THERMAL ECOLOGY OF INVERTEBRATE BIOLOGICAL CONTROL AGENTS: ESTABLISHMENT AND ACTIVITY

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ABSTRACT.

Terrestrial arthropods have limited ability to regulate their body temperature. For this reason, many aspects of the biology and ecology of insects and mites, both pest species and their natural enemies, are influenced by temperature, ranging from macrophysiological effects on their distribution to more localized ‘rate-based’ responses, affecting processes such development, reproduction, activity and survival. In the context of biological control, temperature is a major determinant of establishment, which is essential for classical agents but considered undesirable for species released for inundative control in glasshouses, and also has a direct effect on the activity of predators and parasitoids relative to their target prey. This paper describes advances in methods for assessing the establishment potential and activity of biocontrol agents, a focus of considerable interest in an era of global climate change.

INTRODUCTION.

Insects and mites are poikilothermic animals with limited ability to regulate their body temperature. For this reason, temperature can exert a major influence on such arthropods: at a macrophysiological scale, determining distributions and abundance, and at a more localized level, affecting ‘rate-based’ processes such as development, reproduction, activity and survival. Interest in the thermal biology of insects has increased greatly in recent years, stimulated largely by concerns about the possible impacts of climate warming. There are now well documented reports of shifts in the distribution of indicator species such as butterflies and other taxa of conservation importance (Parmesan et al. 1999; Thomas et al. 2001), as well as the range expansion of many pest species such as the pine beetle Dendroctonus frontalis Zimmerman (Coleoptera: Scolytidae) in North America (Tran et al. 2007), the southern green stinkbug Nezara viridula L. (Hemiptera: Pentatomidae) in Japan (Musolin 2007), the pine processional moth Thaumetopoea pilyocampa (Dennis and Schiff.) (Lepidoptera: Notodontidae) in the Italian Alps (Battisti et al. 2005) and the birch-defoliating moths Epirrita autumnata L. (Lepidoptera: Geometridae) and Operophtera brumata Bkh. (Lepidoptera: Geometridae) in northern Scandinavia (Jepsen et al. 2008). Whilst higher temperatures might be expected to increase rates of development and fecundity and reduce the winter mortality of pest species, similar effects would also impact on natural enemy species, thus the ‘net outcome’ of such interactions in an era of climate change is as yet largely unknown. However, a cautionary conclusion would be that climate warming would allow at least some pest insects and mites to increase in abundance and to expand their range margins beyond current distributions. Such changes are also occurring against the backdrop of the progressive withdrawal of pesticides without replacement, concerns about the environmental impact of GM crops, and relatively high development and registration.
costs for biopesticides. The opportunity to utilize arthropod biocontrol agents is therefore as promising and opportune as at any time in the history of this technology.

Whilst the socio-economic importance of crop losses to pests and the possible exacerbating effects of a changing climate highlight the need to seek ‘accurate predictions’ on the future distributions and abundance of key species, the reliability of such forecasts is compromised by a number of factors. Firstly, it is difficult to predict with any accuracy the impact of future climate scenarios without a detailed knowledge of a species’ ecophysiology and thermal ecology under prevailing climates, and for many species, this information is still lacking. As an example, the environmental risk assessment of non-native biocontrol agents intended for release in the UK in the 1990s rarely contained any direct measure of cold tolerance or winter survival; rather, ‘climate matching’ was used as a proxy for cold hardiness and it was assumed that species of tropical origin would not survive through temperate winters – an assumption now known to be untrue. Secondly, long term datasets (30-50 years) that represent a ‘biological record’ of responses to a changing climate are relatively rare (see earlier examples), especially so for natural enemy species. Also, direct experimental manipulation of climate (solardomes, FACE systems) and modeling approaches have rarely considered the ‘biological control’ dimension, and in any case, suggest that species-specific differences in life cycles, host plants, feeding guild and natural enemy interactions make it difficult to reach any general consensus.

The effects of current and future temperature regimes on pests and their predatory and parasitoid fauna are therefore many and varied, but a case can be made that two of these processes are of fundamental importance to the success and wider environmental effects of biocontrol: establishment and activity. Establishment is a ‘double-edged sword’ in biological control – for classical control, establishment is essential, but for inundative control in glasshouses, the likelihood of escape and establishment in the natural environment is undesirable (because negative impacts on non-target native species may occur), and in some countries would prevent the granting of a licence to release. With regard to activity, all insects, mites and their natural enemies have temperature thresholds below which the organism cannot move (walk or fly); above this threshold, the rate of activity will increase up to an optimum, and then decrease through heat stress. Thus, the relative position of these thresholds between the target pest and its intended control agent and differences in the rates of increase in activity will determine, at least in part, the efficacy of the control.

Establishment and activity are therefore at opposite ends of the ‘thermal spectrum’, the first being a large scale macrophysiological process and the latter governed by more local climatic conditions – but both can be quantified under current climatic patterns, and from such data, informed predictions can be made on how such ecophysiological attributes might be modified by different scenarios of climate warming. This paper reports on recent developments in methods to assess establishment potential and activity thresholds.

**Establishment.**

The recent focus for studies on establishment has been closely linked to the development of methods for the environmental risk assessment (ERA) of non-native...
species, particularly in Europe. The regulatory framework in Europe is fragmented with some countries having stringent conditions for the import and release of non-native species whilst other countries, sometimes direct neighbours, have no restrictions. Among those countries with regulation, there has been a move toward a more evidenced-based risk assessment, characterized by the requirement to generate data from experimentation when such information is not available in the published literature. At the same time, and in recognition of the cost implications for small companies, van Lenteren et al. (2006) have proposed that risk assessment should have a hierarchical structure, whereby candidate agents are filtered through a series of tests, such that ‘risky’ species with little or no prospect of licensing are eliminated at an early stage, allowing resources to be channeled into the most promising agents (Fig 1).

![Flow chart summarising a hierarchical environmental risk assessment scheme for arthropod biocontrol agents (van Lenteren et al., 2006).](image)

**Fig. 1:** Flow chart summarising a hierarchical environmental risk assessment scheme for arthropod biocontrol agents (van Lenteren et al., 2006).

Whilst this approach has advantages for both regulatory authorities and companies, it is dependent on the development of reliable methods, which are either not yet available, in need of refinement, or too costly for industry. Importantly, a reliable method of predicting establishment is equally relevant to both classical and inundative glasshouse control. The first part of this paper therefore reports on
laboratory and field studies on the establishment potential of a number of glasshouse biocontrol agents (released or under consideration for release) in the UK.

Activity.

All insects and mites (pests and natural enemies) have temperature thresholds below which they are unable to develop or move, and in the case of winged insects, fly; and similar effects are seen at high temperatures. Some generalizations can be made about these various thresholds: thus, temperate species usually have lower developmental and activity thresholds than tropical species (Bale & Walters 2001), and can lower these thresholds after a period of low temperature acclimation (Powell & Bale 2008), whereas this is less common in tropical species. Also, for most species, there is a larger temperature differential between the chill coma and lower lethal temperature than between the heat coma and upper lethal temperature.

Various methods are available to determine the activity thresholds of insects and mites, but in general, they require ‘direct observation’ of the organisms as the temperature is increased or decreased. This paper describes a new method of video recording which allows retrospective analysis of data, and has applications in the areas of host range, prey preferences and efficacy.

MATERIALS AND METHODS.

Establishment.

The low temperature tolerance of various life cycle stages of eight non-native biocontrol agents with and without acclimation and in fed and starved conditions was assessed by a range of indices including the freezing temperature (supercooling point or SCP), lethal temperature (often higher than the freezing temperature), and lethal time (at -5º, 0º and 5ºC). The same life cycle stages and treatment groups were also exposed outdoors (with moisture and protection from direct sunlight) and replicate samples retrieved from the field at intervals in winter. Temperatures experienced by the organisms were monitored continuously with dataloggers. The data from the laboratory and field exposures were analysed to identify laboratory indices that were the most reliable predictors of field survival (for full details of species, methods and results see Hatherly et al. 2005).

Activity.

The system comprised of an aluminium block within which a cooling fluid (antifreeze solution) was circulated from a low temperature programmable alcohol bath. An arena was milled into the aluminium block and covered by a thin sheet of clear perspex under which the insects or mites were contained. The activity of the organisms was filmed with a digital video camera (with macro lens) linked to a computer with customized software. A thermocouple is positioned 1mm above the base of the arena and coupled to a digital thermometer so that activity and temperature were recorded simultaneously. A set of interchangeable blocks with different arena diameters and depths enabled experiments to be conducted with organisms of different sizes ranging from microarthropods such as mites to larger species such as predatory mirids. For any selected species, comparisons could be...
made between different life cycle stages and treatments (e.g. acclimated, starved or fed). A permanent record of each experiment is stored electronically, allowing retrospective visualization and analysis of a range of thermal tolerance traits. For small species, the arena can accommodate sample sizes of up to 40 specimens, each of which can be investigated individually by ‘play back’ of the recorded images (for a full description of the system see Hazell et al. 2008).

In this paper example data is presented on non-acclimated adults of the predatory mite *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) and the mirid *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae). To determine the walking speeds of *A. swirskii*, 20 mites were placed in a 25 mm diameter by 7.5 mm deep arena and cooled from 30°C at 0.5°C min⁻¹ to 25º, 20º, 15º and 10ºC and held at each of these temperatures for 5 min after which the walking speeds were measured. The chill coma temperature of *A. swirskii* was assessed by cooling two samples of 20 mites from 25°C to 10ºC at 0.5°C min⁻¹ and then from 10º to -10°C at 0.1°C min⁻¹. In the context of these experiments chill coma is defined as the temperature at which an individual ceases to move i.e. walk. At a lower temperature, the organism will cease movement of all appendages (antenna, leg), and this value is also sometimes described as the chill coma, or critical minimum temperature (CT\text{min}) – see Hazell et al. (2008) for a discussion of these terminologies. With the mirid *N. tenuis*, 10 specimens were placed in a 40 mm diameter by 7.5 mm deep arena at their culture temperature (23ºC) and cooled at 0.5ºC min⁻¹ to 20°C and then at the same rate to 0ºC at intervals of 2.5ºC. At each 2.5ºC interval, the temperature was held for 5 min and the activity recorded at these temperatures. For the chill coma experiments, mirids were placed in the same arena at their culture temperature and the temperature reduced at 0.2ºC min⁻¹ to -15ºC. The video recordings for both experiments were then analysed to generate the data.

**RESULTS.**

**Establishment.**

A series of earlier analyses on five non-native (to the UK) control agents: *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae), *Macrolophus caliginosus* Wagner (Hemiptera: Miridae), *Delphastus catalinae* (Horn) (Coleoptera: Coccinellidae), *Eretmocerus eremicus* Rose and Zolnerowich (Hymenoptera: Aphelinidae) and *Typhlodromips montdorensis* (Schicha) (Acari: Phytoseiidae) identified the lethal time that killed 50% of sample populations (LTime\text{50}) at 5ºC to be strongly correlated with survival time in the field in winter (Hatherly et al. 2005). Further data has since been acquired on the mirids *Dicyphus hesperus* Knight (Hemiptera: Miridae) and *N. tenuis* and the mite *A. swirskii* and the same correlation has been substantiated (Fig. 2). All field experiments were carried out at the University of Birmingham (Birmingham, UK).

There is a strong correlation between survival in the laboratory at 5ºC and under variable winter temperatures in the field, and no species has yet been studied that departs from this trend. Given the importance of possible outdoor establishment in northern Europe of glasshouse biocontrol agents, this relationship is important for two reasons. Firstly, it represents a method that can be incorporated into the environmental risk assessment proposed by van Lenteren et al. (2006). Secondly,
the experiments could be conducted by companies within their own research facilities. The important consideration here is whether the ‘predictive relationship’ across these eight species is now sufficiently robust that laboratory assessment alone could be used to assess establishment potential.

Data on the walking speeds at 20° and 10°C and chill coma temperature of *A. swirskii* and *N. tenuis* are shown in Table 1.
Table 1. Walking speeds and chill coma temperature of non-acclimated adult *A. swirskii* and *N. tenuis*.

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<tr>
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<th><em>A. swirskii</em></th>
<th><em>N. tenuis</em></th>
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<tbody>
<tr>
<td>Walking speed (cm min$^{-1}$) ± SE</td>
<td>20°C</td>
<td>20.8 ± 1.4</td>
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<tr>
<td></td>
<td>10°</td>
<td>7.1 ± 0.5</td>
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<tr>
<td>Chill coma (°C) ± SE</td>
<td>6.2 ± 0.1</td>
<td>3.2 ± 0.1</td>
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The data on *A. swirskii* and *N. tenuis* should not be regarded as directly comparable because the species could be used in different biocontrol situations. However, the data do show that whilst the mobility of both species is greatly reduced at 10°C compared with 20°C, they are still active at the lower temperature. Below 10°C, *N. tenuis* is still capable of some movement at a temperature at which *A. swirskii* has become inactive. This method provides an effective measure of the temperature-mobility interaction which is an important component in the efficacy of biocontrol, particularly with regard to predators. As such, in a screening programme for relative efficacy, this system can provide valuable data, especially when the mobility of candidate agents is compared with that of their prey.

DISCUSSION.

The focus on the establishment potential and wider environmental risks of non-native biological control agents is a paradox, as indicated by van Lenteren et al. (2006). In the long history of biocontrol, with numerous introductions of a large number of species in many parts of the world, there have been remarkably few negative effects. And yet, there has been a trend toward greater regulation, with well organized systems in North America, Australia and New Zealand, and a move toward a more coordinated approach in Europe. It is difficult to identify a single reason that explains these developments, but in general it represents the increasing emphasis on environmental protection within agricultural systems, and a greater awareness of possible threats to native species and ecosystems – the recent expansion through Europe of the predatory ladybird *Harmonia axyridis* being a prime example. Whilst the biocontrol industry has to work within this framework, it is also important that the regulatory requirements are proportionate to the risks (real rather than perceived), and affordable by companies with limited R&D budgets.

The hierarchical risk assessment proposed by van Lenteren et al. (2006) has the dual merits of identifying ‘risky species’ early in the process, thus allowing companies to focus resources on the most promising candidate agents. However, the ERA is dependent on having reliable methods and where possible, techniques that industry can implement within their own facilities. For releases into glasshouses in temperate and colder climates a case can be made for assessing establishment
potential as the ‘first test’, because if it can be shown that a species would be unable
to survive for more than 2-3 weeks in winter in the outdoor environment, there would
be no prospect of wider establishment, and there would be no need to invest in host
range tests that can be expensive. However, if the release was to be made for
instance, in southern Europe, the climate would support year-round development and
reproduction, and host range tests would then be essential. For those species where
risk of establishment is the crucial factor in the application for a release permit, the
studies on 8 natural enemies across a range of taxa suggest that there is a very
strong correlation between laboratory and field survival at low temperature (Fig. 2).
The absence of any exceptions to this relationship suggests that a laboratory
assessment alone (at 5ºC) may now be an adequate basis for assessing
establishment potential. It then becomes relevant to ask whether data obtained in a
UK study be applied to other countries, and how climate warming might impact on
this relationship. By reference to climate records for other European countries (or
parts thereof) and in other parts of the world, it would be relatively straightforward to
identify countries and regions where the climate is either colder (e.g. Scandinavia) or
similar (The Netherlands, northern France and Germany) to the UK and to which the
identified relationship should apply. The impacts of climate warming are more difficult
to quantify. There are a number of examples of pest species extending their northern
range margin by around 50 km over the past 30 years (see Introduction) against a
backdrop of a ~1°C increase in mean or minimum temperatures over the same time
period. The general implication is that climate warming may promote enhanced
winter survival, but whether this would allow species currently regarded as ‘safe’ (see
Fig. 2) to become capable of establishment in a temperate or colder climate seems
doubtful, unless the level of temperature increases were toward the maximum end of
current predictions.

The technique described for assessing activity and chill coma has some major
advantages over previous systems: cooling regimes are flexible and programmable,
there is an accurate ‘real-time’ visual display of temperature that is directly relatable
to the observed insect behaviours, and every experiment produces a permanent
record that can be analysed and re-analysed as and when required. The method
enables the activity and chill coma temperatures of biocontrol agents to be directly
compared, as well as that of their target prey, which is relatable to both glasshouse
climatic regimes and outdoor conditions. The system also has applications in studies
on predator-prey interactions, including variations such as relative densities and the
influence of host plant species.

In summary, temperature has both direct and indirect effects on biocontrol
agents, their target prey and hosts, and the wider ecosystem within which these
organisms interact. These effects vary in scale from the macrophysiological
perspective of distributions and abundance to localized effects on development,
reproduction and activity. In order for the potential of biocontrol to be more fully
realized, it is necessary to develop reliable methods to assess both the
environmental risks of novel agents, and the factors that might affect their efficacy, of
which climate, and particularly temperature, is clearly important. The techniques
described and evaluated in this paper could be immediately utilized by researchers
and companies and contribute toward the overall aim of releasing safe and effective
biological control agents to combat the increasing problems caused by arthropod
pests, which are likely to increase and become more widespread in an era of climate change.

REFERENCES.


