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NOTE

Lethal High Temperature Extremes of the Brown Marmorated Stink Bug (Hemiptera: Pentatomidae) and Efficacy of Commercial Heat Treatments for Control in Export Shipping Cargo¹

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Since its accidental introduction into the U.S.A. in the mid-1990s, the brown marmorated stink bug, *Halyomorpha halys* Stål (Hemiptera: Pentatomidae), has spread rapidly across North America and become an economically significant pest of tree fruits, vegetables, tree nuts, and field crops (Leskey et al. 2012, Rice et al. 2014). Consequently, there has been tremendous interest in better understanding aspects of its biology. Knowledge on the effects of temperature on the bug's biology is critical to developing ecological models (Nielsen et al. 2008), predicting range expansion (Zhu et al. 2012), and for potentially developing pest control tactics using controlled temperature (Hammond 2015). In two laboratory experiments, we determine the lethal high temperature extremes of *H. halys* and efficacy of commercial heat treatments for control of the bug in export shipping cargo.

Experiments were conducted in 2014 and 2015 in a laboratory at Virginia Tech in Blacksburg, VA to determine the lethal high temperature of the brown marmorated stink bug. All insects used in experiments were obtained from a laboratory colony maintained in mesh cages (30.48 cm × 30.48 cm × 30.48 cm) (BioQuip, Rancho Dominguez, CA) at 23°C, 12-h photoperiod, and 50% relative humidity. Bugs were fed fresh seeds from green beans, *Phaseolus vulgaris* L. (Fabaceae), and sunflower, *Helianthus annuus* L. (Asteraceae), and they were supplied with water through a wick. The colony was continuously supplemented with field-collected *H. halys* from trees and crops in Virginia during the summer months or from manmade structures in the fall and winter of each year. Adults of both sexes were haphazardly selected from the colony and placed in groups of ten in 9-cm glass Petri dishes (Corning, Inc., Corning, NY). These groups of bugs were placed in an incubator (Fisher Scientific Thermo Incubator 537D, Fisher Scientific, Waltham, MA) at different temperature-time intervals. Bugs were exposed for 4 h at the following temperatures: 35, 38, 40, 42, and 45°C. Additionally, bugs were exposed for 15-min and 1-h intervals at 40, 45, and 50°C. Mortality was assessed at 1 h and 24 h after exposure. Bugs unable to walk

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Table 1. Average mortality of *H. halys* adults following exposure for different times and temperatures in an incubator located at Virginia Tech, Blacksburg, VA.

Temp. (°C)	% mortality (mean ± SE) ^a		
	15-min exposure ^b	1-h exposure ^b	4-h exposure ^b
35	–	–	5.0 ± 1.4 c
38	–	–	11.7 ± 1.9 c
40	0.0 ± 0.0 b	0.0 ± 0.0 b	38.3 ± 3.7 b
42	–	–	91.3 ± 2.2 a
45	22.5 ± 19.3 b	100.0 ± 0.0 a	100.0 ± 0.0 a
50	100.0 ± 0.0 a	100.0 ± 0.0 a	–

^aThere were four replications, and more than 50 bugs per temperature-time treatment.

^bMeans within columns followed by the same letter are not significantly different (ANOVA, LSD test, $\alpha = 0.05$).

properly and right themselves after being turned over on their backs were considered lethally injured from the heat exposure and were recorded as dead. Each time and temperature combination was replicated at least four times. Percentage mortality at 24 h after exposure were analyzed using analysis of variance (ANOVA) (JMP version 10.0; SAS Institute 2012). Means were separated using Fisher's Protected Least Significant Difference (LSD) at $\alpha = 0.05$.

Temperature had a significant effect on bug mortality for the 15-min ($F = 10.88$; $df = 2, 9$; $P < 0.004$), 1-h ($F = 80,000$; $df = 2, 9$; $P < 0.0001$), and 4-h exposure times ($F = 50.57$; $df = 4, 25$; $P < 0.0001$). All *H. halys* adults that were exposed to 50°C for ≥ 15 min or to 45°C for ≥ 1 h were killed (Table 1). The *H. halys* adults exposed to 35°, 38°, 40°, 42°, and 45°C for 4 h had an observed mean mortality of 5, 12, 38, 91, and 100%, respectively. Mortality at 42 and 45°C was significantly higher than it was at the other temperatures, and mortality at 40°C was higher than it was at either 38°C or 35°C.

A second study was conducted to evaluate the potential of heat treatments for control of live *H. halys* in export cargo. In 2015, a heat-treatment facility at the Port of Savannah, GA, USA was constructed by Willenius Wilhelmsen Logistics (WWL, Lysaker, Norway) to create a usable heated space approximately 6.1 m × 12.2 m × 3 m (Figure 1a). The interior of the facility was coated with a series of spray foam insulation and 5-cm wide foam board insulation to reduce heat loss. One end of the facility allowed for vehicles to enter through a standard exterior grade metal garage door, while the opposite end housed three 500,000 BTU propane heaters (PEST-HEAT, Aston, PA) (Figure 1b) that allowed the heat to move throughout the space. These heaters were supplied by a 3785-liter propane tank located in the rear of the facility. Current capabilities of this space allow for treatment of up to four standard-sized passenger vehicles at one time.

For our studies at the Port of Savannah, *H. halys* field mortality was assessed using a seven-passenger 2003 Chevrolet Astro Extended Wagon. This vehicle had a 4.6-L V6 CPI engine. The same vehicle was used for all experiments. Previous



Fig. 1. (a) Vehicle used for experiments parked inside a container heat treatment facility constructed by Willenius Wilhelmsen Logistics (WWL), and (b) the 500,000 BTU propane heaters used to generate lethal temperature extremes greater than 60°C for heat treatment of vehicles.

testing by WWL identified the engine compartment, under the driver or passenger seats and interior areas that house the spare tire to be the coldest spots during the heat treatment. The test vehicle did not have an interior spare tire compartment; therefore, we were only able to test for bug survival in the engine compartment and under the seat.

The vehicle was parked in the heat treatment facility and subjected to targeted heat treatments of 40°, 50°, and 60°C. Ten adults (collected near Sharpsburg, MD the week prior to testing) were placed in 1-liter mesh bags (Figure 2) and then placed in the engine compartment and under the driver's seat for 15 min once the minimum temperatures in these areas reached the target temperatures. After reaching the minimum target, temperatures were monitored at each location every 60 s for 15 min using a hard-wired thermocouple integrated to a BlueTherm Duo system (Thermoworks, Lindon, UT) integrated with the BlueTherm Pro software (Thermoworks, Lindon, UT) for tablets. Stink bugs were removed and assessed for mortality at 0, 1, and 24 h after the heat treatment. Each temperature treatment was replicated eight times and each replication had a control group of ten *H. halys* that were held at ambient temperature with a mean of 16°C. As with the previous experiment, percentage mortality at 24 h after exposure were analyzed using ANOVA (JMP version 10.0; SAS Institute 2012) and means were separated using Fisher's Protected LSD.

In the second study, temperatures recorded from under the driver's seat were about 2 to 3°C cooler than those recorded in the engine compartment, but both were at least at the minimal targeted temperature generated from the heat treatment (Table 2). Temperature had a significant effect on bug mortality ($F = 21,600$; $df = 2, 21$; $P < 0.0001$). Targeted heat treatment exposures for 15 min at either 50 or 60°C resulted in 100% mortality of the adults tested, regardless of location in the vehicle. Heat treatments at 40°C resulted in very low mortality of adults. All *H. halys* held under ambient conditions during this study resulted in 0% mortality.



Fig. 2. Dead brown marmorated stink bugs in a mesh bag after removal from under the seat of a vehicle exposed to heat treatments at the Port of Savannah, GA.

Table 2. Targeted temperatures, actual measured temperatures, and observed mortality of *H. halys* adults after 15 min exposures to heat treatments applied to a vehicles at the Port of Georgia, Savannah, GA in 2015.

Target temp. (°C)	Actual measured temp. (mean °C ± SE)		% mortality (mean ± SE) ^a	
	Under driver's seat	Engine compartment	Under driver's seat ^b	Engine compartment ^b
40	39.1 ± 0.2	42.2 ± 0.3	2.5 ± 2.5 b	17.5 ± 17.5 b
50	50.9 ± 0.1	54.2 ± 0.1	100.0 ± 0.0 a	100.0 ± 0.0 a
60	65.0 ± 0.7	67.1 ± 0.8	100.0 ± 0.0 a	100.0 ± 0.0 a

^aObserved for 24 h after 15 min of exposure to temperatures.

^bMeans within columns followed by the same letter are not significantly different (ANOVA, LSD test, $\alpha = 0.05$).

Most insects cannot survive exposure to extreme heat ($>50^{\circ}\text{C}$) for even brief durations (Hammond 2015). High temperatures disrupt the function of proteins, metabolic enzymes, and the respiratory and endocrine systems, all of which can lead to insect mortality (Neven 2000). Our results showed that *H. halys* adults are killed after exposure for 15 min to 50°C or 1 h or more exposure to 45°C . These lethal high temperature levels are consistent with many other insects (Hammond 2015). According to Burkes et al. (2000) and Fields & White (2002), most stored-product insect pests (coleopterans and lepidopterans) are effectively controlled under the following time-temperature combinations: 24 h at 40°C , 12 h at 45°C , 5 min at 50°C , 1 min at 55°C , and 30 s at 60°C . Thus, *H. halys* appear to follow a similar pattern of mortality.

These results may have implications on heat treatment control guidelines (restrictions) for shipping cargo. For instance, recent restrictions were placed on the shipping of potential *H. halys*-infested cargo from the U.S. to other countries. During periods of restriction, if vehicles cannot be fumigated prior to transport, they are currently required to be heat-treated at 60°C for 20 min (restrictions set by New Zealand) (Thompson 2014) or 30 min (restrictions set by Australia) (Australian Government 2015) prior to boarding cargo ships. This heat treatment regime has been found to be quite difficult to achieve in an efficient manner for treating large cargo such as multiple vehicles because of the difficulty in constantly maintaining an enclosure at 60°C . Consequently, fumigants have been the primary choice for treatment of stink bugs in export goods mostly because of efficiency (Thompson 2014). However, heat treatments are often less expensive, and do not involve the use of toxic gases, such as phosphine (PH_3) or methyl bromide, which have numerous environmental and human safety issues (Hammond 2015). Based on our research, heat treating export cargo at 50°C rather than 60°C would dramatically improve the efficiency of the process without losing insect control efficacy. Based on our estimates and field testing with WWL at the shipping port in Savannah, GA, such a switch would basically cut the time of heat treatment in half, and thus double the amount of vehicles that could be treated in a day. More efficient heat treatment controls could help to reduce our reliance on fumigation as a means of insect control in exports.

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