from the School of Geography, Earth and Environmental Sciences at the University of Birmingham was highest for *N. californicus*, and this was the only species that could complete a generation during winter (Hart et al., 2002a). Even if *D. catalinae* and *E. eremicus* escaped from the glasshouse during the summer, only two and three generations respectively would be possible before winter intervenes, and therefore the impact on non-target species would be transient. *Typhlodromips montdorensis* may be able to complete up to six generations a year outside the glasshouse, but development would begin later in the year, when populations of both target and non-target prey would be higher.

SCPs, LTemp$_{50}$, LTime$_{50}$ at 5 °C

For all five species, mean SCPs were below −20 °C and therefore well below temperatures likely to be experienced during a UK winter. Pre-freeze mortality was 100% in all species after being cooled to the exposure temperature at 1 °C min^{-1} and held at the minimum temperature for 1 min. The LTime$_{50}$ at 5 °C was longest for *M. caliginosus* and *N. californicus* which was reflected in the field experiments, where survival was much longer than for the remaining three species, that had correspondingly shorter survival times in the laboratory at 5 °C.

Acclimation

The acclimation regime used for all five species was intended to detect a capacity for acclimation rather than to determine the full extent of such a response. The regime used was 7 days at 10 °C, LD 12:12 h. The exception was *E. eremicus*, where individuals were held for 3 days as longer exposure in this species proved to be deleterious (some mortality) and thus may have masked any short term response (Tullett et al., 2004). Under laboratory acclimation, *D. catalinae*, *E. eremicus* and *T. montdorensis* survived slightly lower temperatures and for slightly longer than non-acclimated individuals. In contrast, there was a much stronger acclimation response exhibited by *N. californicus* (Hart et al., 2002a). In the field, acclimated *E. eremicus* survived

slightly longer than non-acclimated individuals; however, the difference was again minimal (Tullett et al., 2004). Acclimated *T. montdorensis* did not survive longer in the field than non-acclimated individuals (Hatherly et al., 2004), suggesting that small acclimation responses in the laboratory may not be indicative of greater survival in the field.

Field survival

Experiments to determine the maximum field survival of each species were started in October and November of each experimental year and further experiments were conducted later in each winter. Temperatures recorded during experiments were 'typical' for winters in the West Midlands region of the UK (data reviewed over 15 years) and a wide range of temperatures were experienced by each species whilst in the field. *Delphastus catalinae*, *E. eremicus* and *T. montdorensis* survived for approximately 4 weeks in the field. Field survival of *T. montdorensis* was not increased by the provision of prey (Hatherly et al., 2004). This suggests that temperature was a more limiting factor for survival than the lack of food. In contrast, whereas unfed *N. californicus* survived for 100 days, when prey was provided, 10% of mites were still alive after 112 days (Hart et al., 2002a). Similarly 3% of fed *M. caliginosus* nymphs survived and continued development for up to 200 days in the field, considerably longer than unfed nymphs (75 days) (Hart et al., 2002b). This suggests that temperature was not entirely limiting for *N. californicus* and *M. caliginosus* and that providing prey was available, survival could be increased to span almost an entire winter.

The ability of species to enter diapause in winter has been investigated in *N. californicus* and *T. montdorensis*. Some strains of *N. californicus* are able to diapause (Jolly, 2000) and it is likely that this species can use a more cold tolerant diapausing trait to aid its winter survival in the UK. No diapause was observed in *T. montdorensis* (Hatherly et al., 2004, 2005), and diapause was not investigated in *D. catalinae*, *E. eremicus* and *M. caliginosus*. *Macrolophus caliginosus* is thought to actively seek shelter during the winter months and is capable of feeding and developing throughout the year in its native habitat. A diapause stage may not therefore be necessary for this species to overwinter successfully in the UK (Hart et al., 2002b).

The laboratory data suggest that *N. californicus* and possibly *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 experiments conducted for all five species involved a number of

laboratory treatments, the most important of which included comparisons between different life stages, acclimated and non-acclimated populations, and fed and unfed groups. The various laboratory indices of cold tolerance of individual treatment groups of comparable life cycle stages and physiological states were compared with field survival of the equivalent group as summarised in Table 1. Pearson's product moment correlation coefficient revealed a strong positive correlation ($p = 0.005$) between the laboratory $LTime_{50}$ at 5 °C and the field survival of unfed adults (larvae for *E. eremicus*) as shown in Figure 1. A Bonferroni correction (Legendre and Legendre, 1998) was applied to control for elevated type 1 errors due to multiple comparisons ($p = 0.03$ after Bonferroni). There were no other significant correlations between the other four predictors of field survival as shown in Table 2.

Neoseiulus californicus had the longest $LTime_{50}$ in the laboratory, with populations surviving for up to 3 months in the field; also, the mites reproduced before dying. Diapause strains of *N. californicus* are able to survive throughout the winter (Jolly, 2000). In contrast, *T. montdorensis* has a low $LTime_{50}$ and field survival is correspondingly short. Field survival of *N. californicus* and *M. caliginosus* was increased by the provision of prey but not in *D. catalinae* and *T. montdorensis* (not tested in *E. eremicus*). If *N. californicus* and *M. caliginosus* are able to utilise wild prey outside the glasshouse, their field survival times are likely to increase. When the $LTime_{50}$ at 5 °C and maximum field survival time are compared (Figure 2), a similar relationship to that shown in Figure 1 emerges, but with less of the variation explained.

Table 2. Pearson product moment correlation and corresponding *p* values of laboratory experiments compared with maximum field survival

Laboratory experiment correlated against maximum field survival (days)	Pearson correlation	*p*-Value
DT (°C)	−0.73	0.16
Thermal budget	−0.02	0.98
Voltinism	0.44	0.46
SCP (°C)	0.6	0.29
$LTemp_{50}$ (°C)	0.006	0.99
$LTime_{50}$ (days) at 5 °C	0.97	0.005

The analysis suggests that laboratory assessment of the $LTime_{50}$ at 5 °C could be a reliable predictor of field survival for five biological control agents, representing different taxonomic groups and trophic guilds. According to Bale (1996) *N. californicus* would be classified as chill tolerant, with some pre-freeze mortality in the laboratory but capable of surviving for a number of months in winter. *Delphastus catalinae*, *E. eremicus* and *T. montdorensis* would be regarded as chill susceptible with extensive pre-freeze mortality in brief exposures. *Macrolophus caliginosus* belongs to an 'intermediate' category. Species that survive down to the SCP, for example the goldenrod gall moth, *Epiblemma scudderiana* Clemens (Lepidoptera: Tortricidae) (1996;
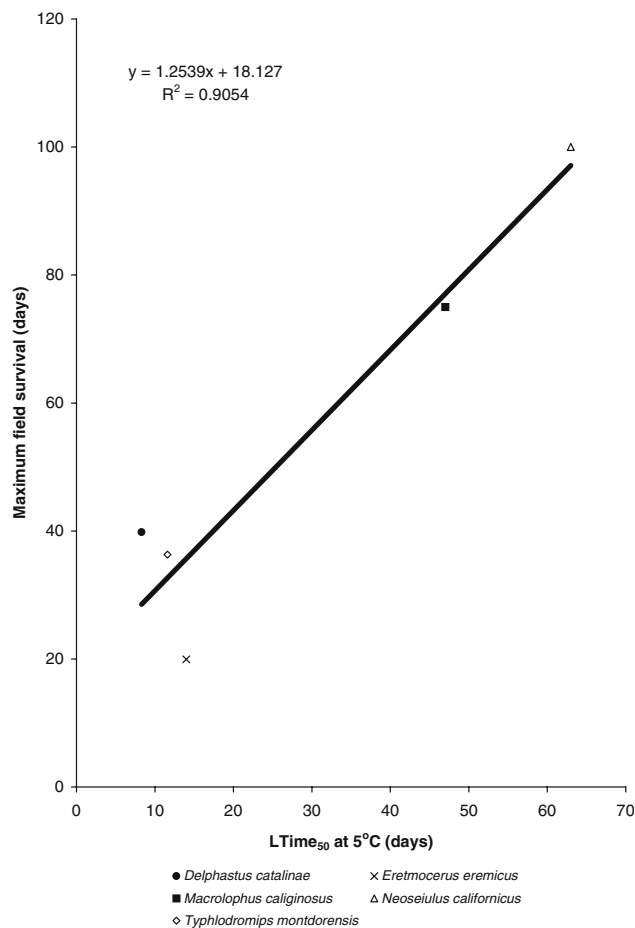

Figure 1. Relationship between maximum field survival (days) and $LTime_{50}$ at 5 °C (days) for five non-native biological control agents (data refer to unfed adults of all species except *E. eremicus* that were exposed as unfed larvae).
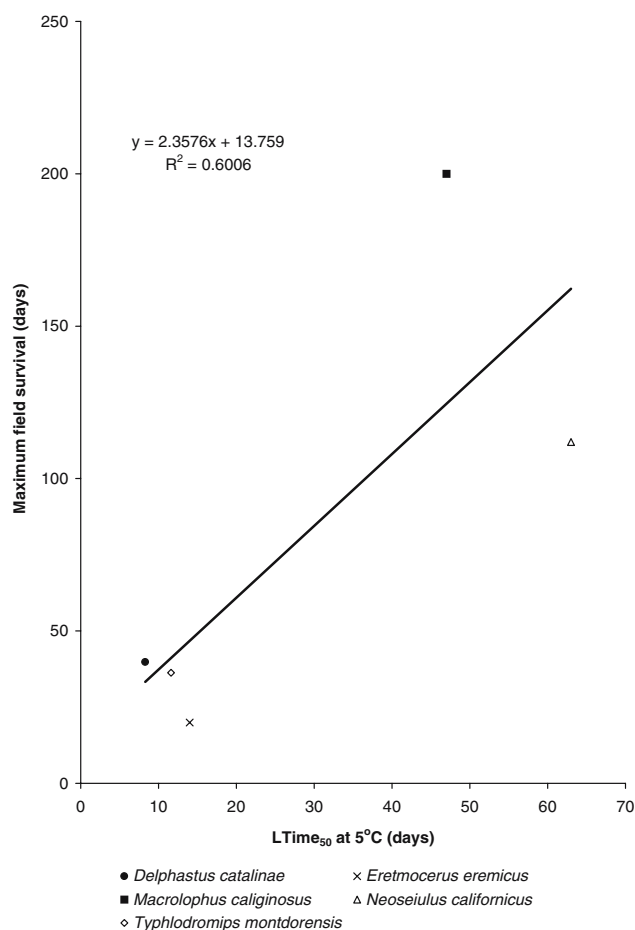
$y = 2.3576x + 13.759$
$R^2 = 0.6006$

Figure 2. Relationship between maximum field survival (days) and $LTime_{50}$ at 5 °C (days) of *N. californicus*, *M. caliginosus* (both fed), *D. catalinae*, *E. eremicus* and *T. montdorensis* (all unfed).

Rickards et al., 1987) and the autumnal moth, *Epirrita autumnata* Borkhausen (Lepidoptera: Geometridae) (Nilssen and Tenow, 1990) are freeze avoding. These species and freeze tolerant arthropods for example the hoverfly *Syrphus ribesii* Linnaeus (Diptera: Syrphidae) (Hart and Bale, 1997) generally inhabit colder regions of the world, and none are used as biocontrol agents. The vast majority of insects show some pre-freeze mortality and in such species there is likely to be a correlation between low temperature survival time in the laboratory and survival in the field. There is every reason to believe that the relationship observed across the five species studied in this investigation will have much wider applicability.

This system may allow candidate biological control agents to be classified into different 'risk categories'. *Delphastus catalinae*, *E. eremicus* and *T. montdorensis* are part of a 'low risk' group, where 100% field mortality occurs within 4 weeks and any establishment is unlikely. An 'intermediate risk' group would contain *M. caliginosus*, where survival may persist for extended periods outdoors in winter with limited establishment. *Neoseiulus californicus* would fall into a 'high risk' group, where some strains are able to overwinter in diapause and non-diapause strains survive long enough to develop and reproduce. Risk indices can then be used in combination with other information to decide whether a non-native species should be released (van Lenteren et al., 2003).

This protocol is a timely response to current discussions within the European Union to increase the use of 'environmentally friendly' biological control without compromising the safety of native ecosystems. If the relationship between $LTime_{50}$ at 5 °C and field survival proves to be consistent across further species, it would also benefit biological control companies that have limited research and development budgets, as field experiments are both time consuming and expensive. The $LTime_{50}$ should be seen as a rapid initial screen for establishment potential, the equivalent of a 'quick scan' identifying any need for further experimentation. For example, if a candidate non-native biocontrol agent has a long $LTime_{50}$ and is therefore likely to be able to survive all winter in the field, it will probably be rejected without the need for further costly work. 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 for predicting establishment potential. However, it may be too early to rely exclusively on a laboratory estimate of the $LTime_{50}$ at 5 °C as a predictor of field survival. Further studies on a wider range of species from other taxonomic groups would be desirable. Additionally, knowledge that a potential non-native glasshouse biological control agent may be able to establish outdoors is not in itself a reason to decline a licence application, as the overall benefits of release may outweigh the risk of an introduction.

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