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## COMPOST TEA REDUCES EGG HATCH AND EARLY-STAGE NYMPHAL DEVELOPMENT OF *HALYOMORPHA HALYS* (HEMIPTERA: PENTATOMIDAE)

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### ABSTRACT

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is an invasive pest causing significant agricultural losses in the USA, particularly among USDA-certified organic agricultural operations that are prohibited from using synthetic pesticides. Recent research indicates that for optimal development, nymphs of *H. halys* require gut microbial symbionts that are acquired from the egg mass. This research investigated the impact of 'compost teas,' biologically-active organic matter emulsions that are commonly applied as foliar sprays for pathogen management in organic agriculture, on *H. halys* during early stages of development. We compared *H. halys* development after misting egg masses and neonates with reversed osmosis water (control), or with a 50/50 mixture of compost teas derived from poultry manure and mushroom waste. The compost tea treatment significantly affected hatch (%) for egg masses of *H. halys* that were treated initially within 24-30 h of deposition, resulting in a 13% reduction in hatch, as compared to the control. Furthermore, significant 2-fold increases in mortality were found for 1st and 2nd instars in the compost tea group, as compared to those in the control group. For egg masses initially treated later (i.e., 2-3 days after deposition), the compost tea treatment resulted in a significant 3-fold increase in mortality (%) for 1st instars, compared to the control. These findings suggest that compost tea holds potential for developing an organic management tactic for *H. halys* and warrant future investigation of potential underlying mechanisms, including antagonistic interactions with microbial gut symbionts and antibiotic compounds that could penetrate the egg chorion.

**Key Words:** brown marmorated stink bug, organic agriculture, gut symbionts, poultry manure, mushroom waste

### RESUMEN

La chinche marrón marmoleada, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), es una plaga invasora que causa pérdidas significativas en los E.E.U.U., particularmente en los establecimientos agrícolas orgánicos certificados por el USDA (siglas en inglés del *United States Department of Agriculture*), a los cuales no se les permiten usar pesticidas químicos. Los resultados de investigaciones recientes indican que para obtener un desarrollo óptimo, las ninfas de *H. halys* requieren la presencia de simbioses microbianos en sus tractos digestivos, los mismos los obtienen de la masa de huevos. El presente estudio investigó el impacto del "té de abono" (emulsiones de materia orgánica biológicamente activas que se aplican en forma de rociador foliar para el manejo de patógenos en establecimientos de agricultura orgánica) en los primeros estadios de desarrollo de *H. halys*. Se comparó el desarrollo de *H. halys* después de rociar las masas de huevos y las ninfas neontas con agua purificada por osmosis inversa (control) o con una mezcla de 50/50 de té de abono producido con guano y residuos de hongos. El tratamiento con té de abono afectó significativamente la eclosión (%) en las masas de huevos de *H. halys* que fueron rociadas entre 24 y 30 horas después de la deposición, resultando en una reducción del 13% en la tasa de eclosión, al compararse con el control. Asimismo, se observó un aumento significativo de la mortalidad, siendo la misma el doble en los estadios de desarrollo 1 y 2 del grupo del té de abono comparado con el grupo control. Para las masas de huevos rociadas inicialmente más tarde (o sea, 2-3 días después de la deposición), el tratamiento con el té de abono resultó en un aumento significativo de tres veces en la mortalidad (%) en los estadios de desarrollo 1 comparado con el control. Los presentes resultados sugieren que el té de abono presenta potencial para ser desarrollado como una forma de manejo orgánico de *H. halys* y justifica el desarrollo de investigaciones futuras relacionadas con mecanismos potenciales subyacentes, incluyendo interacciones antagonistas con simbioses microbianos del tracto digestivo y compuestos con antibióticos que puedan penetrar el corion de los huevos.

**Palabras Clave:** chinche marrón marmoleada, agricultura orgánica, simbioses del tracto digestivo, guano, residuos de hongos

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is an invasive pest causing significant economic losses to farmers in the mid-Atlantic USA due to its broad feeding range and high dispersal capacity in the nymphal and adult stages. Accidentally introduced from Asia to eastern Pennsylvania in the 1990's (Hoebeke & Carter 2003), *H. halys* is now present in 40 states (<http://www.stopbmsb.org/where-is-bmsb/state-by-state>). *Halyomorpha halys* feeds on more than 300 different plants (Bernon 2004), and soybean, corn and all major vegetable and fruit crops are susceptible (Kuhar et al. 2012; Leskey et al. 2012). While conventional *H. halys* management currently relies on high dosage applications of synthetic insecticides (Leskey & Hamilton 2011), this tactic can disrupt effective biological control and integrated pest management programs controlling other pests, and there is no viable management tool for organic producers. Thus, there is a need for management strategies that can be integrated with established organic practices.

Like many other stink bugs in the family Pentatomidae, *H. halys* requires gut microbial symbionts for optimal nymphal development (Prado & Zucchi 2012; Taylor et al. 2014). Ovipositing females smear the surface of newly deposited eggs (Prado & Zucchi 2012), and neonates subsequently acquire the gut symbionts while aggregating on the egg mass throughout the first instar (Lee et al. 2013). Taylor et al. (2014) recently demonstrated that eliminating the gut symbionts of *H. halys* via egg mass surface sterilization not only negatively affects nymphal development rates and survivorship but also reduces survivorship of the second generation progeny. A management tactic that directly targets the gut symbionts of *H. halys* potentially could assist in reducing pest populations.

Compost 'teas' are emulsions produced from composted organic matter and containing a diversity of naturally-occurring microbes and soluble macronutrients (Palmer et al. 2010). Compost teas are commonly used in organic agricultural practices for foliar and soil-borne pathogen management and for foliar fertilizing. Compost teas can suppress a range of microbial pathogens in agricultural production, including common scab (*Streptomyces scabiei*) (Lambert) in potatoes (Al-Mughrabi et al. 2008), powdery mildew (*Oidium neolycopersici*) (Kiss), late blight (*Phytophthora infestans*) [Mont. (de Bary)] and gray mold (*Botrytis cinerea*) (Pers.) in tomatoes (Koné et al. 2010; Pane et al. 2012), apple scab (*Venturia inaequalis*) (Cooke), damping off in peas (*Pythium ultimum*) (Trow), grape downy mildew (*Plasmopara viticola*) (Berk. & M. A. Curtis), tomato bacterial spot (*Xanthomonas campestris*) (Pammel) and various vegetable wilts (*Fusarium* spp.) (See review by Scheuerell & Mahaffee 2002). The diverse

microbial community associated with compost teas is widely attributed with pathogenic microbe suppression via antagonistic interactions (e.g., antibiosis, parasitism and competition for colonization sites or nutrients) (Scheuerell & Mahaffee 2002; Al-Mughrabi et al. 2008; Palmer et al. 2010). The impacts of compost tea foliar application on insect microbial symbionts have not been evaluated. Given the evidence for suppression of a variety of microbial organisms, it is possible that this commonly used organic practice could antagonize the gut symbionts required for optimal development of *H. halys*. However, compost teas also contain macronutrients and metabolites that could potentially augment the gut symbionts of *H. halys* (Scheuerell & Mahaffee 2002; Pant et al. 2012).

The current study explored the impacts of compost tea spray on *H. halys*, specifically targeting the egg and neonate stages in which the gut microbial symbiont is acquired.

## MATERIALS AND METHODS

Two completely randomized design laboratory studies tested the effect of compost tea on egg hatch and nymphal development of *H. halys*.

### Compost Tea Preparation

Separate batches of 'finished' (i.e., mature) composts, one derived from poultry manure (Redbud Farm, LLC) and one derived from mushroom waste (Shenandoah Sand, Inc.) were obtained, and separate non-aerated tea extracts were prepared by mixing 1 part (by volume) compost to 2 parts reversed osmosis (RO) water in an 18 liter plastic bucket without a lid (13 Mar 2013). The tea extracts were permitted to steep for 24 h (in a dark room held at ~20 °C) and subsequently filtered through cheesecloth mesh to remove solids. A 946 mL mixture comprised of both compost teas then was prepared (50/50 by volume), with the intention of optimizing the diversity of both bacterial and fungal communities. Two spray bottles (946 mL, Garden Treasures Model #50-282, L.G. Sourcing, Inc.) were calibrated in advance to deliver ~0.12 mL of liquid per spray. The bottles were filled (25 fl oz [739 mL]) with either reversed osmosis water or the compost tea mixture, for use in the *H. halys* rearing studies, and held at ~20 °C.

### Development Studies

Two laboratory studies were performed using the same methodology but with eggs of *H. halys* provided from different sources. The first study, performed 14 Mar through 19 Apr 2013, utilized colony-reared egg masses of *H. halys* that had been shipped overnight from a professional

rearing facility (University of Maryland, College Park, Maryland) to the laboratory at Shepherd University (Shepherdstown, West Virginia) and were 2-3 days old at time of treatment application. The experimental unit consisted of a single egg mass (avg. 26.3 egg/mass) placed in the center of a 10 mL petri dish. Each experimental unit was misted with either the compost tea mixture or reversed osmosis water via spray bottle positioned ~25 cm above the dish. After randomly applying the treatments (compost tea,  $N = 5$ ; RO water control,  $N = 4$ ) the dish lids were replaced, and all dishes were randomly placed in the growth chamber (Sheldon Manufacturing Inc., Model #2015) on the same shelf and held at  $25 \pm 2^\circ\text{C}$  and 14:10 h L:D. Dishes were observed every 2-3 days for stink bug emergence, and the water or compost tea misting treatments were re-applied to newly emerged 1st instars aggregated on the egg mass to ensure adequate moisture in this stage. No additional misting treatments were applied to instars 2-5. In addition, 1st instars were provided water (RO) via a moistened wick (10 cm) and equal amounts of organic sunflower seeds and organic green beans for adequate nutrition. Dishes were checked subsequently at 2-3 day intervals and examined using the Entovision microscopic imaging system (GT Vision); specimens were checked for nymphal development (i.e., stage) and mortality, which were recorded. For each egg mass the percentage hatch was calculated. Each cohort of nymphs (i.e., siblings hatching from the same egg mass) was tracked through development (day 1 = egg mass hatch) to final stage attained, and the percentage of mortality was calculated by instar, as well as across instars (i.e., 'total mortality'). Cohorts for which there was missing data in a subsequent instar (i.e., due to 100% mortality attained in previous instar) were removed from the analysis for that instar. Following arcsine square root transformation, independent  $t$ -tests were performed to compare hatch (%), mortality (%) within each instar (1-5), and total mortality (%) across instars 1-5 for the treatment and control groups (SPSS Version 20, IBM Corp., Armonk, New York).

The second study was conducted at the USDA-ARS Appalachian Fruit Research Station (Kearneysville, West Virginia) 3 May through 12 Jul 2013 and utilized egg masses of *H. halys* (avg. 27.3 eggs/mass) collected from the onsite laboratory colony within 24 h of deposition. The rearing protocol for the onsite colony of *H. halys* was similar to that of the University of Maryland colony (Taylor et al. 2014), with adult stink bugs held in mesh cages (299 × 299 × 299 cm), provided unlimited organic sunflower seeds, organic green beans, water wick and live soybean and pepper plants, and held at  $25 \pm 1^\circ\text{C}$ , ~70% RH and 14:10 h L:D. The study was performed in the same manner as described earlier except that a different growth

chamber (Convicon, Model TC16) was used, and egg masses were treated initially within 24-30 h of deposition. A total of 19 replicates per treatment was performed in the second study. Since multiple replicates were initiated on different days over several weeks (coinciding with egg mass production from the colony), developmental day 1 began when an egg mass hatched. For each egg mass the percentage hatch was calculated, and the proportion of alive nymphs; hatched nymphs was calculated over time (after day 1). Percentage mortality by stage and across instars was calculated, and statistical analyses were performed in the same manner as described earlier, following arcsine square root transformation (SPSS Version 20, IBM Corp., Armonk, New York).

## RESULTS

There was a significant effect of compost tea on egg hatch (%) for the egg masses treated initially within 24-30 h of deposition ( $t = 2.322$ ;  $df = 36$ ;  $P = 0.026$ ), resulting in a 13% reduction in egg hatch for the compost tea group, as compared to the control group (Fig. 1). No effect on hatch was found for eggs masses treated initially 2-3 days after deposition (Fig. 1). The average proportion of individuals surviving over time (> day 1) is shown by developmental stage for each treatment group (compost tea versus control) in Fig. 2.

For egg masses treated initially within 24-30 h of deposition, the compost tea significantly increased nymphal mortality during instars 1 and 2

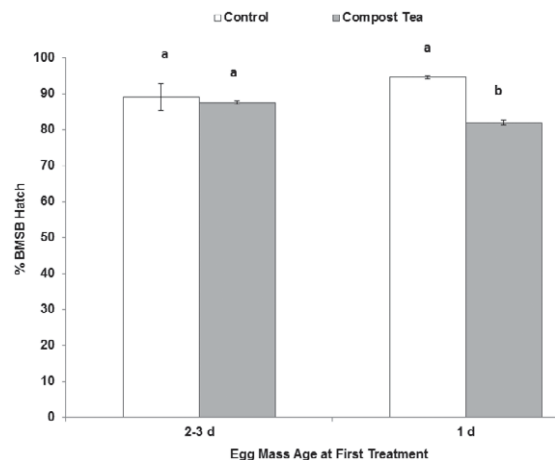


Fig. 1. Percentage hatch of eggs of *Halyomorpha halys* (avg. 27 eggs/mass) following misting with compost tea or reversed osmosis water (control). Two groups of colony-reared egg masses, 2-3 days old and 1 day old, were tested in laboratory experiments. Back-transformed means ( $\pm$ SEM) are plotted. Different letters above bars indicate statistically significant treatment differences within each egg mass age group ( $t$ -test,  $\alpha = 0.05$ ).

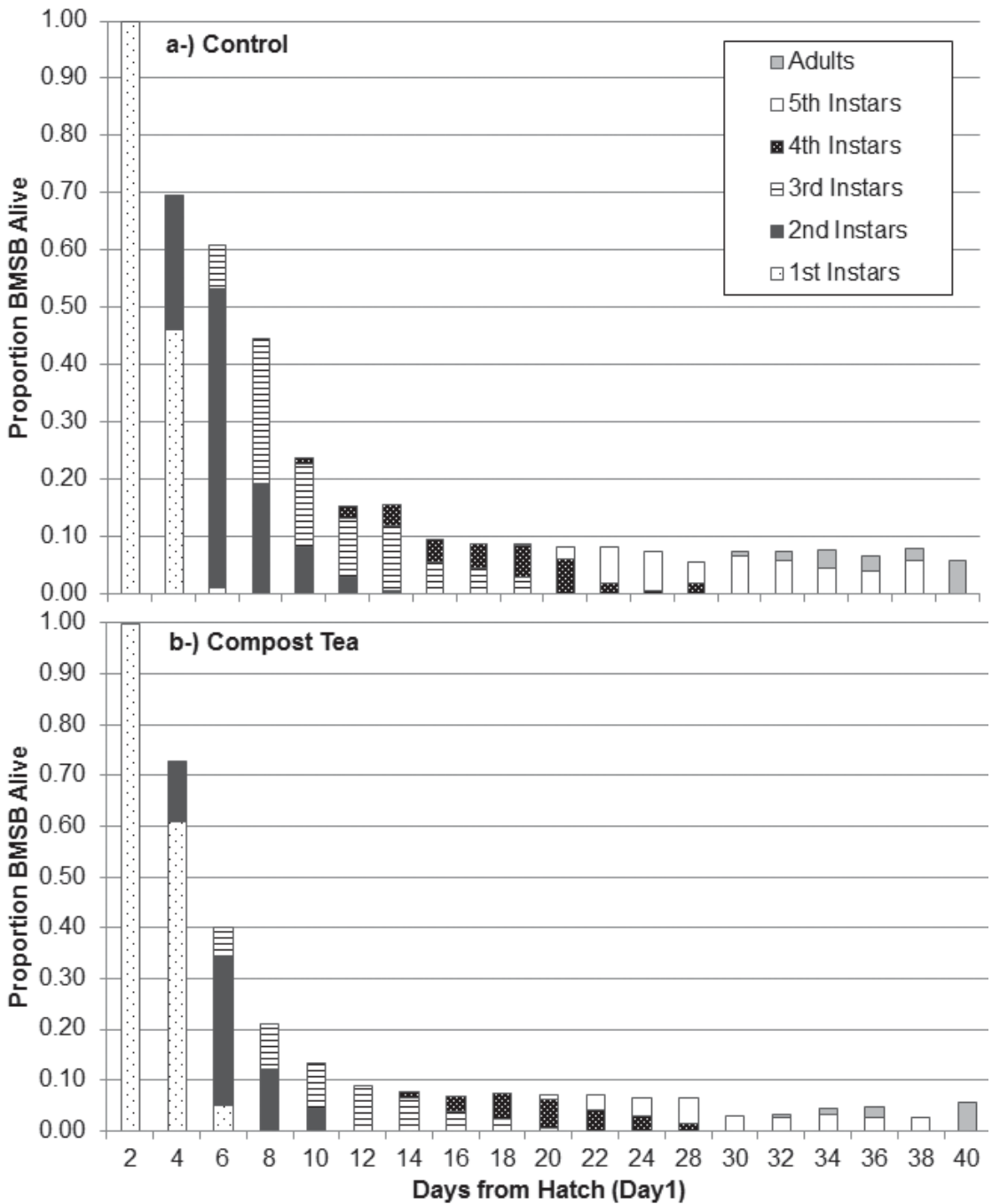


Fig. 2. Development of *Halyomorpha halys* in growth chamber following exposure of eggs and 1st instars to reversed osmosis water control (a) or compost tea (b). The average proportion of living individuals: total hatched per egg mass is shown over time (> day 1) by developmental stage. Treatment was applied to egg masses within 24-30 h of deposition in laboratory colony and repeated after 1st instars emerged.

(Instar 1:  $t = -2.604$ ;  $df = 36$ ;  $P = 0.013$ ;  $N = 19$  per treatment; Instar 2:  $t = -2.219$ ;  $df = 34$ ;  $P = 0.033$ ;  $N = 19$  per control, 17 per compost tea) (Fig. 3). Additionally, total nymphal mortality (%) across

instars 1-5 was significantly affected by the compost tea ( $t = -2.535$ ;  $df = 27$ ;  $P = 0.017$ ;  $N = 19$  per treatment). The mean ( $\pm$  SEM) total nymphal mortality was  $98.4 \pm 0.1\%$  for the control group,

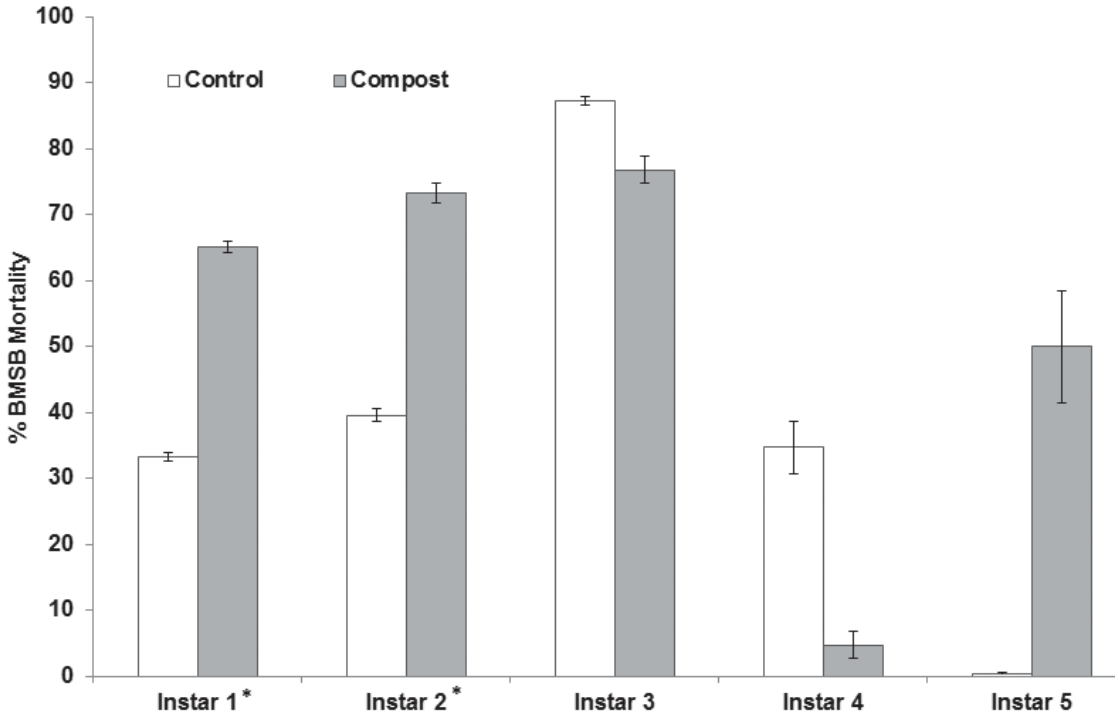


Fig. 3. Effect of misting treatment (reversed osmosis water control or compost tea) on the percentage mortality of *Halyomorpha halys* by developmental stage. Treatment was applied to egg masses within 24-30 h of deposition in laboratory colony and repeated after 1<sup>st</sup> instars emerged. Back-transformed means ( $\pm$ SEM) are plotted. Asterisks indicate statistically significant treatment differences within stage ( $t$ -test,  $\alpha = 0.05$ ).

as compared to  $99.9 \pm 0.03\%$  for the compost tea group.

For egg masses treated initially within 2-3 d of deposition, the compost tea significantly affected nymphal mortality during the 1st and 4th instars (Instar 1:  $t = -3.324$ ;  $df = 7$ ;  $P = 0.013$ ;  $N = 4$  per control, 5 per compost tea; Instar 4:  $t = 5.2$ ;  $df = 3$ ;  $P = 0.014$ ;  $N = 2$  per control, 3 per compost tea) but with variable results (Fig. 4). The compost tea spray resulted in a 3-fold increase in mortality, compared to the control, during the first stage of nymphal development but lower mortality during the 4th instar (Fig. 4). Average total mortality (%) across all instars was  $94.7 \pm 0.8\%$  for the control group and  $96.5 \pm 1.5\%$  for the compost tea group, but this difference was not statistically significant ( $\alpha = 0.05$ ).

#### DISCUSSION

This study provides the first evidence of compost tea impacting development of *H. halys*. The results reveal that misting egg masses with compost tea before eclosion can significantly increase mortality during the 1st instar (Figs. 3 and 4). Furthermore, if egg masses are treated within 24-30 h of deposition, the compost tea significantly

reduces hatch rates (Fig. 1). These preliminary results suggest the potential for foliar applications of compost tea for crop disease or fertility management simultaneously suppressing *H. halys* if timed to coincide with vulnerable stages of pest development (i.e., eggs and early instars), although field-based study is required to examine efficacy under variable environmental conditions.

Although this study did not elucidate underlying mechanisms, it is possible that the compost tea could have penetrated the egg chorion and negatively affected the pre-emergent nymphs directly (e.g., perhaps via antibiosis or infection with a pathogenic organism). Pre-emergent nymphal mortality has been attributed to spinosyns and other classes of foliar insecticides when applied to the egg masses of the stink bugs *Nezara viridula* (Linnaeus) (Brown et al. 2012) and *Chinavia hilaris* (Say) (Koppel et al. 2011). Alternatively, the compost tea could have reduced vertical transmission of the gut symbionts from the egg mass exterior. Taylor et al. (2014) found that sterilizing egg masses of *H. halys* to prevent vertical transmission of its gut symbionts slowed development rates and reduced survivorship of first generation nymphs. In the current study, the compost tea treatment resulted in higher mortal-

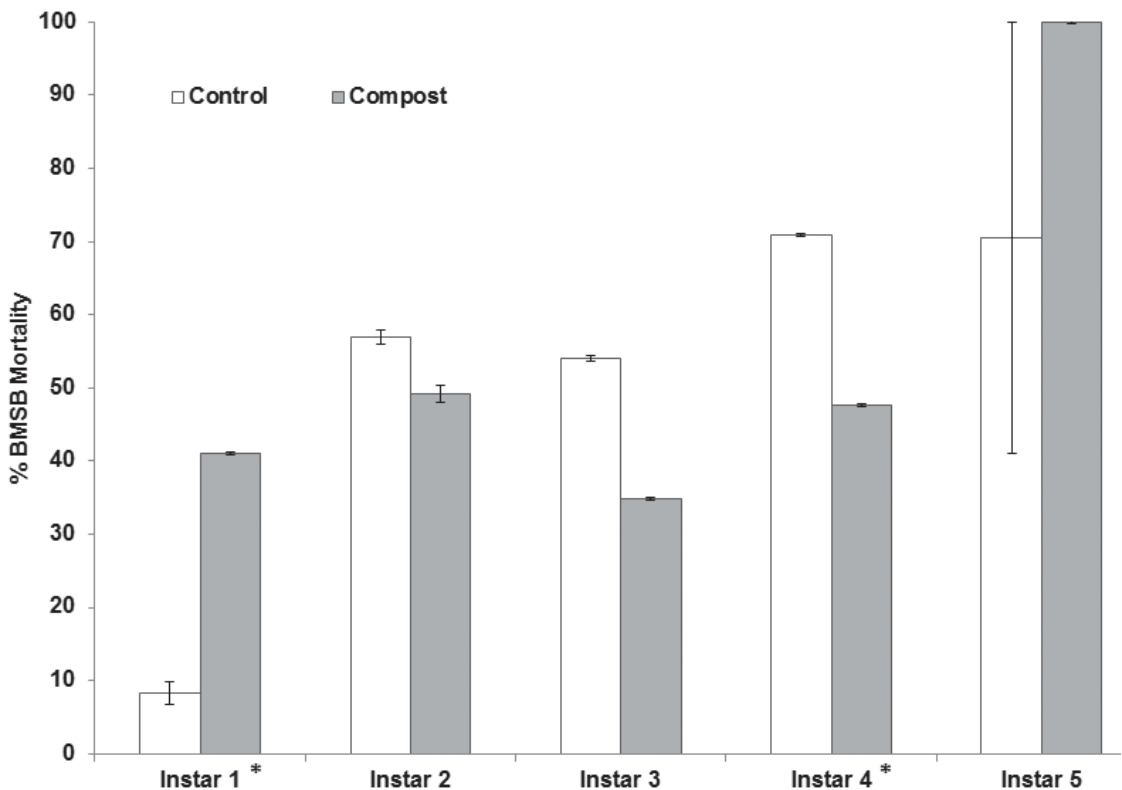


Fig. 4. Effect of misting treatment (reversed osmosis water control or compost tea) on the percentage mortality of *Halyomorpha halys* by developmental stage. Treatment was applied to egg masses within 2-3 d of deposition in laboratory colony and repeated after 1st instars emerged. Back-transformed means ( $\pm$ SEM) are plotted. Asterisks indicate statistically significant treatment differences within stage ( $t$ -test,  $\alpha = 0.05$ ).

ity during the 1st and 2nd instars (Figs. 3 and 4) and delayed development of surviving 1st instars, with a higher proportion of individuals remaining in the 1st instar 4 and 6 days after hatch, as compared the control group (Fig. 2). Taken together, these results suggest that neonates exposed to the compost tea experienced sub-optimal development.

It is important to note that this study used 'finished,' or mature compost stocks that had undergone complete thermophilic biological decomposition, in compliance with the USDA National Organic Production Rule, Section 205.203(c), (USDA National Organic Standards Board 2006). Studies of efficacy of compost teas for crop disease management frequently link plant pathogen suppression to the total living microbial population associated with the tea, and microbial community composition varies depending on stock material (e.g., animal manure or plant waste), stage of compost decomposition and duration of compost tea fermentation (Scheuerell & Mahaffee 2002). For instance, Palmer et al. (2010) demonstrated

that immature compost in the mesophilic stage has more culturable bacteria than fungi. Thus, it may be useful to assess compost teas derived from a variety of stocks and fermentation lengths to determine if the material could be optimized for use against *H. halys*.

The effects of compost tea in this study were limited to a narrow window of development of *H. halys* (i.e., egg mass through 2nd instar); however, there may be metrics beyond first generation survivorship that should be assessed in order to capture potential impacts on population dynamics. As Taylor et al. (2014) noted, preventing vertical transmission of the gut symbionts can negatively affect subsequent generations of *H. halys* through reduced clutch size, hatch rates and survivorship of eggs. In areas where two generations of *H. halys* occur, such as West Virginia, the second generation produces the highest damage to vegetable crops (Leskey et al, 2012), and late-season damage can result in ~100% crop failure in valuable crops such as tomato under organic production (Mathews unpublished). Future field-based

research should address the potential for early season compost tea applications, when adults of the first generation are active, to impact the critical second generation.

In conclusion, our results suggest that there is potential for developing an organic strategy for managing *H. halys* using compost tea. However, further study is required to elucidate the underlying mechanism(s) associated with compost tea and should address potential antagonistic microbial interactions that could reduce the gut symbionts required for optimal development of *H. halys*, as well as antibiotic compounds that could penetrate the chorion of the eggs.

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