Overwintering biology of emerald ash borer in Minnesota

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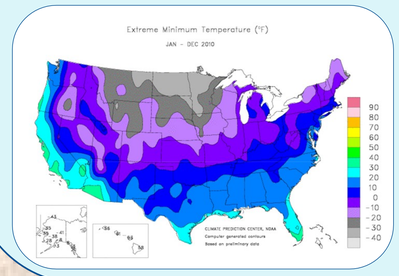
Introduction

Finds of emerald ash borer (EAB, *Agrilus planipennis*) in Minnesota since 2009 have defined the northwest boundary of the insect's distribution in North America. Portions of Minnesota are colder than other areas where EAB has been found (Fig 1). The majority of Minnesota's nearly 1 billion ash trees (*Fraxinus* spp) occur in the northern part of the state. The ultimate distribution and impact of EAB in North America will depend, in part, on the capacity of the insect to survive exposure to extreme cold.

EAB overwinter as larvae and experience little mortality until they freeze (Crosthwaite et al. 2011). EAB avoid freezing by increasing concentrations of glycerol and free amino acids, thereby lowering their supercooling point, the temperature at which an insect spontaneously freezes. In China, EAB supercool between -26.4 to -23.0°C (Wu et al. 2007). Crosthwaite et al. (2011) reported a mean supercooling point of -30.6°C for cold acclimated larvae from Ontario.



The objectives of the current study were to evaluate supercooling points of EAB from Minnesota and to evaluate larval survivorship in logs with different cold treatments.

Figure 1. Extreme minimum air temperatures recorded in the United States during 2010. Minimum temperatures in Minnesota ranged from -10°F (-23°C) to -40°F (-40°C).

Methods

Green ash (*Fraxinus pennsylvanica*) from Minneapolis-St. Paul, MN that were naturally infested with emerald ash borer were cut into bolts in fall and winter of 2009 and 2010.

Supercooling points. Bark was peeled from bolts, and larvae were extracted. Supercooling points were measured with contact-thermocouple thermometry (Fig. 2) following methods modified from Carrillo et al. (2004).

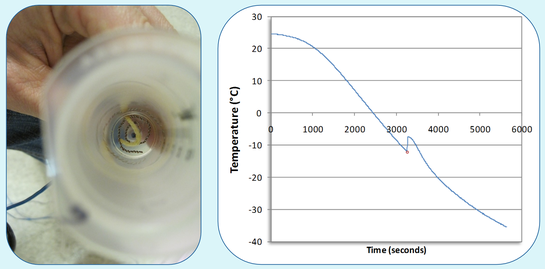


Figure 2. Emerald ash borer attached with high vacuum grease to a copper-constantan thermocouple (left); thermal profile of an insect cooled below 0°C with a supercooling point at -12°C (right).

Overwinter survival. Infested logs were peeled and larval condition assessed before cold treatment. Intact logs were assigned randomly to four temperature regimes: (*6-8 wks*): cold room, St. Paul outdoors, "Grand Rapids"-area outdoors, and (*<12h*) freezer to ca. -36°C. Logs were then peeled and larval stage, color, and movement (over 48h) were noted.

Figure 3. Locations for overwintering studies in Minnesota (left); precautions taken to ensure experimental logs were not taken for firewood (right). Grand Rapids study reviewed and approved by Minn. Dept. Ag.

Results & Discussion

Supercooling points. The mean supercooling point of non-acclimated EAB larvae (summer collection) was -18°C in 2009 and -14°C in 2010; these values did not differ (P>0.05). The mean supercooling point of cold acclimated larvae (late fall/winter collection) was -25°C in 2009 and was not significantly different from the supercooling point of cold acclimated larvae (-28°C) in 2010; these values were significantly different from non-acclimated larvae (Fig. 4).

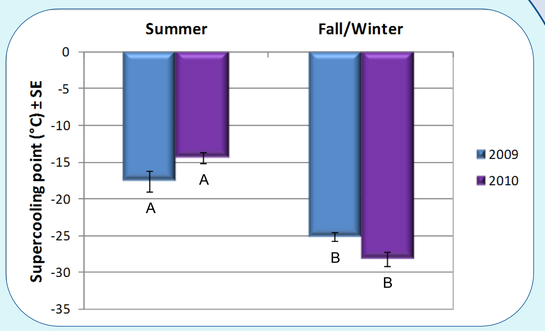


Figure 4. Supercooling points of emerald ash borer larvae collected in the summer (not cold acclimated) or fall/winter (cold acclimated) in 2009 and 2010. Bars with the same letter are not significantly different (P>0.05).

Overwinter survival. Larvae were classified as brown, inactive, or active. Observations of a subset of larvae confirmed that 100% of brown larvae and >80% of inactive larvae were dead. Only 11% of moving larvae were dead. Thus, discoloration or lack of movement are meaningful indicators of mortality.

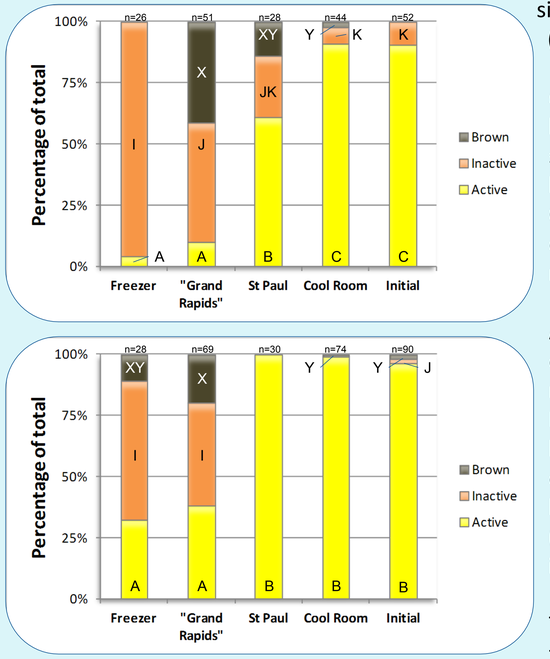


Figure 5. Condition of larvae before and after exposure to cold regimes during the winter of 2009-2010 (top) and the winter of 2010-2011 (bottom). Bars with the same letter are not significantly different (P>0.05).

At the start of the 2009-2010 winter, most larvae (ca. 90%) were active. No brown larvae were observed. Logs held in a walk-in cold room experienced constant temperatures of +4°C. The majority of larvae (90%) from these logs remained active; a small fraction (2%) appeared brown. Logs held outdoors in St. Paul experienced a low temperature of -28°C. Approximately, 40% of larvae from these logs were brown or not moving. Logs held outdoors in the "Grand Rapids" area experienced -36°C and nearly 90% of larvae from these logs were brown or inactive. Most larvae (>95%) from logs chilled to -37°C in a freezer were inactive.

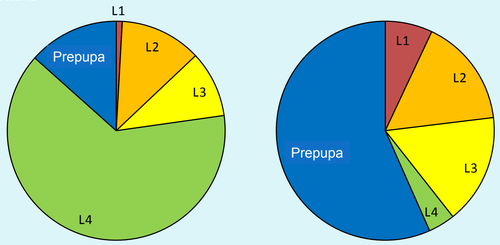


Figure 6. Stage distribution of larvae recovered after the winter of 2009-2010 (left) and 2010-2011 (right).

Trends in the 2010-2011 winter were similar to those in 2009-2010, though the fraction of larvae that remained active was greater in most treatments. In general, the fraction of larvae that were active continued to decline as larvae were exposed to colder conditions. Logs in the cold room experienced a low of +4°C; in St. Paul, -29°C; in Grand Rapids, -37°C, and in the freezer, -35°C. The greatest difference among winters was the proportion of larvae that stayed active in St. Paul; all were active at the end of the 2010-2011 winter.

The reason for the greater survivorship during the 2010-2011 winter is not yet clear. Temperatures during the fall of 2010 were warmer than during the fall of 2009 (data not shown). As a result a greater proportion of larvae developed to the prepupal stage (Fig.6). Prepupae may form pupal cells in the outer sapwood, and thereby increase the thickness of host tissue between the insect and the environment. This layer may provide insulation and protection from short-term drops in temperature (Fig. 7).

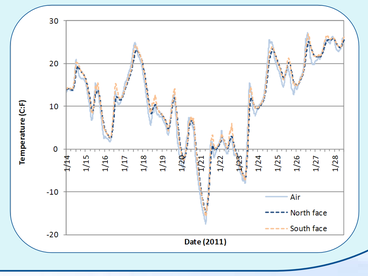


Figure 7. Temperatures recorded in the air and beneath the bark on the north and south faces of a green ash tree in Minneapolis, MN.

Conclusions

- Knowledge of winter low temperatures and EAB supercooling points provides a meaningful forecast of EAB larval survival during the winter.
- Larval mortality from cold ranged from 60-90% in northern Minnesota.
- Cold temperatures may limit the potential for spread or damage from EAB in northern Minnesota where stands of ash, particularly black ash (*F. nigra*), are extensive.

References & Acknowledgments

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