EFFECTS OF TEMPERATURE ON THE ESTABLISHMENT OF NON-NATIVE BIOCONTROL AGENTS: THE PREDICTIVE POWER OF LABORATORY DATA

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ABSTRACT

The European Union (EU) and its constituent governments are committed to increasing the success of biocontrol in Europe, and are currently seeking a pan-European balanced regulatory system to aid this objective. The concept of ‘balance’ recognizes that (a) the complexity of any licensing system must be proportionate to risk, (b) industrial producers of biocontrol agents have limited R&D budgets, but (c) there can be no compromise on environmental safety. Whilst there is common agreement between agencies responsible for environmental protection, regulators and industrial producers, about the ecological information required to assess the establishment potential of non-native species, the research methods by which such data can be generated (if not available in the literature), have not been fully developed or tested. The inappropriate use of ‘climate matching’ between native and introduced ranges as a ‘proxy’ for cold tolerance and overwintering ability is one example of this problem.

Most predatory insects and mites used in glasshouse biocontrol in the UK originate from tropical and semi-tropical climates. For this reason, the licensing system for the introduction of non-native species has operated under the assumption that winter would act as a natural barrier to the establishment of such species outside of glasshouse environments. This view has been challenged by the establishment in the wild of the predatory mite Neoseiulus californicus (McGregor) (Acari: Phytoseiidae) and the discovery of the predatory mirid Macrolophus caliginosus Wagner (Hemiptera: Miridae) outside of glasshouses in winter. Whilst the impact of these species on native ecosystems is unknown, their establishment is considered undesirable. This paper describes a series of experiments used to determine a range of thermal characteristics (developmental threshold, day-degree requirement per generation, supercooling point, lethal times and temperatures, field survival) of five non-native biocontrol agents. A strong correlative relationship was found between the time at which 50% of populations die in the laboratory at 5°C (LTime_{50}) and duration of winter survival in the field. The comparative data provide a retrospective ecophysiological explanation for the establishment of N. californicus and occurrence of M. caliginosus outside of glasshouses, and also indicate that Delphastus catalinae (Gordon) (Coleoptera: Coccinellidae), Eretmocerus eremicus (Rose and Zolnerowich) (Hymenoptera: Aphelinidae) and Typhlodromips montdorensis (Schicha) (Acari: Phytoseiidae) would not survive outdoors in the UK under current climatic condi-
tions, and would therefore be ‘environmentally safe’ introductions. The experimental protocol applied to these species could be used as part of a routine, stepwise testing procedure for ‘establishment potential’ in the licensing system of non-native biocontrol agents in the UK and other parts of the world.

INTRODUCTION

Biological control has a long history of use in pest management, both as a method of control in its own right, and in combination with other techniques as part of IPM programs. In some respects, the importance and success of biological control has been overshadowed historically by pesticides, and more recently, by the prospect of insect-resistant GM crops, both of which have been viewed as a more generic approach to pest management, capable of being targeted against a range of pests in different climatic zones. However, there is now widespread international agreement on the need to reduce over-reliance on chemical pesticides, at the same time as the future of GM crops looks uncertain, particularly in Europe, not least because of public concern over risks to human health and the environment. By contrast, biological control is regarded as safe and environmentally friendly.

The definition of ‘success’ in biological control depends in part, on the environment into which an organism is released. In classical biological control, where relatively low numbers of a non-native predator or parasitoid are released into a new country or region of the world, often against an exotic pest, success can usually be defined by the ability of the introduced species to suppress numbers of the target pest below economic levels, and to become permanently established in the new area, thus reducing the need for re-releases. In inundative biological control, where large numbers of non-native natural enemies are released into glasshouses, pest suppression is again a criterion for success, but there is also the expectation that any organisms that escape from the protected environment will die out rapidly, and not cause any disruption to the native ecosystem. This is the ‘paradox of establishment’: in classical biological control, establishment is a key feature of success, whereas in inundative biological control in glasshouses, establishment outside of the protected environment is considered potentially deleterious.

INTERNATIONAL CONTEXT

Over the last 10-20 years there has been a developing trend toward international regulation for the import and release of non-native biological control agents, including the International Plant Protection Convention and the Convention on Biological Diversity. At the same time, various countries have introduced their own legislation to regulate importation of exotic species (United States, Canada, Australia, New Zealand, United Kingdom). Recently, a number of organizations have developed guidelines for the import and release of non-native biological control agents, in which an environmental risk assessment forms a central component. At the present time, the guidelines previously issued by FAO, EPPO and OECD are being harmonized to provide comprehensive guidance for EU member states and European countries under the auspices of IOBC-WPRS (Bigler et al. 2005) whilst the International Standard for
Phytosanitary Measures (ISPM 3) will soon provide revised advisory guidelines for all introductions of non-native biological control agents worldwide.

It is evident that biological control practitioners, programs and producers will become subject to greater regulation in the future than hitherto. It is however acknowledged that any new regulatory framework should be ‘balanced’, whereby the complexity of the licensing system is proportionate to the risk, without compromising environmental safety. However, there is already an identified problem that may hinder the implementation of new regulations: whilst the guidelines provide clear statements about the range of information that should be included in an environmental risk assessment, they do not indicate the methods by which such information should be obtained, especially when it is not available from the published literature.

ENVIRONMENTAL RISK ANALYSIS

It is self evident, but not always recognized, that there can be no long term negative effects on native species and ecosystems unless exotic species become permanently established in new environments; transient ‘summer only’ survival is unlikely to have any major impact. For this reason, an environmental risk analysis should first focus on the likelihood of successful establishment of non-native species.

The two most important factors affecting the establishment of non-native biological control agents are climate (especially temperature) and availability of prey. This knowledge can be utilized in the design of risk assessment protocols. In a step-wise testing procedure to assess the outdoor establishment potential of non-native species released into glasshouses in cool temperate climates, a case can be made for firstly investigating the effects of temperature on development and winter survival, followed by experiments on host range and non-target effects, in those species that appear to be capable of developing in summer and surviving through winter.

The difficulties of assessing the establishment potential of non-native biological control agents intended for inundative release in glasshouses are well illustrated by recent experience in the UK. Successful biological control has been implemented in glasshouses with the management of the whitefly *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) by the parasitoid *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae), and the spider mite *Tetranychus urticae* (Koch) (Acari: Tetranychidae) by the predatory mite *Phytoseiulus persimilis* (Athias-Henriot) (Acarina: Phytoseiidae). These two schemes have operated successfully over decades without any recorded establishment outside of the glasshouse in the cool climates of western Europe, or any deleterious effects on native fauna. Over the last 10-15 years, a number of ‘new’ species have been licensed for release in UK glasshouses. Although the UK licensing system requires companies to compile an environmental risk assessment dossier containing physiological and ecological information on the subject species, including overwintering ability and host range, this ‘critical information’ is often unavailable. As a classic example, in the absence of any direct assessment of cold tolerance, it has been assumed on the basis of ‘climate matching’, that winter would be an effective barrier to establishment in the UK of species originating from warmer climates. This assumption is incorrect, as evidenced by the outdoor establishment of the predatory mite *Neoseiulus californicus* after a first release...

In the light of the definite establishment of N. californicus and the possible establishment of M. caliginosus, a series of studies were undertaken to investigate the thermal biology of these two species, and two other species that had been licensed in the UK for the same periods of time, and for which there had been no reports of establishment or outdoor occurrence in winter (Eretmocerus eremicus and D elphastus catalinae). The same series of experiments were then conducted on a further species (Typhlodromips montdorensis) that was currently under study as a candidate for release in the U.K. (see H art et al. 2002a,b; H atherly et al. 2004; 2005; T ullett et al. 2004; for full details). It was envisaged that a comparative analysis of the thermal biology of established and non-established species might identify indices with ‘predictive power’ that could be applied to future candidate species in a step-wise risk analysis protocol.

MATERIALS AND METHODS

DEVELOPMENTAL THRESHOLD AND THERMAL BUDGET

Individuals of N. californicus, M. caliginosus, E. eremicus, D. catalinae and T. montdorensis were reared from egg to adult at a range of temperatures (5° to 35°C depending on the species) and the time taken to complete development recorded. The data were analyzed by weighted linear regression and the developmental threshold estimated by extrapolation of the linear relationship between development and temperature to the x (temperature) axis, and the thermal budget (day degree requirement per generation) by taking the reciprocal of the slope (C ampell et al. 1974).

Annual voltinism. The developmental threshold temperature and thermal budget values for each species were compared with daily temperature records over a 10 year period to calculate the annual number of available day degrees and hence the number of generations that could be completed each year. The temperature data were further divided into nominal summer (A pril to September) and winter (O ctober to M arch) periods to indicate if development could continue throughout the year or was restricted to summer.

COLD TOLERANCE

All experiments were carried out on both immature stages (larvae, nymphs) and adult organisms of the five species, with and without a period of prior acclimation (usually 7 days at 10°C). This regime was known to increase the cold tolerance of other species and was intended to identify any acclimation ability, rather than to produce ‘fully acclimated, winter hardy’ populations.

Supercooling points. The freezing temperature (supercooling point or SCP) was measured by cooling the organisms (n = 20 to 50 depending on species) at 1°C min⁻¹ in a Peltier cooling device, alcohol bath or differential scanning calorimeter, depending on the size of the specimens. The SCP was detected by the release of heat (exotherm) when the organisms froze.
Lethal temperatures. Replicate samples (3-5 x 10-50 specimens, depending on species) for each exposure temperature were cooled at 0.5 or 1°C min⁻¹ in a programmable alcohol bath to range of sub-zero temperatures (-5° to -20°C, depending on the species), exposed at the minimum temperature for 1 min, and then warmed back to the rearing temperature at the same rate. Survival was assessed 24h after exposure.

Lethal times. Replicate samples (3-10 x 10-50 specimens, depending on species) were maintained with and without target prey for increasing periods of time (days, weeks or months as appropriate) at -5°, 0° or 5°C, and mortality assessed 24h after return to the culture temperature.

FIELD EXPOSURES

Replicate samples (5 x 40-50 specimens) were placed in the field within sealed ‘quarantine boxes’, with and without prey, for increasing periods of time (days, weeks or months depending on the species), and returned to the laboratory after different exposure periods. Survival was assessed within 24h.

DIAPAUSE

The occurrence of diapause was investigated by maintaining different life cycle stages of *N. californicus* and *T. montdorensis* in various ‘diapause-inducing’ regimes (different LD cycles and temperatures), and monitoring reproduction in the emerging adults after return to normal rearing conditions.

RESULTS

The results for the two predatory mites, *N. californicus* and *T. montdorensis* are presented in Table 1 as examples of the types of data obtained in the range of experiments conducted on the five species. Full details on all species are given in Hart et al., 2002a, b; Tullett et al., 2004; Hatherly et al., 2004, 2005.

The developmental threshold is lower in *N. californicus* than *T. montdorensis*, though both species can complete an average of 6 generations under U.K. summer conditions; a key difference between the species is the ability of a non-diapausing strain of *N. californicus* to both develop and reproduce in winter. The freezing temperatures of adult females of the two species were similar and did not change after a period of acclimation. In both species there was evidence of substantial pre-freeze mortality with L Temp₅₀ values considerably above the mean SCP. However, the most striking differences between the species were in the L Temp₅₀, L Time₅₀ (at 5°C), and maximum survival times in the field in winter. In all these indices, *N. californicus* was clearly the more cold hardy species.

The data in Table 1, together with that for *M. caliginosus*, *E. eremicus* and *D. catalinae* were then analyzed (Pearson product moment correlation with Bonferroni correction for multiple comparisons), to identify any relationship between laboratory indices of development and cold tolerance (developmental threshold, thermal budget, SCP, L Temp₅₀ and L Time₅₀).
and survival in the field in winter. The only significant correlation was between the \( \text{LTime}_{50} \) (at 5°C) and maximum survival time in the field (\( r = 0.97, P < 0.005 \), Fig. 1; Hatherly et al. in press).

In the laboratory, \( N. \) californicus had the longest \( \text{LTime}_{50} \) and survived for the longest in the field (over 3 months); the mites also reproduced before dying. By contrast, \( T. \) montdorensis has a short \( \text{LTime}_{50} \) and died out quickly in the field. Also, provision of prey extended the survival time of \( N. \) californicus, with 10% still alive after about 4 months, when observations ended.

**Table 1.** Ecophysiological data for *Neoseiulus californicus* and *Typhlodromips montdorensis* as part of a risk assessment protocol.

<table>
<thead>
<tr>
<th>Index</th>
<th><em>N. californicus</em></th>
<th><em>T. montdorensis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Development</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Developmental threshold (°C)</td>
<td>8.6</td>
<td>10.3</td>
</tr>
<tr>
<td>Thermal budget (DD)</td>
<td>142.9</td>
<td>108.7</td>
</tr>
<tr>
<td>Mean annual voltinism</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Development in winter</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>Cold tolerance</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean SCP ± SE (°C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acclimated female</td>
<td>-22.2 ± 0.4</td>
<td>-22.4 ± 0.5</td>
</tr>
<tr>
<td>Non-acclimated female</td>
<td>-21.6 ± 0.3</td>
<td>-24.1 ± 0.6</td>
</tr>
<tr>
<td><strong>LTemp50 ± 95% fiducial limits (°C)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acclimated female</td>
<td>-17.7 ± 0.3</td>
<td>-11.5 ± 1.0</td>
</tr>
<tr>
<td>Non-acclimated female</td>
<td>-13.9 ± 0.3</td>
<td>-6.7 ± 1.1</td>
</tr>
<tr>
<td><strong>LTime50 ± 95% fiducial limits (days at 5°C)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acclimated female</td>
<td>65.4 ± 2.5</td>
<td>11.6 ± 1.1</td>
</tr>
<tr>
<td>Non-acclimated female</td>
<td>38.6 ± 1.9</td>
<td>9.5 ± 1.1</td>
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<tr>
<td><strong>Field survival</strong></td>
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<td></td>
</tr>
<tr>
<td>Maximum survival time (days)</td>
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<td></td>
</tr>
<tr>
<td>Without prey</td>
<td>100</td>
<td>35</td>
</tr>
<tr>
<td>With prey</td>
<td>112*</td>
<td>35</td>
</tr>
<tr>
<td>Reproduction in winter</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Ability to diapause+</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

*10% still alive after 112 days, +Refers to tested strain*
Environmental risk assessment (ERA) of non-native biological control agents, regulated by worldwide, European or country-specific legislation, is an inevitable reality over the next 5-10 years. Irrespective of the proven historical safety of biological control with non-native species, the ‘precautionary principle’ is now pervasive across all methods of pest management. The common task of scientists, the biological control industry, regulatory bodies, and environmental agencies, is to design and implement a system where the complexity and level of testing in the ERA is proportionate to the risk, without compromising environmental safety.

The recent IOBC-WPRS guidelines (Bigler et al. 2005), based on similar documentation from OECD (OECD 2004) and EPPO, together with a comprehensive review of risk assessment of non-native biological control agents (van Lenteren et al. in press) have all highlighted an essential requirement for any ERA: the testing should be conducted in a ‘step wise’ manner, such that species that are either demonstrably safe, or likely to establish and impact on native species or ecosystems, are identified early in the process. The likelihood of establishment is clearly a crucial component in an ERA, especially for inundative releases into glasshouses.

Figure 1. Relationship between maximum field survival (days) and $L_{\text{Time}_{50}}$ 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).
In cool temperate climates, two dominant factors will determine the establishment potential of species escaping from glasshouses: overwintering ability and sources of prey. These two factors must therefore be the central focus for any risk assessment. However, the ‘step wise’ concept suggests that the first stage of the assessment should focus on overwintering, because if a species is unable to survive through winter, establishment is impossible, and hence, any consideration of effects on non-target prey becomes irrelevant.

The analysis presented in Fig. 1 indicates that the LTime\textsubscript{50} at 5°C is a reliable predictor of the winter field survival of five non-native biological control agents, representing different taxonomic groups and trophic guilds. It is important to stress that this predictive relationship should not be viewed in isolation; it is one component of an ERA. Also, there is clearly a limit to the sensitivity of the system in terms of estimating maximum survival times in the field. The real value of this approach is that it enables candidate agents be classified into different ‘risk categories’. For example, D. catalinae, E. eremicus and T. montdorensis are representative of a ‘low risk’ group, where 100% field mortality occurs within four weeks and any establishment is highly unlikely. A ‘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.

An indication that a non-native species is able to survive through winter in a new environment is not in itself a reason to reject a licence application. Other forms of risk assessment should then be carried out, on host range and dispersal (van Lenteren et al. 2003; in press), and in the final analysis, it may be decided that the overall benefits of release outweigh the risks.

In critically reviewing the contribution that studies on thermal biology, cold tolerance and overwintering can make to an ERA, there are a number issues to consider, including: the possibility that the observed relationship may have occurred by chance, that other indices have similar predictive power, and the extent to which the system is applicable to insects and mites with different levels of cold hardiness.

There are sound ecophysiological reasons to believe that the observed relationship is based on a representative index of cold tolerance that links the laboratory to the field and is not a ‘chance occurrence’. It is known that the vast majority of insects show some pre-freeze mortality, in some cases, with 100% death above the SCP. For this reason, the SCP temperature in isolation is not a reliable indicator of cold hardiness, and hence, no correlation with field survival would be expected (and was not found). For insects and mites that originate from warm climates, where pre-freeze mortality is extensive, it is intuitive to predict that the duration of survival at low temperatures (0° to 5°C) in the laboratory would be reflected in field survival, and this was shown to be case. A similar relationship has been reported for a range of native and non-native crop pest species in the UK (Bale and Walters 2001).

It is interesting that no other laboratory index of thermal biology was correlated with field survival. In some respects, the most misleading information relates to the estimation of the developmental threshold and annual number of available day degrees. Both N. californicus
and T. montdorensis can complete an average of 6 generations in UK summers, but their winter survival is markedly different. Estimates of annual voltinism are clearly important, but are not a reliable indicator of winter survival or establishment potential.

The final consideration concerns the applicability of this system to other insects and mites with different levels of cold tolerance. The current analysis includes species in which pre-freeze mortality occurs after exposures of days or a few weeks (D. catalinae, E. eremicus and T. montdorensis) up to several months (M. caliginosus and N. californicus). These two groups would be classified as 'chill susceptible' and 'chill tolerant' respectively according to Bale (1996). In terms of the world-wide distribution of insects and mites, there are very few 'true' freeze susceptible species (where there is no mortality above the SCP), and only a small number of freeze tolerant species. These species tend to inhabit the coldest regions of the world, and none have ever been used as biological control agents. In summary, it seems reasonable to conclude that the current protocol is applicable to virtually all insects and mites that are likely to be considered as non-native biological control agents, and can make a valuable contribution to a step-wise environmental risk assessment.

ACKNOWLEDGEMENTS

I am grateful to Andrew Hart, Andrew Tullett, Ian Hatherly and Roger Worland for conducting the experiment work, to Keith Walters for collaborating in various programs, and to Richard Greatrex for the provision of biological material. The research was funded by Defra and CSL, York.

REFERENCES


