

Evaluation of vacuum technology to kill larvae of the Asian longhorned beetle, *Anoplophora glabripennis* (Coleoptera: Cerambycidae), and the emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae), in wood

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Abstract

The potential for using vacuum technology to kill larvae of the Asian longhorned beetle (ALB), *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae), and emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), in solid-wood packing materials (SWPM) and other wood products was assessed. Current regulations require that SWPM be heat treated or fumigated prior to export. Vacuum treatment may be a cost-effective and an energy-efficient alternative that eliminates environmental concerns associated with chemicals used in fumigation. Low pressure, achieved by applying a vacuum to a system, imposes a controlled atmosphere and desiccating environment that results in death of wood-infesting insects. Larval ALB and EAB, either exposed directly to vacuum or inserted into wood at various moisture levels, were subjected to different temperatures and pressures to determine desiccation rates and lethal percentage weight loss. Some ALB and EAB larvae died at 26 percent weight loss, and all were dead at 40 percent weight loss. Desiccation under low-pressure vacuum also killed ALB pupae and eggs. The desiccation rates of both ALB and EAB larvae under vacuum were constant until death, but decreased as larvae approached complete desiccation (approximately 60 percent weight loss). ALB larvae lost weight faster than EAB larvae at 20 mmHg and 20 °C (3.35% weight loss per hour and 2.39 percent, respectively). Lethal vacuum time at 40 percent weight loss was estimated to be 13.2 hours and 18.5 hours, respectively, for exposed ALB and EAB larvae treated at 20 mmHg and 20 °C. Under the same vacuum conditions, lethal time was estimated to be approximately 51.4 hours and 44.8 hours, respectively for ALB and EAB inserted into wood blocks with 16.6 to 21.6 percent moisture content. Temperature, pressure, and relative humidity affected desiccation rate. Larvae desiccated slower with decreasing temperature, increasing pressure, and increasing internal wood moisture.

Wood-packing materials such as pallets and crating made of unprocessed wood can be infested with insects and other organisms (Haack 2006). The spread of pests in solid-wood packaging material (SWPM) can significantly alter ecosystems, hurt regional economies, result in significant remediation costs and limit the international flow of goods (Mumford 2002). The Asian longhorned beetle (ALB), *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae), is an example of such a pest. ALB, a serious tree pest in China (Haack et al. 1997), has been intercepted in warehouses in at least 14 states in the United States (USDA APHIS 2007). ALB poses a threat to American hardwood forests because of its wide host range and its ability to attack and kill apparently healthy trees. It attacks members of the genera *Acer*, *Aesculus*, *Betula*, *Fraxinus*, *Populus*, *Salix*, and *Ulmus* (Haack et al. 1997). In the United States, eradication programs have been implemented in Illinois, New York, and New Jersey

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to prevent the enormous losses that could occur should this beetle thrive and spread unchecked. ALB infestations have the potential to eliminate up to 35 percent of the current canopy cover in North America (1.2 billion trees), with losses of \$669 billion (Nowak et al. 2001). Eradication programs have resulted in the removal of thousands of trees at a cost of millions of dollars (Markham 2004).

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is another exotic beetle now present in the United States that probably arrived in SWPM. It is native to Asia and was discovered in southeastern Michigan near Detroit in the summer of 2002 (Haack et al. 2002). The larvae feed on the inner bark of ash trees (*Fraxinus* sp), disrupting the tree's ability to transport water and nutrients. As of 2006, EAB had killed at least 15 million ash trees in Michigan, Ohio, and Indiana (Poland and McCullough 2006). Unless effective control measures are developed and implemented, EAB could threaten the ash resource nationwide. Both ALB and EAB are of particular concern because they attack and kill apparently healthy trees. Methods to prevent reintroduction of these pests and introductions of other potential pests in SWPM are needed.

An international standard was adopted by the International Plant Protection Convention (IPPC) in 2002 that requires SWPM to be treated with a gas fumigant (methyl bromide) or with heat that achieves a minimum core temperature of 56 °C for 30 minutes (FAO 2002). This International Standard for Phytosanitary Measures was published as Food and Agricultural Organization Publication 15 and is commonly known as ISPM-15. This standard is now being adopted as a regulation by individual countries, including the United States (USDA APHIS 2004). As of 2005, methyl bromide is prohibited for use in developed countries for all purposes except quarantine and preshipment treatment as part of the United Nations Environment Program (1998). Heat treatment is costly due to its high energy input requirements. Thus, there is a need for globally-acceptable treatment alternatives that provide assurance of eliminating the risk of infested wood materials while being cost-effective and environmentally benign. Vacuum treatment may present an economical and energy-efficient alternative (Saththo and Yamsaengsung 2005) that does not release ozone-depleting chemicals or other pollutants, and does not affect the appearance or properties of the wood (Lee and Harris 1984, Harris and Taras 1985). Previous research (Chen et al. 2007) has demonstrated the commercial feasibility of using low-pressure vacuum technology to kill other small species of longhorned beetles (*Monochamus* and *Stenocorus* spp.) and the pinewood nematode (*Bursaphelenchus xylophilus* (Steiner & Buhner) Nickle). The invertebrates die due to desiccation under low-pressure vacuum. Water loss in insects occurs via cuticular transpiration, respiration, secretion from mouth and anus, and via excretion (Hadley 1994). Since larger individuals have a greater initial water mass and higher volume to surface area ratio, which both contribute to longer survival under desiccating conditions (Renault and Coray 2004); we needed to evaluate this method with larger larvae of a species that can be transported in SWPM.

The objective of this research was to evaluate the effectiveness of vacuum treatment against ALB and EAB larvae. Our specific objectives were to determine 1) the lethal time of treatment for mortality of larvae, 2) the lethal percentage

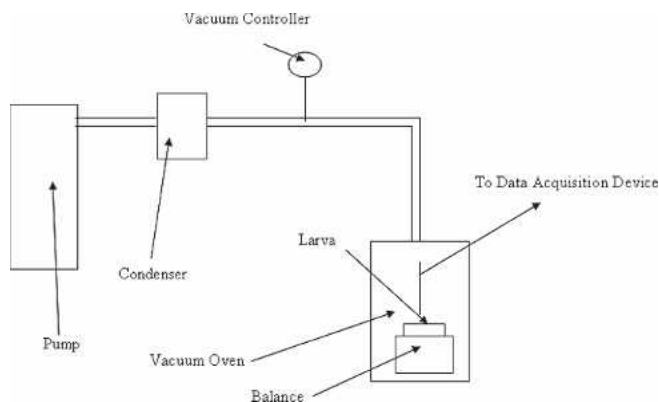


Figure 1. — Schematic diagram of experimental vacuum treatment apparatus.

weight loss of larvae, 3) the desiccation rate of larvae, 4) the relationship between vacuum time and weight loss of larvae, 5) the relation between desiccation rate and wood moisture content (MC), and 6) the effects of temperature, pressure, and relative humidity on the desiccation rate.

Materials

Vacuum treatment equipment

The equipment used consisted of a vacuum pump, condensing system, vacuum control system, vacuum oven, and a balance (Fig. 1). A VacTorr vacuum pump by GCA/Precision Scientific (Winchester, Virginia) was used in these experiments. The vacuum capacity was 1 mmHg. Due to its sensitivity to moisture vapor, a condensing system was added to trap vapor released from the desiccating wood and larvae. A Neslab air-cooled CC-65 condenser was used and consisted of a mechanical refrigeration system that employed a single stage with one compressor. It had a temperature range of -20 to -55 °C and the capacity to remove 120 W of heat at -20 °C. The cold trap was used to condense and collect water vapor from the wood and larvae. The balance used in the experiments had a capacity of 60 g maximum weight with 0.1 mg precision and had a built-in RS-232 interface, which was used to transfer weight data every 5 seconds directly to a computer via a data acquisition system.

Vacuum pressure was controlled between ± 2 mmHg by a HPM -760 Plus Controller (Teledyne and Hastings Co., Hampton, Virginia), vacuum gauge combined with a solenoid valve. Humidity and temperature were measured with a THT-I Electronics relative humidity meter equipped with humidity and temperature sensors (Shinyei Corporation of America, New York). The humidity meter was capable of measuring relative humidity (RH) from 0 to 100 percent with 1.5 percent accuracy over a wide temperature range (-50 to 200 °C) in a vacuum. A Sheldon 1425 vacuum oven was used in the studies (Sheldon Manufacturing, Cornelius, Oregon). The vacuum oven had a stainless steel chamber and could regulate the temperature from 10 °C above ambient to 240 °C.

Supply of insects

Anoplophora glabripennis larvae used in the experiments were reared in the USDA Forest Service quarantine laboratory, Ansonia, Connecticut, according to procedures described

in Keena (2005). ALB larval weight ranged from 0.5 to 2.5 g and about half (the heavier ones) were in their ultimate instar but had not received any chill. EAB larvae were excised from infested logs. Ash trees (*Fraxinus* spp.) infested with overwintering EAB were felled at field sites near Ann Arbor, Michigan, between January and March 2006 and cut into 60-cm-long log sections. Logs were held in a cold room ($5 \pm 2^\circ\text{C}$) for up to 4 months until dissection. Logs were dissected in the USDA Forest Service laboratory in East Lansing, Michigan. Excised larvae were held in a refrigerator ($5 \pm 2^\circ\text{C}$) for up to 48 hours before treatment. EAB larval weight ranged from 0.05 to 0.13 g. Most EAB larvae were in their ultimate instar and near pupation.

Methods

Effectiveness of vacuum treatment and lethal percentage weight loss of larvae

A total of 32 ALB and 75 EAB larvae were held from 6 to 24 hours under 20 mmHg vacuum at 20°C to determine lethal percentage weight loss, lethal time, and the relationship between the two. Up to 10 ALB or EAB larvae were placed in the vacuum oven at one time. One larva was placed on the load cell and its weight was recorded every 5 seconds using data acquisition software. All larvae were weighed before and after treatment in order to calculate individual weight loss and desiccation rate.

To determine the maximum weight loss of larvae, five ALB larvae were held for 64 hours at a vacuum pressure of 20 mmHg at 20°C . Ten EAB larvae were held in a drying oven (Blue M Electric Co., single-wall gravity convection laboratory oven) at 100°C for 94 hours. Five pupae and 11 eggs of ALB were also exposed to a vacuum pressure of 20 mmHg at 20°C for 20 hours to assess response.

After the tests, larvae were considered dead if they did not move when probed within 3 hours of removal from treatment. ALB larvae that survived vacuum treatment were returned to the artificial diet on which they had been reared (Keena 2005), and their survival and development were monitored. Pupae were considered dead when no movement was observed within 3 hours, and they did not complete development to adults within 3 weeks after being held at 25°C and 60 percent RH. After vacuum treatment, the condition of eggs was observed, and they were held at 25°C in a high-humidity environment (as described in Keena 2005) for 3 weeks to determine viability and hatching success.

Vacuum lethal time at different test temperatures and pressures

The relationship between temperature and vacuum lethal time was assessed. Twenty-five ALB larvae were exposed to 20 mmHg pressure at 30°C for 4 to 6 hours and 40 EAB larvae were exposed to 20 mmHg at -10°C for 24 or 36 hours to investigate desiccation of larvae at these temperatures. Fifteen ALB larvae were held at each of two additional vacuum pressures, 10 and 75 mmHg, at 20°C to evaluate the relationship between vacuum pressure and lethal vacuum time.

Vacuum treatment of larvae inserted into wood

Thirty ALB larvae and 20 EAB larvae were inserted into pieces of wood (dimensions given below) with varying MC to determine the relationship between desiccation rate and MC.

Larvae were inserted into holes (4 cm deep) that were drilled in test wood pieces. Holes were 1 cm or 0.5 cm in diameter for ALB and EAB, respectively. The holes were securely plugged after inserting the larvae with pieces of wood dowel. Vacuum treatment was applied within 3 hours of placing larvae in the test wood pieces. Immediately following treatment, larvae were removed from the holes, placed on moist filter paper in petri dishes, and maintained at room temperature. Larvae were considered dead if they did not move when probed within 3 hours of removal.

Ten ALB larvae were placed in separate wood test pieces at each of three wood moisture levels: (1) commercially available Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) lumber, 2.5 cm wide by 10 cm long by 2.5 cm thick at 21.6 percent MC, (2) commercially available Douglas-fir lumber 10 cm wide by 10 cm long by 2.5 cm thick at 31.4 percent MC, and (3) freshly cut Norway maple (*Acer platanoides* L.) 5 cm thick by 10 cm diameter at 89.4 percent MC. Half of the test wood pieces of each type with larvae inserted were held at 20 mmHg and 20°C and the other half at 10 mmHg and 30°C to determine the effect of wood MC on vacuum lethal time.

Twenty EAB larvae were placed in separate wood test pieces cut from commercially available Douglas-fir 2.5 cm wide by 10 cm long by 2.5 cm thick. The MC of the wood was 16.6 percent. The test wood pieces were held at 20 mmHg and 20°C .

Data analysis

Lethal percentage weight loss for ALB and EAB held at 20 mmHg and 20°C was determined by probit analysis (Robertson and Preisler 1992) with Polo Plus (LeOra Software, Berkeley, California). Desiccation mortality response lines for the two beetle species were compared by a Chi-square test (PROC FREQ, SAS Institute 2002–2003). Desiccation rates at 20 mmHg and 20°C of ALB and EAB were compared using a t-test (PROC t-test, SAS Institute 2002–2003). For each species, desiccation rates for larvae held at two different temperatures were compared using a t-test. Differences in desiccation rates of ALB held at three different vacuum levels were compared using analysis of variance (ANOVA, PROC GLM, SAS Institute 2002–2003). The desiccation rates of ALB larvae and pupae were compared using a t-test. For each species, the desiccation rates of larvae exposed directly to vacuum at 20 mmHg and 20°C were compared to larvae inserted into wood and exposed to the same vacuum and temperature conditions by a t-test (PROC t-test, SAS Institute 2002–2003). Desiccation rates of ALB larvae inserted into wood were compared for the three different wood MC levels by ANOVA followed by the Ryan-Einot-Gabriel-Welsh (REGW) multiple comparison procedure (PROC GLM, SAS Institute 2002–2003).

Results and discussion

Effectiveness of vacuum treatment and lethal percentage weight loss of larvae

The desiccation mortality response line (Fig. 2, solid line, intercept -23.2 ± 5.3 , slope 15.6 ± 3.6 , probit mortality vs. log % weight loss) for ALB predicts that 50 percent of the larvae will be dead after 31.2 percent weight loss and that virtually all (probit 9) the larvae will be dead after 56.4 percent weight loss. The desiccation mortality response line (Fig. 2, dashed

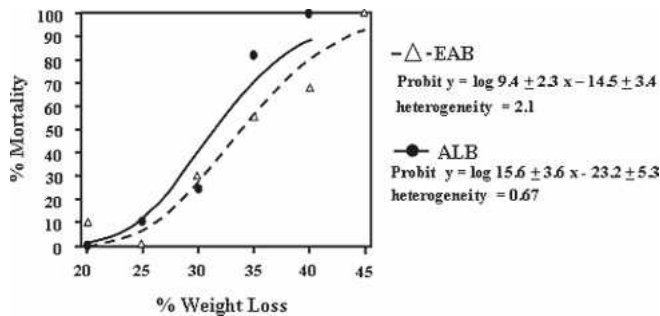


Figure 2. — Percentage *A. glabripennis* (ALB, solid line) and *A. planipennis* (EAB, dashed line) larval mortality in response to increasing percentage weight loss ($n = 55$ ALB, $n = 57$ EAB). Heterogeneity = $\chi^2 / \text{degrees of freedom}$ (Russell et al. 1977).

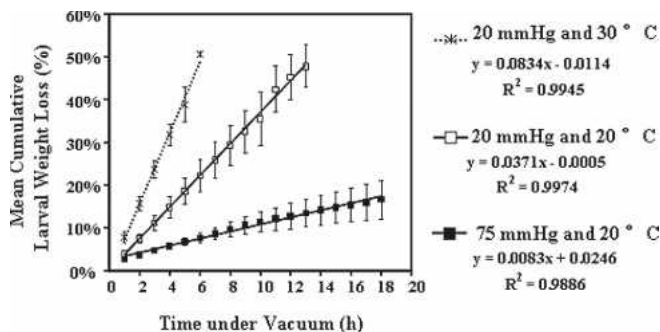


Figure 3. — Mean ($\pm$ SE) cumulative desiccation curves for *A. glabripennis* (ALB) larvae exposed to 20 mmHg at 20 °C ($n = 5$) and 30 °C ($n = 3$), and 75 mmHg at 20 °C ($n = 3$).

line, intercept -4.5 ± 3.4 , slope 9.4 ± 2.3 , probit mortality vs. log % weight loss) for EAB predicts that 50 percent of the larvae will be dead after 34.5 percent weight loss and that virtually all (probit 9) the larvae will be dead after 91.9 percent weight loss. The desiccation mortality curve for EAB does not provide as good a fit to the data so the estimates for the lethal percentage weight loss, and predicted mortality based on the probit mortality curves is not as accurate. The response lines for the two beetle species are not significantly different from each other ($\chi^2 = 3.64$, $df = 2$, $p = 0.162$).

These results demonstrate that both ALB and EAB larvae can be killed by low-pressure vacuum treatment through evaporative removal of body water. ALB larvae died after losing as little as 26 percent total body weight, and all larvae in the trials that lost ≥ 35 percent weight died (Fig. 2). EAB larvae died after losing as little as 28 percent total body weight and all larvae that lost >39 percent weight died (Fig. 2). Similar percentage dehydration is tolerated by other beetles; certain tenebrionids tolerated >50 percent, chrysomelids survived up to 46 percent water loss, and two other cerambycids survived 35 to 40 percent weight loss (Gehrken and Sømme 1994, Chen et al. 2007).

The desiccation curves (mean cumulative percentage weight loss vs. time) for both ALB (Fig. 3) and EAB (Fig. 4) larvae were linear during the first part of the desiccation process. The desiccation rate for ALB decreased after about 30 hours at 20 mmHg and 20 °C as weight loss reached approximately 50 percent and larvae approached complete desiccation. Complete desiccation for ALB larvae required about 50 hours at 20 mmHg and 20 °C. The maximum weight

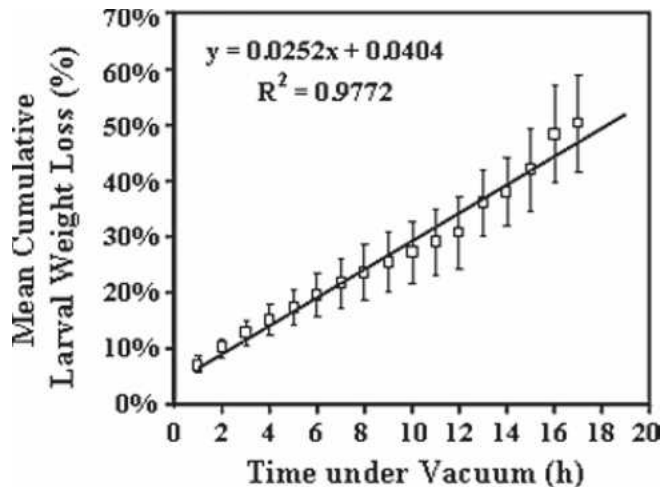


Figure 4. — Mean ($\pm$ SE) cumulative desiccation curve for *A. planipennis* (ALB) larvae exposed to 20 mmHg at 20 °C ($n = 5$).

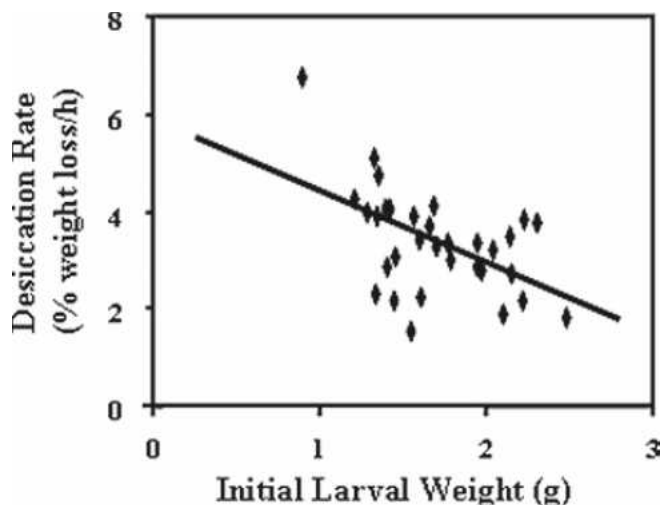


Figure 5. — Desiccation rate vs. initial larval weight for *A. glabripennis* (ALB) larvae exposed to 20 mm Hg at 20 °C ($n = 32$).

loss under 20 mmHg at 20 °C for ALB larvae ranged from 60 percent to 67 percent with an average of 62.8 percent. The maximum weight loss for EAB larvae was similar and ranged from 58.5 percent to 63.1 percent with an average of 60.9 percent. Terrestrial arthropods usually contain 65 to 75 percent water by weight (Hadley 1994). Linear desiccation rates also have been observed for some tenebrionid beetles (Renault and Coray 2004).

The desiccation rate for ALB larvae was negatively correlated with initial larval weight (Fig. 5, desiccation rate = $-1.5 \times \text{Initial weight} + 5.9$, $r^2 = 0.28$). Similarly, the desiccation rate for EAB larvae was negatively correlated with initial larval weight (Fig. 6, desiccation rate = $-17.7 \times \text{Initial weight} + 3.78$, $r^2 = 0.22$). ALB larvae desiccated significantly faster (3.35 % weight loss per hour, $t = -5.57$, $df = 105$, $p < 0.0001$) than EAB larvae (2.39 % weight loss per hour) under 20 mmHg at 20 °C.

It took longer to kill EAB larvae than ALB larvae under vacuum, despite the fact that EAB larvae are smaller than ALB larvae and thus have a higher surface area to volume ratio. It is possible that ALB and EAB differ in cuticular

composition and/or respiratory rate. Both factors are related to transpiration and desiccation in insects (Addo-Bediako et al. 2001). The EAB used in these experiments were prepupal larvae that had completed feeding, were overwintering, and were held in cold storage. On the other hand, the ALBs used in these experiments were laboratory-reared on artificial diet at room temperature and had not yet reached the pupal stage or completed feeding. Overwintering insects have adaptations to cope with the potential for ice formation in their body fluids, including the production of sugars (trehalose) and antifreeze (glycerol) in the haemolymph (Lee Jr. 1989). Production of osmolytes not only protects against freezing, but also decreases the gradient of water vapor pressure and thus provides some protection against desiccation (Kærsgaard et al. 2004). Moreover, the presence of trehalose protects membranes from morphological damage during drying allowing organisms to survive high levels of desiccation, a condition known as anhydrobiosis (Crowe et al. 1992). Because the EAB larvae used

in these experiments were collected from their overwintering sites, they may have had a higher concentration of trehalose and osmolytes than the laboratory-reared ALB and thus may have been somewhat protected from desiccation through a reduced desiccation rate and an increased ability to survive higher levels of desiccation.

Vacuum lethal time at different test temperatures and pressures

Desiccation rate was directly related to temperature during vacuum treatment. Desiccation rate increased significantly at higher temperatures ($t = 9.86$, $df = 55$, $p < 0.0001$). For example, ALB larvae lost weight twice as fast at 30 °C (6.17 % weight loss per hour) compared to 20 °C (3.35 % weight loss per hour) (Table 1). Similarly, for *A. planipennis* larvae, the desiccation rate at 20 °C (2.39 % weight loss per hour) was significantly higher than at -10 °C (0.13 % weight loss per hour) ($t = 20.30$, $df = 113$, $p < 0.0001$). After 36 hours under 20 mmHg at -10 °C all of the EAB larvae were still alive. Mbata and Phillips (2001) also found that higher temperatures resulted in significant reductions in vacuum lethal time for stored-product insects. As temperature increases so does respiration which is one of the avenues through which water is lost in insects (Hadley 1994). At below-freezing temperatures, factors involved in cold tolerance may have also contributed to increased lethal time and protection from desiccation. Regulation of internal osmotic pressure by means of sugars protects against freezing (Lee Jr. 1989) and desiccation (Kærsgaard et al. 2004). Furthermore, when insects are exposed to below-freezing temperatures, the haemolymph becomes concentrated by formation of ice until the vapor pressure of the liquid fraction equals that of ice at the same temperature. The haemolymph of frozen insects in a closed frozen hibernaculum is therefore in vapor pressure equilibrium with the air in the frozen hibernaculum, thus these insects do not lose water during winter (Lundheim and Zachariassen 1993). The slower desiccation rate at below freezing temperatures was not likely due to drier conditions because relative humidity in the vacuum chamber was similar at 20 °C (2.7%) and -10 °C (2%).

For ALB there were significant differences between the desiccation rates at the three vacuum levels tested. Desiccation rate increased significantly under greater vacuum (i.e., lower pressure mmHg) ($F = 87.36$; $df = 2, 59$; $p < 0.0001$); the desiccation rate was 4.82, 3.35, and 0.64 percent weight loss per hour under 10, 20, and 30 mmHg, respectively, at 20 °C (Table 1). Similarly, Mbata et al. (2004) found that stored product insects died more rapidly under greater vacuum (i.e., lower pressure mmHg).

Six ALB pupae were tested under 20 mmHg at 20 °C. Five of them died and one was badly damaged by the test. The pupae lost 26 to 46 percent total body weight.

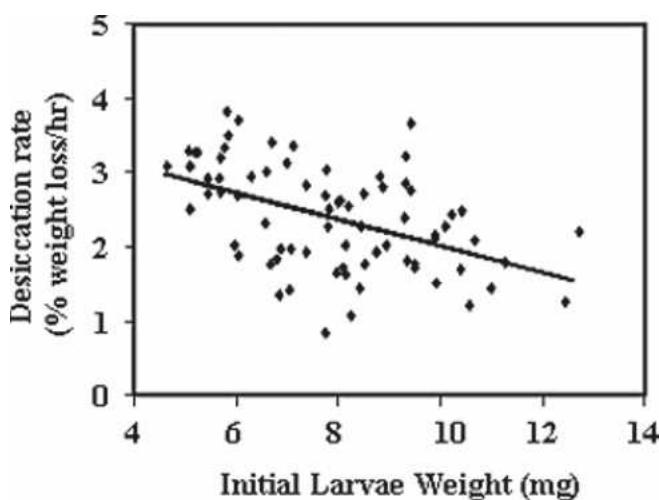


Figure 6. — Desiccation rate vs. initial larval weight for *A. planipennis* (EAB) larvae exposed to 20 mm Hg at 20 °C ($n = 74$).

Table 1. — Mean ($\pm$ SE) desiccation rates and estimated vacuum time for 40% weight loss for *A. glabripennis* (ALB) and *A. planipennis* (EAB) larvae held under different vacuum and temperature conditions and either directly exposed to vacuum or inserted into wood with different moisture levels held under vacuum.

Insect	Wood MC (percent)	Pressure (mmHg)	Temperature (°C)	n	Desiccation rate (percent/hr $\pm$ SE)	Estimated time to 40 percent weight loss ¹ (hr $\pm$ SE)
ALB	Directly exposed	20	20	32	3.35 $\pm$ 0.19	13.2 $\pm$ 0.8
ALB	Directly exposed	75	20	15	0.64 $\pm$ 0.07	78.3 $\pm$ 11.5
ALB	Directly exposed	10	20	15	4.82 $\pm$ 0.22	8.5 $\pm$ 0.4
ALB	Directly exposed	20	30	25	6.17 $\pm$ 0.22	6.7 $\pm$ 0.2
ALB	21.60	20	20	5	0.94 $\pm$ 0.17	51.4 $\pm$ 13.2
ALB	31.40	20	20	5	0.41 $\pm$ 0.06	109.4 $\pm$ 20.2
ALB	89.40	20	20	5	0.23 $\pm$ 0.06	246.7 $\pm$ 81.3
ALB	21.60	10	30	5	2.02 $\pm$ 0.29	22.0 $\pm$ 4.0
ALB	31.40	10	30	5	0.61 $\pm$ 0.17	85.4 $\pm$ 18.0
ALB	89.40	10	30	5	0.33 $\pm$ 0.20	410.9 $\pm$ 182.0
EAB	Directly exposed	20	-10	40	0.13 $\pm$ 0.01	530.5 $\pm$ 70.6
EAB	Directly exposed	20	20	75	2.39 $\pm$ 0.08	18.5 $\pm$ 0.8
EAB	16.60	20	20	20	0.94 $\pm$ 0.05	44.8 $\pm$ 2.3

¹Time to 40 percent weight loss for each larva was estimated using individual desiccation rates.

The desiccation rate for ALB pupae (1.88 % weight loss per hour) was significantly slower than for larvae (3.35 % weight loss per hour, $t = -3.07$; $df = 35$; $p = 0.004$). Similarly, Mbata and Phillips (2001) found that the pupae of stored-product insects were more tolerant to low pressure than larvae.

Eleven *A. glabripennis* eggs held individually in the wells of a 24-well plate were exposed to 20 mmHg at 20 °C. Two tests were conducted; one using five eggs and the other using six eggs. The eggs all appeared to have collapsed after exposure to the same vacuum treatment as the pupae; however, one egg did hatch after they were returned to a high humidity environment.

Vacuum treatment of larvae inserted into wood with different MCs

Wood MC must be taken into account when developing a treatment scheme for SWPM. Relative humidity inside the oven or container used for the vacuum treatment will increase when wetwood is placed inside the chamber. For instance, relative humidity in the vacuum chamber was 2.7 percent when only larvae were placed in the chamber, while it was 28.1 percent when wood blocks containing larvae were placed in the chamber under the same temperature (20 °C) and vacuum pressure (20 mmHg) conditions.

The desiccation rate of *A. glabripennis* larvae subjected directly to 20 mmHg at 20 °C (average 3.35 percent weight loss per hour) was significantly higher than for larvae inserted into wood (average for all wood MC levels 1.42 percent weight loss per hour, $t = 9.86$, $df = 55$, $p < 0.0001$). For both vacuum levels tested, desiccation rates of ALB larvae inserted into wood were higher when MC of the wood was lower. At 20 mmHg and 20 °C, ALB larvae inserted into wood with 21.6 percent MC desiccated more rapidly (0.94 ± 0.17 percent weight loss per hour) than larvae inserted into wood with 31.4 or 89.4 percent MC (0.41 ± 0.06 and 0.23 ± 0.06 percent weight loss per hour, respectively; $F = 11.01$, $df = 2, 14$, $p = 0.0019$). Similarly, at 10 mmHg and 30 °C, ALB larvae inserted into wood with 21.6 percent MC desiccated more rapidly (2.02 ± 0.39 percent weight loss per hour) than larvae inserted into wood with 31.4 or 89.4 percent MC (0.61 ± 0.18 and 0.34 ± 0.20 percent weight loss per hour, respectively; $F = 15.72$, $df = 2, 14$, $p = 0.0004$). *Stenocorus lineatus* Oliv., sawyer beetles (*Monochamus* sp.), and pine wood nematodes (*Bursaphelenchus xylophilus*) all were killed after 24 hours at 20 mmHg at 20 °C when placed in wood with >30% MC (Chen et al. 2007). ALB larvae placed in the test wood pieces at 21.6 percent MC, lost weight (0.94 % weight loss per hour) but at a slower rate than larvae that had been directly exposed (3.35 % weight loss per hour) to the same vacuum treatment (20 mmHg vacuum pressure at 20 °C). Approximately 50 percent of the ALB larvae held in wood under these conditions had lost more than 40 percent body moisture after about 20 hours and were dead. ALB larvae in the wood that had 89.4 percent MC lost weight even more slowly. The results for EAB larvae were very similar to those for ALB. The desiccation rate of EAB larvae directly exposed to 20 mmHg at 20 °C (2.39 % weight loss per hour) was significantly higher than for larvae inserted into wood (0.94 % weight loss per hour, $t = 9.14$, $df = 93$, $p < 0.0001$).

Vacuum treatment can kill ALB and EAB larvae even when inserted into wood; however, lethal time increases with increasing wood MC (Table 1).

The work reported here is a critical step in evaluating the usefulness of vacuum treatment for killing important non-native wood-dwelling pests that can be transported in SWPM. Further work to determine the exact treatment conditions for wood material in a commercial facility will be necessary if this method is to be considered for adoption as part of ISPM-15 used by the U.S. and its trading partners.

Conclusions

ALB and EAB larvae die under low pressure vacuum due to desiccation. *A. glabripennis* pupae and eggs were also susceptible to desiccation under low pressure vacuum. The lethal percentage total body weight loss for both ALB and EAB larvae was determined to be around 30 to 40 percent, and the percentage mortality response curves were not significantly different from each other. The desiccation rate for both ALB and EAB larvae under vacuum is constant before death, but ALB larvae had 1.4 times higher desiccation rate than EAB larvae under the same vacuum and temperature conditions of 20 mmHg at 20 °C. The desiccation rate was positively correlated with the temperature; at higher temperatures larvae lost weight faster than at lower temperatures. Vacuum pressure also affected the desiccation rate; at lower pressures the desiccation rate was higher. When the relative humidity inside the vacuum container was higher, the desiccation rate decreased, which was the case when larvae were placed in wood with varying MCs.

Further testing with larger commercial ovens and vacuum equipment to test logs and dimensional lumber are required to determine the feasibility of using vacuum technology for regulatory treatment of solid wood packing material, firewood, logs, and other wood products moved through commercial trade. In addition, other wood-infesting insects and organisms should be tested in order to develop standardized guidelines for treatment conditions. If treatment conditions can be optimized to reduce the required processing time, vacuum treatment could prove to be a practical and cost-effective regulatory treatment for wood.

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