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Effect of temperature and host tree on cold hardiness of hemlock looper eggs along a latitudinal gradient

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ABSTRACT

The hemlock looper, *Lambdina fiscellaria*, is an economically important insect pest of Canadian forests which overwinters as eggs. Although the hemlock looper causes extensive damages, no information on the mechanisms related to its cold tolerance is known. The objective of this study was to determine the effect of temperature and exposure duration on hemlock looper winter survival but also to identify seasonal supercooling capacity and cryoprotectant levels of three populations along a latitudinal gradient. As host plant may contribute to offspring overwintering success, cold tolerance of hemlock looper eggs from parents whose larvae were fed on three different tree species was also measured. Mean supercooling point (SCP) of hemlock looper eggs was lower than −30 °C from October through the following spring with values being as low as −47 °C in February. Trehalose was the most abundant sugar found in hemlock looper eggs with a peak concentration of 0.3 μg mg^{-1} DW^{-1}. Glycerol, a polyol, was more often absent in eggs of the different populations and tree species tested in the study. When exposed to different temperature regimes for various periods of time, significant mortality of hemlock looper eggs occurred at higher temperatures than the mean SCP. Thus, hemlock looper could be considered as a chill tolerant species. No clear pattern of population and host plant effects on SCP and cryoprotectants was detected in this study. However, when exposed to different winter temperatures and exposure duration, hemlock looper from higher latitudes survived better (survival rates ranging between 0 and 89% at −20 °C) than those from lower latitudes (survival rates ranging between 0 and 56% at −20 °C). Our results may contribute to a better understanding of hemlock looper winter biology and thus facilitate predictions of outbreaks and range expansion.

1. Introduction

In temperate and cold regions, insects have developed cold hardiness strategies to survive prolonged periods of subzero temperatures. Two main strategies are well known: the freeze avoidance and the freeze tolerance (Zachariassen, 1985; Leather et al., 1993; Bale and Hayward, 2010). Because freezing is lethal to freeze avoiding insects, these species have the capacity to supercool, *i.e.* to maintain their body fluids in a liquid state below their melting point by removing ice nucleators, synthesizing antifreeze proteins, and accumulating sugars and polyols (Zachariassen, 1985). On the other hand, freeze tolerant species can survive the formation of ice initiated by ice nucleators in the haemolymph or gut (Leather et al., 1993). As for the freeze avoiding insects, freeze tolerant species also synthesize sugars and polyols which limit freeze damage (Baust, 1973; Zachariassen, 1985). Whether they are freeze avoiding or freeze tolerant, the majority of insects of temperate and cold regions enter diapause, a genetically programmed response to changing seasons allowing them to escape harsh environmental conditions (Denlinger, 1991; Bale and Hayward, 2010). Even if basal metabolism of insects is slowed down during diapause, notable changes happen in cold hardiness, supercooling capacity, and in the level of antifreeze products such as low molecular weight sugars and polyols (Denlinger, 1991; Han and Bauce, 1995).

In northern North America, most insects exposed to sub-zero temperatures in nature are freeze avoidant (Leather et al., 1993; Denlinger and Lee, 2010). To survive winter, they produce and accumulate antifreeze products (*e.g.* glycerol) as temperature decreases (Leather et al., 1993). For phytophagous insects, the amount of energy reserves stored depends on the availability and on the quality of their host plant (Hough and Pimentel, 1978; Rossiter, 1991; Röder et al., 2008). As the production and the accumulation of cryoprotective substances depend on energy reserves, host plant quality will affect insect overwintering success (Storey and Storey, 1991).

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The hemlock looper, *Lambdina fiscellaria* (Guenée) (Lepidoptera: Geometridae), is a widespread neartic species and because it can cause rapid and extensive tree mortality, it is one of the most economically damaging defoliator of North American coniferous forests (MacLean and Ebert, 1999; Bordeleau, 2000; Hébert and Jobin, 2001). The hemlock looper is highly polyphagous, feeding on both deciduous and coniferous trees but balsam fir, *Abies balsamea* ((L.) Mill.) is the most heavily damaged tree species (Carroll, 1956; Hébert and Jobin, 2001). Young larvae feed on current-year foliage while older larvae feed on old foliage (Carroll, 1999). The most important outbreaks have been reported in the northern part of its distribution (Martineau, 1984) and they usually appear and disappear suddenly, rarely persisting more than 3 years at a specific location (MacLean and Ebert, 1999; Hébert and Jobin, 2001; Bordeleau, 2002). Adults are active from mid-August to early October and females lay scattered eggs almost everywhere in the forest but mainly on trunks and branches (De Gryse and Schedl, 1934; Watson, 1934; Carroll, 1956). Eggs are often exposed to harsh winter conditions for several months and go through three developmental stages: a pre-diapause which lasts about 2 weeks after oviposition, the diapause itself which is completed by the end of December, and a post-diapause and/or quiescence where eggs are ready to hatch when conditions become favorable (Carroll, 1956; Delisle et al., 2009). Thus, the only source of energy allowing the production and accumulation of cryoprotective substances in hemlock looper eggs is provided by the parental generation.

Even though hemlock looper causes substantial damages to forests (in 2000, near 1 million ha were severely defoliated in the province of Québec; Bordeleau, 2000) and that winter survival is an important component of insect population dynamics, no information is currently available on its cold hardiness. Therefore, this study aims to establish seasonal trends in supercooling points and in the levels of major cryoprotective substances in hemlock looper eggs. Because egg content is expected to vary according to female "quality", we studied the effect of two important variables known to influence female fitness: host tree and population along a latitudinal gradient (Berthiaume, 2007). Previous studies have shown that offspring overwintering success and insect cold tolerance differed along a latitudinal gradient (Leather et al., 1993; Turnock et al., 1998). Moreover, as winter survival also depends on the exposure duration of an insect to sub-zero temperatures (Bale, 1987), eggs were exposed to different temperatures for various periods of time.

In this study, we predicted that overwintering eggs originating from parents reared on a more suitable host and coming from a higher latitude population should show stronger cold hardiness potential (lower supercooling points, higher level of cryoprotectants, and higher survival rate at low temperatures) than those coming from parents fed on a less suitable host and coming from a lower latitude population.

2. Materials and methods

2.1. Insect material and rearing

The experiment was carried out with hemlock looper eggs coming from established colonies which were reared on balsam fir foliage for 7 years (Berthiaume, 2007); during winter, eggs were kept in a field insectary at the Laurentian Forestry Center in Québec City (46°78′N, 71°28′W). In order to evaluate cold hardiness of different hemlock looper populations with respect to their original latitude, eggs from three populations across the province of Québec, Canada, were used: Rivière-Ouelle (47°26′N, 70°01′W), Québec City (46°51′N, 71°16′W), and Sawyerville (45°20′N, 71°33′W). Also, to measure the influence of host tree on hemlock looper cold hardiness, larvae of each population were reared on either balsam fir or white spruce, *Picea glauca* [Moench] Voss (two conifers) or on white birch, *Betula papyrifera* Marsh. (a deciduous tree). These tree species are mostly found across the boreal forest and often grow in the same stands, with balsam fir as the dominant species with white spruce and white birch as companion species (Victorin, 1964; Farrar, 1995). During hemlock looper outbreaks, balsam fir is the most severely damaged tree species.

At the end of May 2009, more than 1000 hemlock looper eggs of each populations were placed at 23 °C under a 16L:8D photoperiod to promote hatching. Newly hatched larvae were immediately placed on balsam fir, white spruce, or white birch foliage in 6-L Omni^{TM} plastic boxes (#008632; A.B.M. Canada Inc., Brampton, ON, Canada). Larvae were reared in a growth chamber at 18 °C, 60% R.H. and a 16L:8D photoperiod from the beginning of June to the end of July 2009. Branches of each tree species were collected from mature trees (n = 10/tree species) which had a 10–30 cm diameter at breast height (DBH) in a tree stand located in Québec City, Québec, Canada (46°48′N, 71°23′W). Twigs were cut and placed into plastic florist's tubes filled with water. To keep tubes upright, a piece of polystyrene was fixed at the bottom of the rearing boxes. For coniferous trees, foliage provided to larvae included current and 1-year-old foliage while shoots bearing young leaves were provided for white birch. Foliage in each box was replaced every 2 days to provide a continuous source of fresh foliage.

Once emerged, moths of each population that were fed on the same tree species were placed in screen cages for mating. Mated females were then placed individually in 140 ml screened plastic bottles with a foam strip as oviposition substrate and a 8% water–sugar solution (Hébert et al., 2003). Females were kept in a growth chamber at 20 °C, 40% R.H. and 16L:8D photoperiod. After oviposition was completed, eggs were kept under the same conditions for 2 weeks. Following this period, foam strips containing eggs were placed at 15 °C for 1 month followed by 2 weeks at 3 °C before being stored on 22 October 2009 in the field insectary. Fertile eggs (turgescent brown eggs) were used for estimating supercooling points and cryoprotectant contents as well as for running cold hardiness experiments in fall 2009 and winter 2010.

2.2. Supercooling points

For measuring supercooling points, individual hemlock looper eggs were placed and fixed with an adhesive tape on thermocouples (copper/constantan Type T; Physitemp Instruments, Chicago, IL, USA) that were attached to a mount to keep them in place. The mount was placed in a multi layers polystyrene box which was put in a freezer (maximum freezing capacity of −55 °C). A Type T thermocouple digital thermometer was used to measure temperature changes. The decrease of temperature was nearly linear in the first 100 min to reach ~ −35 °C and then temperature decreases at a lower speed with an average of less than 0.1 °C/min. The lowest temperature reached before observing the heat release produced by crystallization was considered to be the supercooling point, also known as the spontaneous crystallization temperature (Leather et al., 1993). Supercooling points were measured for 15 individual eggs (5 eggs per measurement (rep), 3 replicates per population and tree species) at six sampling dates: October and December 2009, January, February, March and April 2010.

2.3. Sugar and polyols contents

Because of their importance in insect physiology and their roles as cryoprotectants (Zachariassen, 1985), trehalose, glucose, glycerol, and glycogen contents were measured in hemlock looper eggs. Trehalose, glucose, and glycerol were quantified using the method described by Izumi et al. (2005). Three replicates of 25