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All or nothing: Survival, reproduction and oxidative balance in Spotted Wing Drosophila (*Drosophila suzukii*) in response to cold

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ABSTRACT

Winter severity and overwintering capacity are key ecological factors in successful invasions, especially in ectotherms. The integration of physiological approaches into the study of invasion processes is emerging and promising. Physiological information describes the mechanisms underlying observed survival and reproductive capacities, and it can be used to predict an organism's response to environmental perturbations such as cold temperatures. We investigated the effects of various cold treatments on life history and physiological traits of an invasive pest species, *Drosophila suzukii*, such as survival, fertility and oxidative balance. This species, a native of temperate Asian areas, is known to survive where cold temperatures are particularly harsh and has been recently introduced into Europe and North America. We found that cold treatments had a strong impact on adult survival but no effect on female's fertility. Although only minor changes were observed after cold treatment on studied physiological traits, a strong sex-based difference was observed in both survival and physiological markers (antioxidant defences and oxidative markers). Females exhibited higher survival, reduced oxidative defences, less damage to nucleic acids, and more damage to lipids. These results suggest that *D. suzukii* relies on a pathway other than oxidative balance to resist cold injury. Altogether, our results provide information concerning the mechanisms of successful invasion by *D. suzukii*. These findings may assist in the development of population models that predict the current and future geographic ranges of this species.

1. Introduction

The development of international trade and intercontinental transportation has accelerated the movement of species, breaking down biogeographic barriers that had isolated populations for thousands of years (Mooney and Cleland, 2001; Williamson and Fitter, 1996). Recent global changes promote the expansion of geographic ranges for a number of species, through effects such as corridor creation (Resasco et al., 2014) and winter warming (Clements and Ditommaso, 2011). The economic impact of invasive species may be considerable (Pimentel et al., 2005). Such species can negatively impact the functioning of invaded ecosystems (Facon et al., 2006; Mack et al., 2000; Mooney and Cleland, 2001; Suarez and Tsutsui, 2008). Understanding the distribution and population dynamics of invasive species is a challenging goal of both fundamental and applied research. Physiological factors, life histories and environmental factors that determine the fundamental and realized niches of such species requires elucidation (Kearney and Porter, 2009). The integration of physiological approaches into invasion studies is emerging and promising. Physiological data can explain the mechanisms underlying observed survival and reproductive capacities and can be used to predict the response of organisms to environmental perturbations (Evans et al., 2015; Fefferman and Romero, 2013). Among the abiotic factors affecting the physiology of most terrestrial organisms, temperature is probably the most important. Temperature has a direct influence on cellular processes and metabolism (Gillooly et al., 2001; Marshall and Sinclair, 2010; Pörtner et al., 2006) that, in turn, affect individual performance (Hochachka and Somero, 2002; Huey et al., 2012). This is particularly true for ectotherms, which represent more than 90% of the terrestrial invasive animal species in Europe (DAISIE, 2015).

Winter conditions have been known to be explicative factors in species distribution and dynamics (Crozier, 2003). One example is

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the southern green stinkbug *Nezara viridula*, which originates from sub-tropical areas. Since the 1960s, the range of this species has been expanding northward in temperate regions of Japan and Europe. This is probably due to reduced mortality resulting from milder winters (Tougou et al., 2009; Yukawa et al., 2009). Low temperatures impose a number of physiological challenges, depending on the strategy of animals to face freezing. Different physiological mechanisms are known to be involved in cold injury; these include loss of membrane fluidity (Lee et al., 2006; Overgaard et al., 2008; Shreve et al., 2007), loss of ion homeostasis (Kostál et al., 2004; Koštál et al., 2007; MacMillan et al., 2015a), the induction of cell death pathways (Yi et al., 2007) and the production of reactive oxygen species (ROS) (Gao et al., 2013; Joanisse and Storey, 1996; Lalouette et al., 2011; Rojas and Leopold, 1996). Cold can decrease mitochondrial membrane fluidity, which may lead to an increase in the production of ROS (Hazel, 1995). Ice can form in tissues and disturb the oxidative balance by changing the partial pressure of oxygen (Hermes-Lima and Storey, 1993). At physiological concentrations, ROS play a major role in immune systems and are involved in cell signalling pathways. However, the effects of ROS are dose dependent. An unbalanced concentration of ROS induces oxidative stress when the concentration overloads the detoxifying machinery. When antioxidants and repair mechanisms are overloaded, cellular components such as proteins, lipids and DNA are damaged (see reviews by Halliwell and Gutteridge (2007), Dowling and Simmons (2009) and Selman et al. (2012)).

Drosophila suzukii (Matsumura 1931) (Diptera: Drosophilidae) is native to Southeast Asia and was originally described in Japan in 1916 (Kanzawa, 1939). This invasive species was observed for the first time outside Asia in California (Hauser, 2011) Spain and Italy (Calabria et al., 2010) in 2008. The species then quickly spread throughout North America and Europe (Asplen et al., 2015; Cini et al., 2012). In contrast to the vast majority of *Drosophila* species, *D. suzukii* is able to lay eggs on healthy, ripening fruits that are still attached to the plant using its serrated ovipositor. Damage is caused by larvae feeding on the pulp inside fruits and berries. *D. suzukii* also facilitates secondary fungal and bacterial infections that contribute to fruit deterioration. It is thus a pest which deserves attention. The economic impact on berries and stone fruits may reach $500 million in US dollars in annual losses in the production areas of the western United States (Goodhue et al., 2011). Because of this remarkable invasive success and its economic effects, the biology of *D. suzukii* has been increasingly studied over the past few years. However, little information is available concerning the overwintering capacities of *D. suzukii* adults in invaded areas. This species is more tolerant to cold as an adult than at the pupal stage (Kimura, 2004) and has recently been classified as chill-susceptible (Jakobs et al., 2015; Stephens et al., 2015). Previous studies have been mostly descriptive and restricted to survival or chill coma recovery time. Therefore, the underlying physiological mechanisms remain unexplored.

The aim of this study was to examine the effects of low temperature on ecological parameters, such as survival, fertility and physiological parameters such as oxidative balance in *D. suzukii*. The oxidative stress has been recently described as mediators of life history traits variations (Monaghan et al., 2009). Positive correlations between reproductive effort and oxidative stress were observed in several taxa, including insects (see Alonso-Alvarez et al., 2004; Bergeron et al., 2011; Rojas and Leopold, 1996; Wiersma et al., 2004). We expected that the survival of *D. suzukii* would be modulated both by temperature and the duration of exposure. We predicted that the coldest temperature and the longest duration of exposure would have a negative impact on survival and fertility. We also hypothesized that ROS production would increase with exposure to cold, resulting in an oxidative challenge that may in turn trigger a compensatory response. The activation of the antioxidant system during cold exposure may occur in anticipation to the over-generation of ROS. If no energy is allocated to antioxidant defences, an increase in oxidative damage due to cold exposure would appear and have a subsequent effect on adult survival and fertility.

2. Materials and methods

A strain of *D. suzukii* was generated from approximately 20 mated females collected in Sainte-Foy-lès-Lyon (France; North: 45°44′23.9″, East: 4°47′26.7″) in May 2012. Insects were mass reared at 21 °C and 60% humidity at a 12 h/12 h light/dark photoperiod in climate chambers (SANYO, type MLR-351H; temperature fluctuation: ±0.3 °C). Insects were fed on artificial diet composed by 30 g Agar–Agar, 230 g brewer's yeast (Lynside@nutri 16MKS), 220 g corn flour, 13 g Nipagin: all these compounds diluted into 2.3 L of water (David and Clavel, 1965).

2.1. Experimental procedure

In Japan and Europe, *D. suzukii* is apparently known to overwinter as adults below leaf litter (Kanzawa, 1939; P. Gibert, personnal communication). Therefore, we decided to work on adults only. Three days after emergence, mated males and females were separated after diethyl ether anaesthesia and kept for 48 h in the same controlled conditions used for rearing. The following temperatures were used for the cold treatments, typical of winter regional temperatures (MeteoFrance, 2015) in leaf litter: −4 °C, −2 °C, 0 °C and 2 °C. Temperature and humidity were controlled in a 566L PRECISION TM Low Temperature Incubator 815 from THERMO Scientific (temperature fluctuation: ±0.3 °C). Humidity was maintained between 42 and 45%. For each temperature treatment, preliminary experiments (not shown) allowed us to determine four durations of exposure (Table 1) that generated different mortality rates. A group of flies not exposed to cold was used as a control. For traits measurements, the control group was the same age as the first exposure group at each temperature. For the 2 °C cold treatment, a second control group was used that was the same age as the longest exposure group (144 h, 6 days). This was necessary to ensure that no absolute age effect compromised the results for physiological traits. For each condition and sex, we placed four groups of 15 individuals in plastic boxes (9 cm × 6 cm × 5 cm) containing a slice of artificial diet. In total, 60 individuals were used for each condition. Due to unexpected survival results at 2 °C, we replicated the exposure at this temperature two times to elicit potential experimental artefact effect. Since similar trend has been observed, the datasets have been pooled leading to the use of 90 (6 groups of 15 individuals) for each condition at 2 °C. For controls males under 2 °C conditions, only five groups of 15 individuals were used.

2.2. Measured traits

2.2.1. Survival

After cold treatment, the number of living flies was counted after 3 days maintained in a climatic chamber at 21 °C (the same

Table 1
Duration of exposure for each cold treatment.

Cold treatments	Duration of exposure			
−4 °C	8 h	12 h	24 h	36 h
−2 °C	12 h	24 h	48 h	72 h
0 °C	24 h	48 h	72 h	96 h
2 °C	48 h	72 h	120 h	144 h