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Attraction of Adults of *Halyomorpha halys* (Stål, 1855) and *Nezara viridula* (Linnaeus, 1758) (Hemiptera: Pentatomidae) by Artificially Heated Shelters

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Abstract: The brown marmorated stink bug (*Halyomorpha halys*) and southern green stink bug (*Nezara viridula*) (Hemiptera: Pentatomidae) are invasive species in Europe. They move into overwintering sites (tree bark, buildings) in the temperate latitudes in the autumn, which is induced by the change of photoperiod in the autumn. The aim of this study is to examine whether heating has an impact on their behaviour in the pre-overwintering period and whether the two species can be attracted with artificially heated shelters. A heated box shelter and the same shelter as structure and size but unheated (as control) have been made and the numbers of stink bugs entering into them were recorded. Significantly higher numbers of *H. halys* and *N. viridula* specimens have been registered in the heated shelter than in the unheated one. This indicates that temperature has an impact on their overwintering site choice and thus heated shelters can attract the two species.

Key words: brown marmorated stink bug, southern green stink bug, overwintering, shelters, heating

Introduction

The brown marmorated stink bug *Halyomorpha halys* (Stål, 1855) and the southern green stink bug *Nezara viridula* (Linnaeus, 1758) (Hemiptera: Pentatomidae) are present in Europe as invasive species (Colazza et al. 1996, Rédei & Torma 2003, Wermelinger et al. 2008, Salisbury et al. 2009, Vétek et al. 2014, Cesari et al. 2015, Dioli et al. 2016). Both species are polyphagous and cause serious damages on agricultural crops (Todd 1989, Bariselli et al. 2016). In addition, their mass presence at winter diapause sites in buildings makes them a nuisance pest (Inkley 2012).

In temperate latitudes, the most stink bug species overwinter in adult stage and a few as eggs or nymphs (Saulick & Musolin 2012). Diapause is generally controlled by photoperiodic events, i.e. shorter day length (Musolin & Numata 2003, Saulick & Musolin 2012). *Halyomorpha halys* and *N. viridula*, similarly to other pentatomids, overwinter as adults in natural or artificial shelters, e.g. under bark of dead trees or in buildings (Musolin 2012, Rice et al. 2014, Lee et al. 2014a). In the autumn, they move to aggregate in protected overwintering sites and stressors such as low temperatures, desiccation and nutrition state equally influence the overwintering success (Cira et al. 2016, Ciancio 2018, Skillman et al. 2018).

Halyomorpha halys can fly 2–5 km to find suit-

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Fig. 1. Box shelters and experimental site. Heated box shelter (left). Heated and unheated (control) shelters (right).

able overwintering sites (Lee & Leskey 2015) and prefers tight spaces in protected shelters (Lee et al. 2014a, b). Chambers et al. (2019a) revealed that *H. halys* avoids places with corpses of freshly dead conspecifics. A long spell of temperatures under -6°C can cause severe mortality in overwintering *H. halys* (Cira et al. 2016, Lowenstein & Walton 2018).

Overwintering of *N. viridula* go together with a reproductive diapause (Esquivel 2011). The change of photoperiod indicates reversible reddish-brown colour change of *N. viridula* adults in this period of the season (Musolin & Numata 2003) and they do not require low temperatures to trigger the overwintering diapause. Short day-lengths alone are enough to trigger the behavioural and physiological changes needed for successful winter survival (Musolin et al. 2007). The winter mortality is relatively high in the temperate latitudes (50-60%) and every 1°C decrease in temperature results into c. 16% increase of mortality (Coombs 2004, Kiritani 2011).

The aim of the present study was to clarify whether the two species enter artificially heated shelters in greater numbers than unheated ones. This can provide underpinning knowledge for future studies on the attraction of stink bugs to artificially heated shelters during the pre-overwintering period.

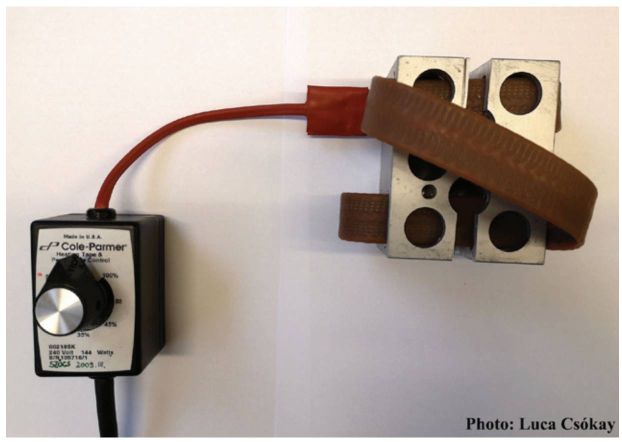

Fig. 2. Heating apparatus.

Materials and Methods

Collection and experimental sites

Adults of *H. halys* and *N. viridula* were collected in Budapest, Hungary (GPS 47.5599735 N, 18.921735 E) from their host plants (*Vitis vinifera* L., *Solanum lycopersicum* L., *Humulus lupulus* L. and *Rubus fruticosus* L.) by insect nets and insect collector vials. We also collected adult *N. viridula* from other populations in Törökszentmiklós (GPS 47.1793568 N, 20.409153 E) and Szentistván (GPS 47.7714601 N, 20.6633045 E). Collections were carried out during September and October 2019, when stink bugs started to move to their overwintering sites. The collected individuals of each species were kept in a separate insect-rearing cage under natural conditions in the garden of the Experimental Station of Plant Protection Institute (Budapest, Hungary), in an open-wall greenhouse under outdoor temperatures (13–19°C), where experiments were carried out in October 2019 in the afternoon hours (12:30–16:30).

Selection between heated and unheated box shelters

Tests were done in a 600 x 600 x 1600 mm transparent cage with insect screen sites. We made 150 x 150 x 200 mm, 3 layers, 3 mm wall thickness cardboard (Frogpack s.r.o., Zlín, Czech Republic) box shelters. Ten mm from the top of the shelters was cut a 20

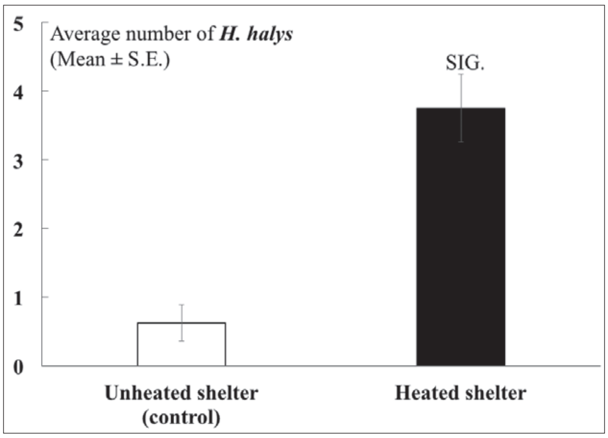

Fig. 3. Average number of *Halyomorpha halys* adults (± S.E.) in the heated and unheated shelters. Welch's t-test, $P \leq 0.05$.

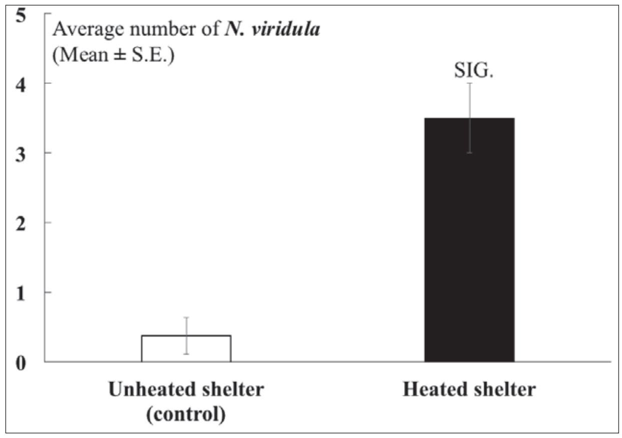

Fig. 4 Average number of *Nezara viridula* adults (± S.E.) in the heated and unheated shelters. Welch's t-test, $P \leq 0.05$.

mm tall and 100 mm long slot. Before starting the experiment, the cardboard shelters in the cage were put on a 1 m tall table inside the cage. The distance between the shelters was ca. 150 mm, and their position was randomly changed in the cage between experiments (Fig. 1).

A silicone rubber heating tape (Cole-Parmer Instrument Company, LLC., Vernon Hills, IL United States) with percentage control manual thermostat and equipped with an aluminium body was placed into one shelter (Fig. 2). The other shelter was not heated and thus served as control. The outside temperature and temperature of the heated shelter were continuously measured during the experiments. The temperature of the heated shelter was adjusted in order the internal side of the slot to be always 35 °C. The slot of the shelters was faced to the bright side of the greenhouse. Under the shelters, a 5 mm tall and 15 mm wide iron grille was placed.

When the required conditions were achieved, 10 female and 10 male *H. halys* or 8 female and 8 male *N. viridula* adult specimens were placed into the cage in one experiment. In an experimental day, *H. halys* and *N. viridula* were tested, separately by species and following one another. The tested specimens were removed from the cage after the experiments. The shelters were changed between experiments to avoid the potential contamination from aggregation pheromones. A test was lasted for 2 hours and 8 repetitions were made with both species (N=160 *H. halys* and N=128 *N. viridula*, sex ratio 1:1).

Statistical analyses

To analyse the homogeneity of variance, we used Levene's test. Homogeneity consists in the case of *N. viridula* data. However, the score of analyses with *H. halys* data did not show homogeneity. For comparison of the number of stink bugs in the heated and unheated (control) box shelters, we used Welch's t-test (GraphPad2018 Software Package, GraphPad Software Inc., San Diego, CA) (α was set to 0.05). Chi-square ($\chi 2$) test was made with Yates correction to evaluate the quantitative differences between the two species in the artificially heated shelters (α was set to 0.05) (GraphPad2018 Software Package).

Results

Our recordings showed that *H. halys* (Fig. 3) and also *N. viridula* (Fig. 4) moved in significantly larger numbers into the heated box shelter than into the unheated one.

Significantly more specimens of *H. halys* were found in the heated shelter than in the control (P=0.0002, df=10). The average number of *H. halys* adults in the heated shelter was 3.75 ± 0.5, while in the control only 0.63 ± 0.26 (N=30 in the heated and N=5 in the unheated box) (Fig. 3). Twenty-eight percent of the adults in the heated box were female.

Significantly more individuals of *N. viridula* were found in the heated shelter than in the control (P=0.0003, df=10). The mean number of *N. viridula* in the heated box was 3.5 ± 0.5, while in 0.375 ± 0.26 adults in the control (Fig. 4). Twenty-eight *N. viridula* adults were staying in the heated and three specimens used the control shelter. Sixty-two percent were females in the heated one.

Six among 10 individuals of both species were under or right next to the heater body of the heated shelter.

The chi-square ($\chi 2$) test did not show significant differences between the number of *H. halys* and *N. viridula* individuals in the heated box shelters (*P*=0.7261, df=1).

Discussion

We show that artificially heated shelter during the pre-overwintering aggregation period attracted significantly more adults of both sexes of *H. halys* and *N. viridula* than unheated one, highlighting that temperature plays an important role in the choice of their overwintering sites. Inkley (2012) has reported that walls of human-made structures heated by sunshine can induce movement to overwintering locations of *H. halys,* facilitating invasion of human-made structures by this species. This behaviour can lead to a reduction of winter mortality (Kiritani 2007, Rice et al. 2014, Cira et al. 2016).

In this study, both species chose the heated shelters in similar numbers (*N*=30 *H. halys* and *N*=39 *N. viridula*), or 19% of *H. halys* and 22% of *N. viridula* adults entered into the heated shelter. Only 3% of *H. halys* and 2% of *N. viridula* chose the unheated shelters. The low mean numbers in shelters are possibly the result of the relatively short experimental time (2 h). However, longer experimental time might have been skewed by aggregation pheromone production, causing mass attraction.

Numerous overwintering shelter traps were designed and made for stink bug, mostly for *H. halys* management. These traps, which are usually made from cardboard or wood, have holes or slit entries of around 3.0-9.5 mm (Watanabe et al. 1994, Bergh et al. 2017, Chambers 2017). Chambers et al. (2019b) found that 9–10 mm hole size or 4–5 mm wide slots were optimal entrance sizes for *H. halys*, and our boxes with 20 mm wide slots under 5 mm tall and 15 mm width grids were thus also suitable. Chambers et al. (2019b) used a heat application but not with the same scope as we did. They triggered escape after chill stunning with heating. Our observations emphasise the importance of combining heating and overwintering shelter structures, which result in increased catches.

Aigner & Kuhar (2016) reported 5.0 ± 1.4% mortality at 35°C after 4 hours exposure time in the case of *H. halys*. We did not experience any mortality at this temperature within 2 hours in the case of both species.

Overwintering shelter tarps are successfully used for trapping and monitoring stink bug adults. Despite of this, in some cases smaller number of adults have entered into these traps than expected (Bergh et al. 2017, Chambers 2017). None of these traps used thermal equipment or other heating apparatus. Therefore, according to our results, a combination of heating and overwintering shelter boxes can enhance catches.

According Kobayashi & Kimura (1969) and Lee et al. (2014a), many adult stink bugs move to sun-warmed places or heating elements. However, the attraction by warm overwintering spaces has been remained contradictory so far and has not been clarified satisfactorily (Chambers 2017). Our results reveal that warm places can attract stink bugs to overwintering sites.

Our examination gives a platform to develop a novel method to monitor stink bug numbers during the pre-diapause period. It was not our aim to clarify the operability of this method in field conditions but it would be essential for future works.

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