COLD HARDINESS OF EMERALD ASH BORER, *AGRILUS PLANIPENNIS*: A NEW PERSPECTIVE

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Abstract

This study was designed to assess the cold hardiness of emerald ash borer larvae, the overwintering stage of the insect. We began by measuring larval supercooling points, the temperatures at which larvae freeze. We found that larvae collected from naturally infested trees in St. Paul, MN between late October and early December had an average supercooling point of -25°C (-13°F). Research elsewhere indicates that when these insects freeze, they die. Our laboratory assessments of cold hardiness were confirmed during field tests. Naturally infested logs were held outdoors in St. Paul, MN (low winter air temp=-28°C) and near Grand Rapids, MN (-34°C) for ca. 5.5 weeks. Approximately 40% of larvae from logs in St. Paul were inactive or brown, both evidence of death; approximately 90% of larvae from logs near Grand Rapids were inactive or brown, compared with the approximately 10% that showed evidence of death prior to exposure or after being held under cool, non-lethal conditions. Overwintering mortality may help to minimize the damage caused by emerald ash borer in areas with extremely cold winter climates.

Emerald ash borer, *Agrilus planipennis* Fairmaire was detected in North America initially in 2002. It was detected for the first time in Wisconsin in 2008 and in Minnesota in 2009. The insect is now poised to move into some of the coldest regions of North America, especially northern Wisconsin, northern Minnesota, and North Dakota. Cold stress has proven to be a significant constraint during invasions by other species. Although regions where emerald ash borer has been found in North America could be considered "cold," with an average low January temperature between -29 to -18°C (-20 to 0°F), colder regions have an average low January temperature frequently between -40 to -34.5°C (-40 to -30°F). The potential for emerald ash borer to survive and cause damage under these extreme conditions is not known.

Previous work suggests emerald ash borer will typically overwinter as a pre-pupa. Earlier instars may also overwinter, perhaps as part of a 2-year lifecycle. Prepupae will overwinter in a pupal cell commonly formed in the outer sapwood, but other life stages may be found in the phloem or bark.

Much of our current understanding of the cold tolerance of emerald ash borer is inferred from the presumed distribution of the insect in Asia, not careful observations of the insect. Preliminary reports on the cold hardiness of the insect in Ontario suggest that cold causes very little mortality until the insect actually freezes (Sobek et al. 2009). The temperature at which an insect freezes is known as its supercooling point. Sobek et al. (2009) indicate that the average supercooling point of emerald ash borer larvae recovered from Ontario was -30.6°C (-23°F). However, Wu et al. (2007) suggest that the supercooling point of larvae from China falls between -26.4 to -23.0°C (-15.5 to -9.4°F).

The objectives our study were to measure supercooling points for emerald ash borer larvae recovered from naturally-infested green ash (*Fraxinus pennsylvanica*) in St. Paul, MN. We then developed an equation to relate the predicted extent of mortality to the lowest temperature experienced by the larvae. Finally we measured the survival of larvae in infested logs exposed to different cold regimes.

Methods

Supercooling points. Larvae were collected at random from infested trees felled in St. Paul, MN in June 2009. Supercooling points were measured using copper-constantan thermocouples (24 gage, non-stranded) following the methods of Carrillo et al. (2004). These larvae (n=11) were presumed not to be winter acclimated and provided a summer baseline. Larvae were again collected and supercooling points measured from late October to early December 2009 (n=62) and were presumed to be fully acclimated. Supercooling point data were analyzed in @Risk (Palisade Corp., Ithaca, NY) to determine whether the observations were normally distributed.

Larval winter survivorship. Three green ash trees that were naturally infested with emerald ash borer were felled in St. Paul, MN on 28 Dec 2009. Logs (ca. 0.6 m in length) were taken from upper-, mid- and lower-canopy braches. Logs (n=20) from each tree and canopy level were sealed with paraffin wax and randomly assigned to each of five batches. Bark was peeled from logs of the first batch immediately to determine initial larval densities and condition. Each of the other batches was assigned to one of four treatments: (i) logs cooled to a target of -35°C in a sub-zero freezer; (ii) logs held outdoors in northern Minnesota near Grand Rapids; (iii) logs held outdoors in St. Paul, MN; and (iv) logs held in a walk-in cool room at approximately 4°C. We drilled 5.6-mm (7/32 in.) holes at an oblique angle to a depth of approximately 5 cm on the future north and south face of the smallest and largest diameter log within batches to be kept outdoors or in the walk-in cool room. We inserted a thermistor from a Hobo Pro v2, 2 ext temp (Onset Computer Corp., Bourne, MA) into each hole and sealed the hole with high vacuum grease. The data logger was programmed to record temperature once every five minutes. We also screwed eye-bolts into the logs that were held outdoors, chained the logs together, and secured them with a padlock to prevent unauthorized removal. Logs held near Grand Rapids were further secured behind a locked gate. All handling procedures in northern Minnesota were reviewed and formally approved by the Minnesota Department of Agriculture. With the exception of the freezer treatment, all logs were exposed to their respective treatments for 5-6 weeks. For the logs destined for the freezer, we drilled 2.8- mm (7/64in) holes on the lower (in contact with a layer of polystyrene insulation) and upper (exposed to the air) surfaces of each log. We inserted a 24 gage copper-constantan thermocouple into each hole to record log temperatures. Logs were chilled to ca. -35°C and moved to the walk-in cold room for less than 72 hrs. For all treatments, after cold exposures were complete, bark was peeled from logs, larvae counted, and the condition of each larva noted. Larvae that did not move after repeated observations over 24 hrs were considered dead. Larvae that were not damaged during the peeling process were saved for additional observation and testing. Data were analyzed by using logistic regression (PROC LOGISTIC in SAS).

Results and Discussion

Supercooling points. Winter-acclimated larvae (collected between Oct-Dec) had a mean supercooling point of -25°C (-13°F), which was significantly colder than the supercooling point of non-acclimated larvae (-18°C; 0°F; $t=5.1$, $df=13$, $P<0.01$). Supercooling point observations from the winter-acclimated larvae were not significantly different from a normal distribution (Chi-square = 5.7; $P>0.1$). From our simple model that related the coldest temperature experienced by emerald ash borer larvae to the extent of mortality, we predicted that when larvae reach -17.8°C (0°F), 5% will die; at -23°C (-10°F), 34% will die; at -29°C (-20°F), 79% will die; and at -34°C (-30°F), 98% will die.

Larval winter survivorship. Coldest temperatures recorded for logs in each of the treatments are reported in Table 1.

More than 90% of the larvae that were recovered at the start of the experiment were of "good" color (buff-yellow) and actively moving (Fig. 1). A statistically equivalent proportion was moving when extracted after ca. 5.5 weeks in the walk in cold room, a treatment where we predicted no mortality (Table 1). A significantly lower proportion of the larvae moved after being extracted from logs held outdoors in St. Paul. Only 5-10% of larvae moved after being extracted from logs held outdoors near Grand Rapids, MN or chilled in a sub-zero freezer. Approximately 40% of the larvae from near Grand Rapids had turned brown, clear evidence of tissue damage and strong additional evidence for mortality.

Follow-up observations confirmed that 100% of the larvae that were brown were clearly dead 3weeks after extraction. Between 75-85% of larvae that were inactive at the time of extraction were dead 3weeks after extraction, while only 5-15% of the larvae that were active at the time of extraction were dead after the same amount of time.

Minnesota winters, especially in the northern part of the state, may cause substantial mortality of emerald ash borer larvae. However, even with the extreme cold air temperatures that were experienced near Grand Rapids, MN, some emerald ash borer larvae survived. Thus, cold temperatures may not completely eliminate the insect. However, cold temperature may help to keep populations from building quickly and may give ash trees some time to recover from initial attacks.

We have also learned that air temperatures, recorded at standard meteorological weather stations, are not necessarily the most reliable measure of the temperature experienced by overwintering emerald ash borer larvae. Trees warm considerably on sunny days through radiant heating. Larvae that are able to form a pupal cell in the outer sapwood may be afforded some protection against brief drops in temperature.

These results have significant implications for predictions of the future range of emerald ash borer, spread rates of the insect in areas with a harsh winter climate, and the time required for these insects to kill a tree.

References

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Table 1. Coldest temperatures recorded in largest and smallest diameter logs used in larval winter survivorship studies and predicted range of larval mortality in each of the treatments.

Log (diameter)	Temperature (°C)			Predicted mortality (%)
	Air Temp	Warm face	Cool face	
Freezer				
F5 (10.9 cm)	NA	-34	-37	97-99
F2 (25.5 cm)	NA	-25	-33	48-96
"Grand-Rapids" area				
GR4 (10.9 cm)	-34	-36	-36	99
GR8 (35.6 cm)	-34	-32	-32	93
St. Paul				
SP2 (13.0 cm)	-28	-28	-28	73
SP17 (35.6 cm)	-28	-26	-28	57-78
Cooler				
C6 (10.2 cm)	4	NA	4	0
C1 (40.6 cm)	4	NA	3	0

Figure 1. Initial condition of emerald ash borer larvae and after exposure to different cold treatments. The number of larvae that were recovered is reported above each bar.