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Review

An invitation to measure insect cold tolerance: Methods, approaches, and workflow

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ABSTRACT

Insect performance is limited by the temperature of the environment, and in temperate, polar, and alpine regions, the majority of insects must face the challenge of exposure to low temperatures. The physiological response to cold exposure shapes the ability of insects to survive and thrive in these environments, and can be measured, without great technical difficulty, for both basic and applied research. For example, understanding insect cold tolerance allows us to predict the establishment and spread of insect pests and biological control agents. Additionally, the discipline provides the tools for drawing physiological comparisons among groups in wider studies that may not be focused primarily on the ability of insects to survive the cold. Thus, the study of insect cold tolerance is of a broad interest, and several reviews have addressed the theories and advances in the field. Here, however, we aim to clarify and provide rationale for common practices used to study cold tolerance, as a guide for newcomers to the field, students, and those wishing to incorporate cold tolerance into a broader study. We cover the 'tried and true' measures of insect cold tolerance, the equipment necessary for these measurement, and summarize the ecological and biological significance of each. Finally, we suggest a framework and workflow for measuring cold tolerance and low temperature performance in insects.

Contents

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1. Introduction

Temperature constrains the geographic distribution and seasonal activity of insects (Chown and Nicolson, 2004), and therefore can directly or indirectly affect the spread and impact of invasive pests, the success of species introduced for biological control, and the dynamics of native insect populations (Bale and Hayward, 2010). In temperate, polar and montane habitats, the majority of insects spend a large proportion of their life in an overwintering stage, and must survive the low temperatures and accompanying environmental stressors that are associated with winter (Leather et al., 1993; Williams et al., 2015). Similarly, insects in deserts and tropical high mountains can also be regularly exposed to potentially-lethal freezing conditions (Sømme, 1995; Sømme et al., 1996; Sømme and Zachariassen, 1981). Thus, low temperature biology is a key component of insect fitness, and one of the best determinants of insect distribution (Andersen et al., 2015b).

Most insects are ectotherms, and as such, their body temperatures are generally similar to the ambient microclimate temperature, and changes in ambient temperature can thus have drastic effects on the physiology of an insect. Thus, measuring low temperature performance (which we refer to loosely here as 'cold tolerance') is an excellent way to incorporate the pervasive effects of temperature in studies ranging from ecological (e.g. van Dooremalen et al., 2013) to molecular (e.g. Reis et al. (2011)). In the cold, many insects enter a reversible state of paralysis, called chill coma, at the critical thermal minimum (CT_{min}) (MacMillan and Sinclair, 2011a). At sub-zero temperatures, insects risk freezing of the body fluids, as well as a host of other low temperature injuries (Denlinger and Lee, 2010). The ability of an insect to survive at low temperatures is referred to as its cold hardiness, and their responses to low temperature have generally been categorized as chill-susceptible, freeze-avoidant, and freeze-tolerant (Fig. 1, see Section 3) (Bale, 1993).

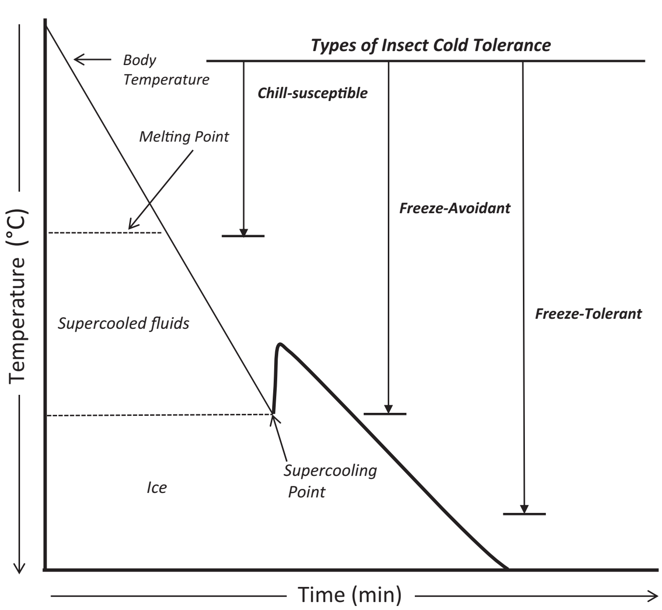

Fig. 1. Classifications of insect cold tolerance. Insect cold tolerance is divided into three main classifications, based on survival of low temperatures and extracellular ice formation. Chill-susceptible insects die of cold exposure unrelated to freezing, whereas freeze-avoiding insects maintain their body fluids in a supercooled state and die when ice formation occurs (i.e. at the supercooling point). Freeze-tolerant insect are able to withstand extracellular ice formation. Adapted from Lee (2010).

There is a long history of the study of insect cold tolerance (Ring and Riegert, 1991; Sømme, 2000), and the sub-discipline has consequently developed its own semantic and methodological traditions. Although there are many excellent reviews on the subject (e.g. Asahina (1969), Bale (2002), Block(1982a), Denlinger and Lee (2010), Lee (1991), Salt (1961), Sinclair et al. (2003b), Zachariassen (1985)), a unified summary of the methods and approaches used in insect cold tolerance is not available. Nevertheless, although care must be taken in the design and interpretation of experiments, measuring insect performance at low temperatures is by no means arcane, and many measurements require no specialist equipment. Our purpose in this review is to explain some of the common measures of insect low temperature biology from a methodological viewpoint, with the intention of making these methods more accessible. We try to identify some of the diverse measurements that are comparable among studies, and our ultimate goal is to reduce some of the trial-and-error inherent in learning a new set of techniques and measurement. Our intended audience is newcomers to the field, students, and (particularly) those who are interested in incorporating low temperature performance into their existing studies, and are looking for an overview of common practices. While there is an unavoidable bias towards the methods or approaches used over the past two decades by the first author, we have tried to encompass alternative approaches wherever possible, and also provide (hopefully) lucid explanations of why we favor one approach over others. In some cases, we give examples of equipment that has been used in these studies, but we do not intentionally endorse any manufacturer or model.

2. Technical and apparatus considerations

Studying insect low temperature performance requires some form of temperature control and measurement. We conclude this section by discussing how to identify and measure an insect's supercooling point (SCP), as measuring the SCP is fundamental to many other measures of insect cold tolerance and provides a useful application of measuring an insect's body temperature during cold exposure.

2.1. Temperature control

The simplest cold exposures involve constant low temperatures, and the equipment needed for these exposures is often readily available. For example, an ice–water slurry is at a constant (and precise) 0 °C, many laboratory and domestic refrigerators and cold rooms are held at approximately +4 °C, and domestic freezers are usually somewhere between −12 and −20 °C. Similarly, refrigerated incubators or refrigerated baths can be easily set to a single temperature. Ultra-low freezers (usually set somewhere between −70 and −90 °C), dry ice (−80 °C), a dry ice–acetone slurry (−78 °C) and liquid nitrogen (−196 °C) all provide constant low temperatures with reasonable precision. Although these latter temperatures are of limited biological relevance when studying living insects, they can be useful if extreme rapid cooling is required.

More commonly, insects are cooled using specialised cooling equipment, such as rate-controlled incubators (e.g. Ju et al. (2011)) or refrigerated circulators (e.g. Marshall and Sinclair (2011)). These are often programmable, allowing precise cooling, hold ('soak' in engineering terminology), and warming programmes. Refrigerated