Halyomorpha halys (Hemiptera: Pentatomidae) as a Potential Risk for Early Vegetative-Stage Sweet Corn[1]

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Abstract *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) is an invasive species in the United States representing a great threat to crops of economic importance, such as soybean and corn. Due to the lack of information about its damage to early vegetative-stage corn, this study was conducted to provide information about *H. halys* damage to sweet corn seedlings. In the field experiment, caged sweet corn seedlings were exposed to sexed *H. halys* adults of densities of 0, 1, or 2 insects per plant for 7 d. In a complementary greenhouse experiment, caged sweet corn seedlings were exposed to 0 or 2 nonsexed *H. halys* at different stages (second to fifth instars and adult) per plant for 14 d. In both experiments, we evaluated plant fresh and dry weights, plant height, stalk diameter and plant injury (using a rating scale, 0 to 5). In the field experiment, plant injury based on the rating scale was greater in plants exposed to insects compared with the control. In the greenhouse experiment, fresh and dry weights, height and diameter of seedlings were lower for those exposed to fourth instars. This stage also caused greater injury based on the rating scale. In general, our results indicate that *H. halys* can feed on sweet corn seedlings, and that fourth instars cause more injury. The rating scale adapted here can be used for early identification of *H. halys* occurrence and to assess its injury in the field.

Key Words: brown marmorated stink bug, plant injury, *Zea mays*

Sweet corn, *Zea mays* L., is harvested in all 50 states of the United States, both for fresh and processed products. It is the second largest processing crop in terms of production and value, concentrated in the upper Midwest and Pacific Northwest of the United States (USDA 2012).

Stink bugs (Hemiptera: Pentatomidae) are important insect pests worldwide, causing severe damage to crops of economic importance, such as sweet and field corn (Kuhar et al. 2012, Koch et al. 2017, Leskey et al. 2012a, Lucini and Panizzi 2017, Sappington et al. 2018). Injury from stink bugs may be conspicuous due to the injection of toxic saliva and mechanical injury (Bardner and Fletcher 1974).

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Stink bug species that occur in the United States, such as *Euschistus variolarius* (Palisot de Beauvois), *E. servus* (Say), *Nezara viridula* (L.) and *Chinavia hilaris* (Say) (Hemiptera: Pentatomidae), have been reported feeding on developing ears and kernels of corn and also on corn seedlings (Cottrell and Tillman 2015, Koch and Pahs 2015, Koch et al. 2017, Negrón and Riley 1987, Ni et al. 2010, Reisig 2011, Townsend and Sedlacek 1986). In later stages of plant development, *N. viridula*, for instance, can destroy or cause abortion of young ears (Negrón and Riley 1987). Also, corn in the reproductive stages fed on by *E. servus* adults have been reported with significant yield and grain quality losses (Ni et al. 2010). Corn seedlings fed on by *E. variolarius* and *E. servus* presented a yield loss around 75% and 20% plant mortality (Apriyanto et al. 1989).

Cultivated plants are generally more vulnerable to stressors immediately after germination (Bardner and Fletcher 1974), and the impact of early-season insect feeding on seedlings may significantly reduce yield (Sappington et al. 2018). Stink bug nymphs and adults feeding on corn seedlings cause linear feeding holes with yellow necrotic halos leading to plant shortening, tillering, wilting of whorl leaf and even plant death (Annan and Bergman 1988, Townsend and Sedlacek 1986). The presence of stink bugs on seedling corn creates a management challenge, because there is little time to intervene between insect attack and irreversible injury to the plants (Sappington et al. 2018).

Early-season pest management in corn could be further challenged by invasive species. The brown marmorated stink bug, *Halyomorpha halys* (Stål), is a polyphagous insect native to Japan, Taiwan, China, and Korea (Hoebeke and Carter 2003). This species was first detected in North America in Allentown, PA, in 1996, but it was only properly identified in 2001 (Hoebeke and Carter 2003). *Halyomorpha halys* is rapidly spreading through other states and has become a serious agricultural pest in the Mid-Atlantic Region of the United States, feeding on hosts of economic importance (Rice et al. 2014). Stink bugs prefer to feed on reproductive parts of their host plants (McPherson and McPherson 2000). Among host crops targeted by *H. halys*, sweet corn is strongly preferred in reproductive stages (Cissel et al. 2015, Kuhar et al. 2012, Leskey et al. 2012b, Rice et al. 2014). However, it is not known if *H. halys* is capable of feeding on vegetative growth stages of corn like the other stink bugs mentioned earlier.

Thus, we performed complementary field and greenhouse experiments to assess *H. halys* feeding on corn seedlings. This study was conducted to (a) provide information about the potential of *H. halys* to cause injury to sweet corn seedlings and (b) compare impacts of feeding by different *H. halys* life stages on corn seedlings. Results of this study will help pest management practitioners as *H. halys* continues to invade corn-producing regions of the United States.

Material and Methods

Insect stock colony. Insects used in these experiments were sourced from a colony maintained at the University of Minnesota. A stock colony of *H. halys* was started with egg masses collected from Wyoming, MN, and maintained under laboratory conditions (26 $\pm$ 2°C and photoperiod = 14 h), with similar methodology as described by Cira et al. (2017). Nymphs and adults were kept in mesh cages

(BioQuip, Rancho Dominguez, CA) of 38 × 38 × 61 cm and 61 × 61 × 91 cm, respectively. In the cages, insects were fed with a diet consisting of green pods of common beans (*Phaseolus vulgaris* L.) and baby carrots (*Daucus carota* L. subsp. *sativus* L.) replaced three times per week, and sunflower (*Helianthus annuus* L.) and soybean (*Glycine max* (L.) Merrill) seeds replaced twice per week. One pot with 13 common bean plants was included in the cages and watered twice a week to help meet humidity requirements inside cages and provide shelter and oviposition sites for the insects. Egg masses were collected every weekday from inside the cages and placed inside Petri dishes (4-cm diameter) lined with filter paper at the bottom. After developing to second instars, nymphs were transferred to cages and reared using the methodology described.

Field experiment. To evaluate if *H. halys* adults can feed on and injure corn seedlings under field conditions, an experiment was conducted in an experimental field at the Agricultural Experiment Station of the University of Minnesota, Saint Paul, MN in 2018. Corn seed used was “Allure” Sweet Corn Sh2xSE variety without seed treatment. Seed was sown in small plots of four seeds arranged in a 2 × 2 grid (four seeds total) with 5-cm spacing between plants. Plots were arranged in a completely randomized design with a 3 × 2 factorial treatment structure with three levels of *H. halys* density (0, 1, or 2 stink bug adults per plant) and *H. halys* sex (female or male), and with eight replications of each treatment combination. Plots were spaced 0.5 m apart from one another and each plot was enclosed by a cage. The cage frames were made with three pieces of polyvinyl chloride (PVC) pipe (60 cm long) evenly spaced around the outside of each plot and pounded vertically into the soil leaving 50 cm of pipe above ground. The cage frames were covered with a No-See-Um mesh (Quest Outfitters, FL, USA) that was buried in the soil around the base of the cages.

Halyomorpha halys used in this assay were adults at 10 d of age. Prior to experimentation, adults were moved from the stock colony cage to a new cage with no food or potted bean plants to starve them for 24 h. Seven days after plant emergence, when plants were at V3 corn growth stage (Ritchie and Hanway 1966), *H. halys* adults were released inside the cages in the field. The cages were assessed daily to monitor survival of *H. halys*, with dead individuals replaced (insects from the same generation) to maintain constant densities of *H. halys* adults over time. At 7 d after infestation, *H. halys* were removed from all cages and cages were closed again to protect plants from other herbivores.

At 23 d after plant infestation (30 d after plant emergence), each plant was rated for injury using a rating scale modified from Roza-Gomes et al. (2011), with 0 = plant without injury; 1 = leaves with holes and no impact on the plant size; 2 = leaves with holes and impact on the plant size; 3 = plant whorl slightly injured; 4 = severe injury to the whorl or tillering; 5 = plant with dry or dead whorl (Fig. 1). At this time, corn plants in each cage were also evaluated for the following: above-ground fresh weight, plant height (ground to upper boundary of the highest leaf), and stalk diameter. In addition, dry weight of above ground plant material was evaluated after plants dried for 5 d in an oven at 60°C. For each cage (experimental unit), the measured response variables were averaged across the plants in the cage for use in the analyses. In each of three cages from the control, one plant died for unknown reasons, not related to stink bugs feeding, so cage-level averages from those cages were calculated from three plants.

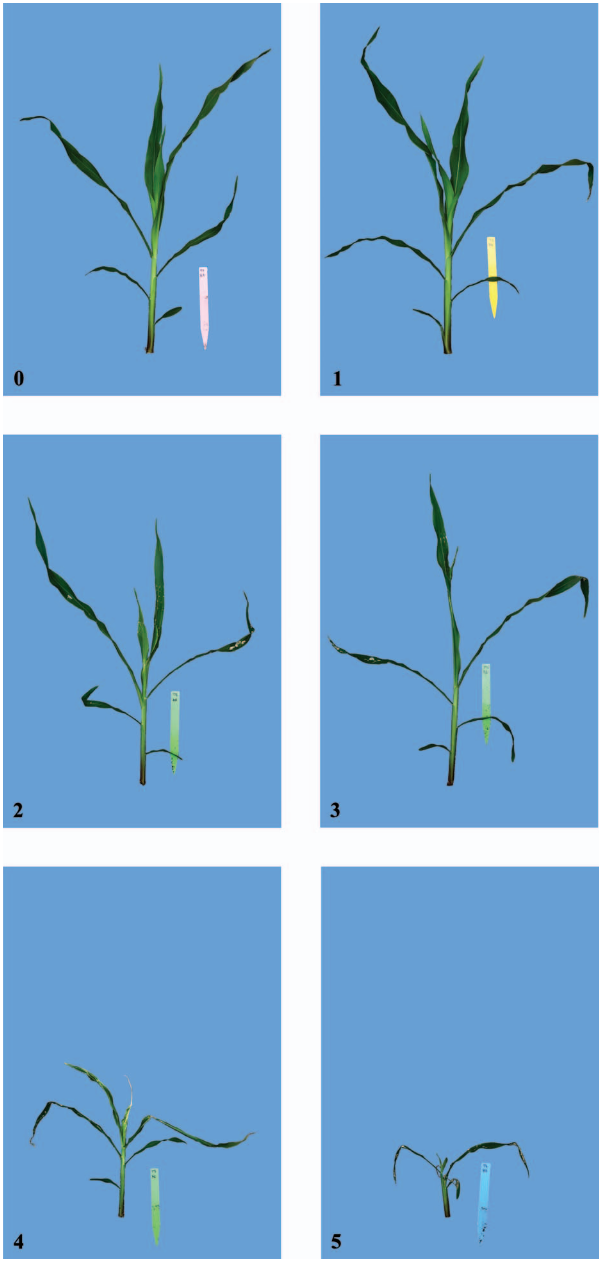

Fig. 1. Injury rating scale demonstration, being: 0 = plant without injury; 1 = leaves with holes and no impact on the plant size; 2 = leaves with holes and impact on the plant size; 3 = plant whorl slightly injured; 4 = severe injury to the whorl or tillering; and 5 = plant with dry or dead whorl. Stakes used for comparison are 15 × 1.5 cm.

Greenhouse experiment. To more thoroughly investigate *H. halys* injury to corn seedlings, an experiment was conducted in a greenhouse at the Plant Growth Facility of the University of Minnesota, Saint Paul, MN in 2018. In particular, the experiment aimed to assess the injury caused to corn seedlings by second to fifth instars and adults. First instars were not assessed because they generally do not feed on host plants and stay aggregated on the egg mass until their first molt (Leskey et al. 2012a). Corn seed used was "Allure" Sweet Corn Sh2xSE variety. Three seeds were sown per 3-L plastic pot and only one seedling per pot was retained after thinning. Each pot was surrounded by a metal cage frame (galvanized tomato cage, Gilbert and Bennett, 137.16-cm long) covered with a No-See-Um mesh. Cages, which were considered experimental units, were arranged in a completely randomized design with six treatments (i.e., four nymphal stages, adults and a control) and with eight replications. Noninfested plants were used as control.

One week after plant emergence, when seedlings were at V3 stage, two *H. halys* from each stage (recently molted) were released inside the cages. Prior to experimentation, insects were starved for 24 h, as previously described. Nonsexed adults were used because effects of sex or its interaction with treatment were not observed in the previous experiment. The cages were assessed daily, with dead or molting individuals replaced to maintain constant densities of individuals at the same stage over time. At 14 d after infestation, *H. halys* were removed from all cages and cages were closed again to protect plants from other herbivores. We increased the time the stink bugs stayed in the cages from the field to the greenhouse experiments in order to more thoroughly investigate the potential injury of *H. halys* to sweet corn seedlings.

At 23 d after plant infestation (30 d after plant emergence), each plant was rated for injury using the rating scale previously described. At this time, corn plants were also evaluated for above ground fresh and dry weight, plant height, and stalk diameter as in the previous experiment.

Statistical analyses. All analyses were conducted in R version 3.5.1 (R Core Team 2018) and RStudio Desktop version 1.1.463 (RStudio Team 2016). Data from the field experiment were analyzed using an unbalanced two-way analysis of variance (ANOVA) (R package, *command*: car, *Anova*; Weisberg; Fox 2011) using a general linear model (stats, *lm*; Wilkinson and Rogers 1973). Treatment, sex, and their interaction were included as explanatory variables in the linear model. For the greenhouse experiment, data were analyzed using ANOVA (stats, *anova*; Chambers et al. 1992) with a general linear model with treatment as an explanatory variable. Means were compared using Tukey's test at $\alpha = 5\%$ (agricolae, *HSD.test*; Mendiburu 2019) for significant effects. The variable height and rating scale for the greenhouse experiment were Box-Cox transformed $y\lambda = [((y + 1)\lambda - 1) / \lambda]$; $\lambda = 3$ and 0.04242, respectively; Mass, *boxcox* (Venables and Ripley 2002) to follow assumptions of normality (Jarque-Bera: JB = 1.26, $P = 0.394$, and JB = 1.06, $P = 0.476$, respectively [normtest, *jb.norm.test*, Jarque and Bera 1987]) and heteroscedasticity (Levene's test: $F_{5,42} = 0.93$, $P = 0.472$, and $F_{5,42} = 2.18$; $P = 0.074$, respectively; car, *leveneTest*; Weisberg and Fox 2011).

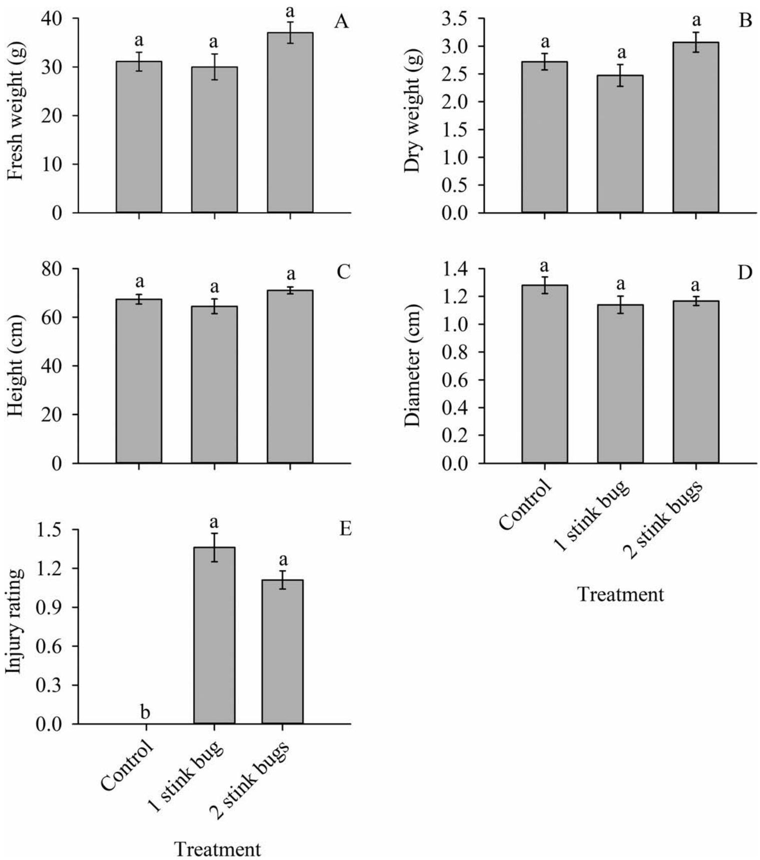

Fig. 2. Mean (± standard error) fresh (A) and dry (B) weights, height (C), stem diameter (D), and injury rating at 23 d after infestation (E) for sweet corn seedlings exposed to *Halyomorpha halys* at densities of zero (i.e., control), one or two insects per plant, in the field. Plants were infested 1 week after emergence and insects were maintained on plants for 7 d. Different letters indicate significant differences among treatments (Tukey's test, $\alpha = 5\%$).

Results

Field experiment. Effects of *H. halys* density, sex, and their interaction were not significant for plant height ($F_{5,16} = 1.41$, $P = 0.274$), stem diameter ($F_{5,16} = 2.38$, $P = 0.085$), and fresh ($F_{5,16} = 1.77$, $P = 0.176$) and dry ($F_{5,16} = 1.81$, $P = 0.168$) weights of plants (Fig. 2). For plant injury ratings at 23 d after infestation, effects of *H. halys* sex ($F = 0.84$, $df = 1$, $P = 0.362$) and its interaction with *H. halys* density ($F = 1.94$, $df = 2$, $P = 0.151$) were not significant, but there was a significant effect of *H. halys* density on injury ratings ($F = 40.87$, $df = 2$, $P < 0.001$). Injury ratings from cages

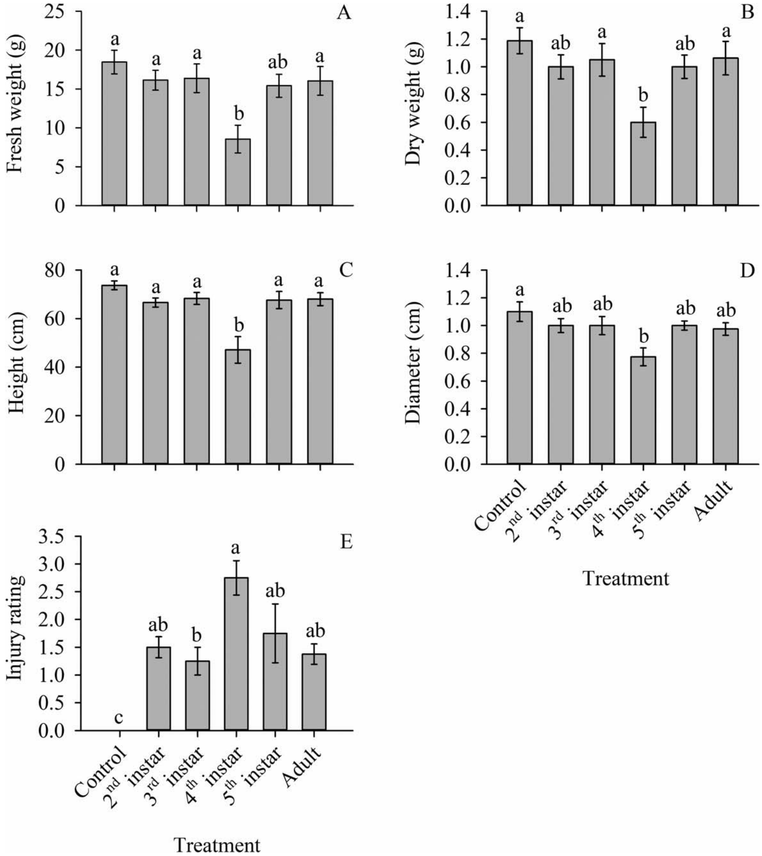

Fig. 3. Mean (± standard error) fresh (A) and dry (B) weights, height (C), stem diameter (D), and injury rating at 23 d after infestation (E) for corn seedlings exposed to second, third, fourth, and fifth instars and adults of *Halyomorpha halys* at densities of two insects per plant or zero insects (i.e., control), in greenhouse. Plants were infested 1 week after emergence and insects were maintained on plants for 14 d. Different letters indicate significant differences among treatments (Tukey's test, $\alpha = 5\%$).

with one or two bugs per plant were similar to one another (1.1–1.36) and significantly higher than the control cages with 0 bugs (Fig. 2E).

Greenhouse experiment. A significant difference among treatments was observed for all evaluated response variables (fresh weight: $F_{5,42} = 4.31$, $P = 0.003$; dry weight: $F_{5,42} = 3.76$, $P = 0.006$; height: $F_{5,42} = 7.40$, $P < 0.001$; stem diameter: $F_{5,42} = 3.61$, $P = 0.008$; injury rating: $F_{5,42} = 9.29$, $P < 0.001$). Fresh weight of corn seedlings was significantly lower for plants exposed to fourth instars in comparison with second and third instars, adults, and the control (Fig. 3A). Dry weight of plants exposed to *H. halys* was not significantly different from any other

Fig. 4. A fourth instar *Halyomorpha halys* feeding on a sweet corn seedling and associated plant injury 4 d after infestation (11 d after plant emergence) under greenhouse condition.

treatment (Fig. 3B). Plants exposed to fourth instars had significantly lower plant height than all other treatments (Fig. 4C). Plant heights of the other treatments did not differ (Fig. 3C). Stem diameter of corn seedlings was significantly lower for plants exposed to fourth instars compared with the control (Fig. 3D). Stem diameter of the plants exposed to other life stages did not differ from the control or fourth instars (Fig. 3D). At 23 d after infestation, injury ratings were significantly higher for all infested plants compared with the control. Among infested plants, injury ratings for plants infested with fourth instars ($>$2.5) was greater than the rating for plants infested with third instars (Fig. 3E).

Discussion

Sweet corn is a horticultural vegetable that is highly consumed and of great economic importance in the United States (Khan et al. 2018), and emerging pests such as *H. halys* threaten this crop by causing direct damage to the ears and kernels (Cissel et al. 2015, Kuhar et al. 2012, Leskey et al. 2012b). Despite the concern of *H. halys* on reproductive-stage corn, it was not known if this species could feed on and cause injury to corn seedlings. To our knowledge, this is the first study that assessed the potential impact of *H. halys* on corn seedlings.

The *H. halys* injury observed here did not differ significantly between female or male individuals, which has been observed for other stink bug species (Apriyanto et al. 1989, Simmons and Yeargan 1988). With the exception of the injury rating scale, all evaluated variables for plants exposed to *H. halys* in the field experiment showed responses similar to the control. This may have occurred because we used just adults during this experiment. As shown in the greenhouse with plants exposed to different stages of *H. halys*, adults caused similar injury to the control for all variables except for the injury rating scale, which supports the results obtained on the field. Mean injury ratings of infested plants in the field experiment were around 2, which means the injury was caused by stink bugs feeding on leaves, without severe damage extending to the whorl. Injuries from stink bug feeding that did not affect plant height and weight were also observed in *E. heros* (F)., *N. viridula*, and Diceraeus (Dichelops) furcatus (F.) (Hemiptera: Pentatomidae) feeding on corn seedlings (Roza-Gomes et al. 2011). This may have happened because stink bugs cause more damage when they feed on the stalk, near to the soil, in an upside down position (Apriyanto et al. 1989, Panizzi and Lucini 2018), a preferred feeding behavior observed in some stink bug species (Ávila and Panizzi 1995, Panizzi and Machado Neto 1992).

In the present experiments, we generally observed *H. halys* feeding on leaf surfaces (Fig. 4) and not on the stem near the soil surface. In spite of stink bugs being found feeding on vegetative structures of host plants (Ávila and Panizzi 1995, Chocorosqui and Panizzi 2004, Sappington et al. 2018, Townsend and Sedlacek 1986), they usually prefer to feed on reproductive parts, especially immature seeds (Olson et al. 2011). This may be due to the fact that the seed contents meet essential nutrients needed for the insect development (Lucini and Panizzi 2017). In the absence of a more suitable food source, stink bugs can feed of leaves and stems in order to maintain their development (Panizzi et al. 2012). Thus, despite the likely preference of *H. halys* for reproductive parts of corn, it was able to feed on seedlings of sweet corn.

Irreversible damage caused by stink bug feeding near seedling emergence can occur in a short amount of time (Sappington et al. 2018). Thus, despite the fact that the adult stage caused low levels of injury to corn seedlings when compared with other stages, the injury rating scale could serve as a tool to assess early-season infestations, as shown in our study. In this sense, studies to develop sampling plans for management decision-making for *H. halys* and other stink bugs in early-season sweet corn may be desirable.

In the present work, all evaluated life stages caused injury to corn seedlings and that injury varied by stink bug developmental stage. In general, there are differences among insect stages regarding insect feeding behavior because of their ecological role (Cook and Neal 1999). Younger insects are usually less mobile than later developmental stages (Cook and Neal 1999), and the later stages tend to cause more damage to plants as already described for *E. servus* and *E. variolarius* (Ni et al. 2010, Sedlacek and Townsend 1988). This is to be expected because nutritional requirements usually increase with insect growth (Browne 1995, Gordon 1998). In addition, development often comes with an increase in size, including larger mouth parts, which represents greater potential mechanical injury and food intake (Browne 1995, Valles et al. 1996). However, here the fourth instar of *H. halys* generally caused more injury than other life stages (Fig. 3). Acebes-Doria et al. (2016), evaluating *H. halys* damage to apple and peach, observed that adults cause more injuries than nymphs. Despite the fact that they did not evaluate injuries caused by each stage separately, fourth and fifth instars caused more injury than second and third instars, which generally matches responses in our study. The peculiarity of fourth instars causing higher levels of injury observed in this work may be related to reduced feeding frequency or duration by later insect stages (Bernays 2001, Raubenheimer and Browne 2000). Other potential explanations may include differences in salivary amount or activity, but these were not evaluated in the present study. Further investigation is needed to better understand why fourth instars caused greater levels of injury to corn seedlings.

Our results indicate that *H. halys* confined with corn seedlings can feed on and injure the plants. Scouting for this insect may be necessary in early plant stages, if high populations are observed. In addition, the adapted injury rating scale used in this study could be adopted for early detection of stink bug infestations and for assessment of their injury in the field. Nevertheless, further observations are needed under open field conditions to determine conditions conducive for *H. halys* feeding on sweet corn seedlings.

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