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Short research contribution

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A METHOD FOR SIMPLE ASSESSMENT OF COLD SENSITIVITY IN INSECTS

ABSTRACT: A simple index of sensitivity to frost in insects for the general use in ecological studies was introduced. The index is based on a cooling experiment that leads to the estimation of the subzero temperature at which 50% of a sample of particular species life form individuals are killed within 24 hours. By analogy to toxicology, such an estimate was called $LTemp_{50}$. We present the method using our results from a series of simple cooling experiments conducted on larval gooseberry sawflies *Nematus ribesii* (Scopoli) and imagine queens, workers and males of the hornet *Vespa crabro* L. $LTemp_{50}$ indices were -7.2°C for gooseberry sawfly larvae and ranged from -5.0°C for male hornets to -9.1°C for queen hornets. These differences seem to reflect reasonably the specific thermal environment adaptations of the studied insects.

KEY WORDS: cold sensitivity, insects, $LTemp_{50}$

In the temperate climate, insects at different life stages are influenced by longer or shorter periods of low temperature, especially while overwintering and during spring (Barnes *et al.* 1996). Responses of particular species and stages to cold weather are interesting as important aspects of their autecology, but also in a wide ecological context,

because of potential effects of those species on grazed plants, competitors and predators (Sinclair *et al.* 2003). Ecological and physiological mechanisms of low temperature tolerance have been extensively studied and quite well understood (Lee Jr. and Denlinger 1991, Sinclair *et al.* 2003, Bowler and Terblache 2008).

Even if there are many detailed studies for different insects, thermal tolerance is not typically characterised by any simple index that would be useful to non-specialist ecologists. We propose such an index based on a short-term cooling experiment. This method leads to estimating a temperature at which 50% of individuals of a studied species or life-stage die. This value provides a simple measure of sensitivity to subzero temperature. To check its applicability we used two species of common insects, the gooseberry sawfly *Nematus ribesii* (Scopoli) (Tenthredinidae, Symphyta, Hymenoptera) and the hornet *Vespa crabro* L. (Vespidae, Aculeata, Hymenoptera). Because our principal aim is to introduce a simple preliminary index, we do not consider acclimation or other factors that can complicate the responses of the studied insects.

For this study we used field-collected caterpillar-like larvae of the gooseberry sawfly at

Table 1. Analysis of probit-transformed mortality of the larvae of gooseberry sawflies *Nematus ribesii* (Scopoli) and queen, worker and male hornets *Vespa crabro* L. in freezing experiments. Chi-squared statistics were non-significant.

Species/life form	Subzero temperature (°C)	Log subzero temperature	Actual mortality (%)	Probit-transformed actual mortality	Expected probit	Expected mortality (%)	Chi-squared statistic	Degrees of freedom
<i>Pteronidea ribesii</i> larvae	10	1.00	80	5.84	5.82	79	0.83	5
	9	0.95	70	5.52	5.54	71		
	8	0.90	70	5.52	5.26	60		
	7	0.85	40	4.75	4.97	49		
	6	0.78	30	4.48	4.58	34		
	5	0.70	20	4.16	4.13	19		
<i>Vespa crabro</i> queens	4	0.60	10	3.72	3.56	7.5	1.16	3
	11	1.04	90	6.28	6.02	84.5		
	10	1.00	70	5.52	5.54	70.9		
	9	0.95	50	5.00	4.94	47.9		
	8	0.90	20	4.16	4.34	25.1		
<i>Vespa crabro</i> workers	7	0.85	10	3.72	3.74	10.4	0.75	3
	7	0.85	70	5.52	5.69	75.5		
	6	0.78	60	5.25	5.34	63.1		
	5	0.70	60	5.25	4.94	47.9		
	4	0.60	30	4.48	4.44	28.9		
<i>Vespa crabro</i> males	3	0.48	10	3.72	3.84	12.4	1.26	4
	8	0.90	90	6.28	6.09	86		
	7	0.85	70	5.52	5.82	79		
	6	0.78	60	5.25	5.43	67		
	5	0.70	60	5.25	4.99	50		
	4	0.60	30	4.48	4.44	29		
	3	0.48	10	3.72	3.79	11		

different instars and imagines of the hornet belonging to all three castes: queens, workers and males. Separate freezing experiments were conducted for all these categories of individuals.

To test for thermal tolerance we used an 85 L freezer equipped with a system of electronic adjustment of temperature to the nearest 0.5°C. For every run of a particular freezing experiment a sample of 10 individuals of a given category (sawfly larvae, hornet males, queens or workers) was randomly drawn from the cage and put into the freezer set to given temperature for 24 hours. After that time the sample was kept for 24 hours at room temperature and then surveyed for mortality rate. Samples with 0 or 100% mortality were excluded from the further analysis. Freezing experiments started from the temperature 0°C and were continued in the same way with decreasing temperature treatment steps of 1°C down to the temperature causing 100% mortality.

By analogy with the methodology of assessing the lethal dose of a toxic substance killing 50% of individuals (LD_{50}) applied in

toxicology (Klassen *et al.* 1986, Seńczuk 1990) we performed an analysis of subzero temperature to estimate the temperature killing 50% of individuals ($LTemp_{50}$) by the probit analysis (Finney 1971). Following the probit analysis procedure, the subzero temperatures influencing samples were transformed to logarithms. Mortality rates expressed as percentages were transformed to probits and in this form regressed on *log* subzero temperatures by the least-square method, which ensured a linear relationship. The line of regression was used to calculate the expected probits of mortality rates and to back-calculate the temperature killing 50% of individuals $LTemp_{50}$. 95% confidence intervals of $LTemp_{50}$ were computed according to the method for inverse prediction described by Sokal and Rohlf (1995). The fit of empirical probits to expected probits was examined by the chi-squared test of the goodness of fit.

Our freezing experiments show that there is some variation between insect species/life forms in their mortality in response to subzero temperatures, with a high degree of agreement between actual and expected pro-

bits, as shown by non-significant chi-squared test values (Table 1). The $LTemp_{50}$ as a proposed index of cold tolerance shows that hornet males and workers are more sensitive to subzero temperatures than queens (Table 2). Gooseberry sawfly larvae are in the middle of the range of cold tolerance between hornet queens and workers/males.

The main idea of this paper was to get a simple index of thermal tolerance in insects. The need for such an index arose from our studies of both insects and birds feeding on insects. In the climatic conditions of Poland, during spring (April–May), there often occur cold spells that may influence insects in a different way, depending on their relative tolerance to low temperature. Some level of cold tolerance is an obvious adaptation to living in changing environment of temperate and boreal geographic areas. Variation in sensitivity to subzero temperatures may occur both between and within species. The ecology and physiological mechanisms of thermal tolerance in insects have been extensively studied (Schmidt-Nielsen 1990, Lee Jr. and Denlinger 1991, Sinclair *et al.* 2003, Bowler and Terblache 2008). The results presented in this note do not concern mechanisms but rather suggest a simple way to characterize sensitivity of particular insect species and their life forms at different stages to thermal stress. A simple index of sensitivity may be useful as a background in studies of the biology of particular species and in study systems in which insects are a component.

Because cold tolerance engages specific physiological mechanisms, it must be expensive and avoided when not necessary (Lee Jr. and Denlinger 1991, Sinclair *et al.* 2003). As a consequence we could expect that some species and life-forms will be non-randomly more sensitive to cold than others, which would be associated with the average

conditions of their activity. This was exactly the case with hornets and sawflies used as examples in this study. The values of $LTemp_{50}$ were the lowest for queen hornets, suggesting that they are relatively resistant to subzero temperatures. Female hornets after overwintering in low temperature undertake searching for adequate sites to establish a colony at the time when frost spells happen, especially at night and some degree of cold tolerance seem to be adaptive in this case (cf. Barnes *et al.* 1996). Workers and males appear later and occur in colonies most of their lives and, thus, do not need much cold tolerance.

Tree-foliage caterpillars and caterpillar-like larvae of Symphyta comprising important items of food of tits, especially blue tits *Cyanistes caeruleus* and great tits *Parus major* (Marciniak *et al.* 2007), are most abundant at the stage of the host-plant leafing. As shown in this paper, at least some Symphyta species are considerably tolerant to frost. Even less subzero-temperature-sensitive cases have been reported for sawflies (Salt 1966). However, frosty weather during the larval stage of insects can reduce the numbers of some taxa larvae. Frosty weather in early spring may influence both the start of tit breeding and clutch size decisions (Zajac 1995). If frost happens in May, when tit breeding has already been advanced, a consequence may be poor trophic conditions for nestling feeding. In such a case, the knowledge of relative sensitivity of different insects to subzero temperature may be very helpful in explaining the effects of weather conditions on the food of nestlings and, consequently, on breeding success.

To sum up, the value of $LTemp_{50}$ seems to be an interesting index of low temperature sensitivity that may be very useful in various ecological studies on insects.

Table 2. Estimated values of the temperature (with 95% confidence interval, CI) at which 50% mortality ($LTemp_{50}$) of individuals occurs of a given species/life form of gooseberry sawflies *Nematus ribesii* (Scopoli) and hornets *Vespa crabro* L.

Species/life form	$LTemp_{50}$	95% CI ($LTemp_{50}$)
<i>Pterinidea ribesii</i> larvae	-7.2°C	1.29
<i>Vespa crabro</i> queens	-9.1°C	1.27
<i>Vespa crabro</i> workers	-5.1°C	1.59
<i>Vespa crabro</i> males	-5.0°C	1.11

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