

# Influence of cold temperature and exposure time on egg overwintering survival in the white-whiskered grasshopper (Orthoptera: Acrididae)

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## Abstract

The effect of cold temperatures and exposure time on egg survival and hatching success were examined in the white-whiskered grasshopper, *Ageneotettix deorum*. Temperature treatments ranged from 4 °C to -35 °C, with treatment times ranging from 48 to 240 hours. Both decreasing temperatures and exposure time negatively affected egg survival, with a temperature below -25 °C being lethal. Similar lethal temperatures are known for several North American grasshopper species. The relatively shallow location of *A. deorum* egg pods would result in increased vulnerability of eggs to cold temperatures in the absence of snow.

## Keywords

*Ageneotettix*, egg pods, hatching success, lethal temperature

## Introduction

Cold winter air temperatures and snow cover have been assumed to play an important role in grasshopper overwintering egg survival in northern latitudes in North America, but the exact role cold temperatures have on egg survival and population dynamics remains poorly understood (Riegert 1967, Pickford 1970, Mukerji and Braun 1988, Qi et al. 2007). Mukerji and Braun (1988) conducted the only North American quantitative study on grasshopper egg survival and found significant mortality occurred only with temperatures below -15 °C for three *Melanoplus* spp. and *Camnula pellucida*. Snow cover generally reduces the impact of cold temperatures on below-ground overwintering insects (Somme 1999, Irwin et al. 2003, Marshall and Sinclair 2012), and few grasshoppers hatched following a winter with temperatures below -30 °C and little snow cover in Saskatchewan, Canada (Riegert 1967). Although Mukerji and Braun (1988) argued that cold air temperatures rarely affect egg survival due to insulation from snow, climate change is predicted to result in reduced snow cover duration at northern latitudes while periods of extreme cold will continue (Marshall and Sinclair 2012).

Investigations of chill injury and death can improve the understanding of how extreme cold weather events affect overwin-

tering egg survival (Pang et al. 2014). Cold temperature exposure time, along with super cooling points, is frequently used to assess cold hardiness and mortality of insect eggs (Somme 1999, Pang et al. 2014). In this study, cold temperature effects on egg survival were examined in the white-whiskered grasshopper, *Ageneotettix deorum*, a species that lays egg pods parallel to the soil surface and in the top 0.6 cm (Onsager and Mulkern 1963, Branson 2006). The white-whiskered grasshopper is a widely distributed egg-overwintering grasshopper typically not found at higher elevation sites, with its northern distribution reaching central Alberta and Saskatchewan (Pfadt 2002). The experiment was conducted to determine if eggs of the white-whiskered grasshopper are more vulnerable to temperature extremes than species that lay deeper egg pods or if selection for cold hardiness has reduced vulnerability to temperature extremes, both of which could influence population dynamics (Jing and Kang 2003, Branson and Vermeire 2007).

## Materials and methods

Adult grasshoppers were caught in the field and placed in insect rearing cages containing oviposition trays with a mix of soil, vermiculite, and sand. Cages were maintained at 30 °C with a naturally varying light/dark cycle at the USDA Agricultural Research Service Lab in Sidney, Montana, USA (47°43'33"N, 104°9'4"W). Grasshoppers were fed wheat seedlings and wheat bran *ad libitum*, with romaine lettuce added as a supplement. A given set of oviposition trays remained in a cage for ~2.5 weeks starting on August 10<sup>th</sup>, August 27<sup>th</sup>, and September 16<sup>th</sup>, 2010. Trays were then kept at 30 °C for two additional weeks to promote egg development. The egg pods were then removed from the trays and 16 egg pods were placed in cups with vermiculite; there were an equal number of egg pods from each removal date and eight replicates per treatment. Environmental test chambers (Model SD-505, Associated Environmental Systems, Ayer, MA, USA) were utilized for temperature treatments, with temperatures verified using dataloggers. Temperature treatments were 4 °C (control), -20 °C, -25 °C, -30 °C, and -35 °C, with exposure times of 48, 120, and 240 hours. Temperatures and exposure times were chosen based on Parker (1930), with the lowest (-35 °C) temperature treatment

chosen specifically due to the shallow egg pod depth in this species. Cups were incubated at cold temperatures for a minimum of 4 months to break obligate diapause (Henry 1985), with cups initially placed in a refrigerator maintained at 4°C, moved to test chambers for cold temperature treatments, and then returned to 4°C. Egg pods in the control treatment remained at a constant 4°C. After egg cups were placed in a test chamber, the chamber was held at 4°C for 48 hours before ramping to the desired treatment temperature over a 15-minute period and then maintained at a constant temperature until the end of the exposure time. Cold treatments began on February 16<sup>th</sup> and March 2<sup>nd</sup> using half of the replicates for each treatment on each date. On March 28<sup>th</sup>, water was added to all cups and cups were then placed at 30°C to begin a 16h:8h (light:dark) cycle. Plastic cages with a wire mesh top were placed on each cup and hatching grasshoppers were counted and removed daily. Egg pods were removed and dissected once no hatching had occurred for 10 days.

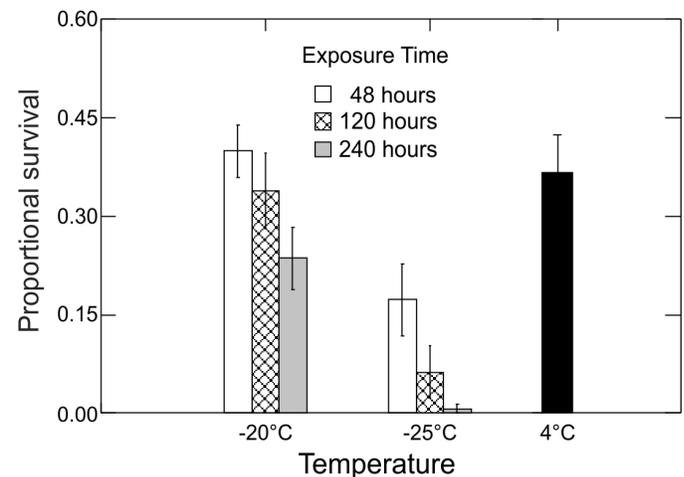
The proportion of eggs hatched was used for statistical analysis after arcsine transformation of the proportion data. As no eggs survived in any exposure time treatment at -30°C and -35°C, those temperatures were not statistically analyzed. For -20°C and -25°C treatments, analysis of variance was used to examine if temperature and exposure time significantly affected the proportion of eggs that hatched. A split plot analysis was used to account for the replicates being evenly divided into separate temperature exposure chamber runs. Additional pairwise comparisons were used to examine treatment differences using Tukey's test. Additional pre-planned pairwise comparisons were used to examine if hatching was reduced in cold temperature treatments relative to the control (4°C) treatment using Tukey's test. Statistical analyses were conducted using Systat 13 (Systat Software 2009).

## Results and discussion

Both temperature and exposure time significantly affected egg survival in the -20°C and -25°C treatments, with the temporally separated chamber runs accounting for very little variation in hatching (Table 1, Fig. 1). For both temperatures, the proportion of eggs hatching decreased with time of exposure (Table 1), with only 3 eggs hatching in the 240 hour -25°C treatment. Survival was lower in the 120 hour exposure than in the 48 ( $P = 0.035$ ), lower in the 240 hour exposure than in the 48 ( $P < 0.001$ ), and lower in the 240 hour exposure than in the 120 ( $P = 0.04$ ). No eggs hatched in any time exposure treatments at -30°C and -35°C, indicating 100% egg mortality occurred at those temperatures. Egg survivorship did not differ significantly between the control (4°C) treatment and 48 hours at -20°C ( $P > 0.3$ ), trended towards sig-

**Table 1.** Results from an ANOVA model examining the effect of temperature and exposure time on proportion of eggs hatching for the -20°C and -25°C treatments, using a split plot analysis to account for treatment replicates being divided between two chambers (Block). Proportion data was arcsine transformed prior to analysis.

ANOVA source	SS	df	F-Ratio	P value
Time	0.651	2	12.63	$P < 0.001$
Temperature	1.791	1	69.54	$P < 0.001$
Time*Temperature	0.076	2	1.48	$P = 0.240$
Block	0.004	1	0.17	$P = 0.680$
Error	1.061	41		



**Fig. 1.** Proportional survival (mean  $\pm$  SE) of white-whiskered grasshopper eggs by temperature (-20°C, -25°C, and 4°C control) and exposure time (48, 120, and 240 hours) treatments (8 replicates with 16 egg pods per replicate). No eggs survived in any exposure time treatment at -30°C and -35°C.

nificance at 120 hours at -20°C ( $P = 0.09$ ), and was significantly lower at 240 hours at -20°C ( $P < 0.01$ ) where survival was reduced by 51%. Egg survival was significantly lower in all three exposure times at -25°C compared to the 4°C control ( $P < 0.001$ ). Egg survival was reduced by 18% with a 48 hour exposure to -20°C relative to 4°C, while survival was reduced by nearly 65% with a 48 hour exposure to -25°C. As no eggs hatched at -30°C, the lethal low temperature for eggs exposed to 48 hours of low temperatures was between -25 and -30°C.

In contrast to the shallow depth egg pods laid by *A. deorum*, *Melanoplus sanguinipes*, examined in cold temperature studies by Parker (1930), Riegert (1967), and Mukerji and Braun (1988), oviposits vertical egg pods with a midpoint depth of  $\sim 2$  cm. Sub-surface temperatures were found to be colder at the depth of *A. deorum* egg pods (0.6 cm) than at the depth of species such as *M. sanguinipes* that lay vertically oriented egg pods, during a cold winter period when snow and litter were removed from the soil surface (Branson unpublished data). Thus, white-whiskered grasshopper eggs would be exposed to colder minimum temperatures than many other grasshoppers when snow cover is limited (Marshall and Sinclair 2012). Mukerji and Braun (1988) tested low temperature impacts on egg mortality in three *Melanoplus* species and *C. pellucida*, but the lowest temperature they tested was -18°C. In a less rigorous study, Parker (1930) found that *M. sanguinipes* and *C. pellucida* egg hatching declined at -25°C, while 100% mortality occurred with varying exposure times at -30°C. Thus, the eggs of several grasshopper species laying egg pods at a range of depths in North America have similar lethal temperatures of -25°C to -30°C, indicating that sustained extremely cold temperatures are required to significantly reduce grasshopper egg survival. Although temperatures were kept constant in this study, winter air temperatures fluctuate and repeated cold exposure events have been shown to modify temperature impacts on eggs (Colinet et al. 2018, Marshall and Sinclair 2018). Due to its shallow egg pod location, the white-whiskered grasshopper may have an increased vulnerability to extreme cold temperature events that could contribute to its northern distribution boundary.

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