RESEARCH ARTICLE

Electronically Monitored Labial Dabbing and Stylet ‘Probing’ Behaviors of Brown Marmorated Stink Bug, *Halyomorpha halys*, in Simulated Environments

Nik G. Wiman¹*, Vaughn M. Walton¹, Peter W. Shearer², Silvia I. Rondon³

¹. Department of Horticulture, Oregon State University, Corvallis, Oregon, United States of America, ². Mid-Columbia Agricultural Research and Extension Center, Oregon State University, Hood River, Oregon, United States of America, ³. Hermiston Agricultural Research and Extension Center, Oregon State University, Hermiston, Oregon, United States of America

*nik.wiman@oregonstate.edu

Abstract

Brown marmorated stink bug, *Halyomorpha halys* (Stål), (Hemiptera: Pentatomidae) is an invasive polyphagous agricultural and urban nuisance pest of Asian origin that is becoming widespread in North America and Europe. Despite the economic importance of pentatomid pests worldwide, their feeding behavior is poorly understood. Electronically monitored insect feeding (EMIF) technology is a useful tool in studies of feeding behavior of Hemiptera. Here we examined *H. halys* feeding behavior using an EMIF system designed for high throughput studies in environmental chambers. Our objectives were to quantify feeding activity by monitoring proboscis contacts with green beans, including labial dabbing and stylet penetration of the beans, which we collectively define as ‘probes’. We examined frequency and duration of ‘probes’ in field-collected *H. halys* over 48 hours and we determined how environmental conditions could affect diel and seasonal periodicity of ‘probing’ activity. We found differences in ‘probing’ activity between months when the assays were conducted. These differences in activity may have reflected different environmental conditions, and they also coincide with what is known about the phenology of *H. halys*. While a substantial number of ‘probes’ occurred during scotophase, including some of the longest mean ‘probe’ durations, activity was either lower or similar to ‘probing’ activity levels during photophase on average. We found that temperature had a significant impact on *H. halys* ‘probing’ behavior and may influence periodicity of activity. Our data suggest that the minimal temperature at which ‘probing’ of *H. halys* occurs is between 3.5 and 6.1 °C (95% CI), and that ‘probing’ does not occur at temperatures above 26.5 to 29.6 °C (95% CI). We estimated that the optimal temperature for ‘probing’ is between 16 and 17 °C.
Introduction

Phytophagous stink bugs (Hemiptera: Pentatomidae) can be highly destructive pests on a wide range of crops worldwide [1]. Their feeding can cause many different types of crop damage, and is particularly destructive on the fruiting parts of the plant [2]. Each time the stylets are inserted into plant tissues, a puncture will result, and secondary infection from incidental or vectored yeasts can result in tissue decay or necrosis [3, 4]. Pentatomidae can vector trypanosomatid flagellate pathogens as well as phytoplasms and fungi that can cause disease in host plants [5]. Salivary secretions, particularly enzymatic watery saliva, dissolve plant tissues and cause subsurface corking damage on fruits, vegetables, and nuts [11]. The saliva can also be considered phytoaggressive in plants and it can enzymatically disrupt function of plant tissues [6–8]. Feeding by stink bugs can also cause other deformities such as discoloration, or dimpling and “catfacing” on the surface of the fruits such as peaches [9]. Typically this damage occurs when undamaged fruit tissue continues to grow and expand around damaged tissue at a feeding site. In cotton, feeding by the stink bug *Nezara viridula* (L.) can cause blister damage [10]. Stink bug feeding can also cause tillering or stunting of developing corn and soybean plants, which negatively affects yield [11]. Feeding during the early stages of fruit or nut development can cause shriveling or abortion [2, 12]. Finally, maturation of crops can be affected by stink bug feeding damage, with soy showing delayed maturity [13], and tomato showing premature maturation [14].

Brown marmorated stink bug, *Halyomorpha halys* (Stål), (Hemiptera: Pentatomidae) is an invasive polyphagous agricultural and urban nuisance pest of Asian origin [15, 16]. Many regions of the world are environmentally suitable for this insect, and this pest has potential to severely damage a wide range of crops [17]. Nuisance problems occur when the insects aggregate en masse in structures for diapause, or are destructive on ornamental hosts or gardens [17–19]. The current range of this pest includes parts of Europe, Canada, and most US states [20, 21]. To date, the most significant economic effects in the US have been in tree fruit crops such as apples and peaches in Mid-Atlantic States [20]. *Halyomorpha halys* has been known from Oregon since 2004, and it now occurs in commercial tree crops, small fruits, vegetables, and generates many homeowner complaints [22]. The economic impact of *H. halys* is expected to increase dramatically as populations continue to spread and increase in other specialty crop production regions in the Western US and the world.

With the increasing economic importance of Pentatomoidea as a result of the spread the invasive pests *H. halys* [20, 21], *N. viridula* [23], *Megacopta cribraria* (Fabricius) [24, 25], and *Bagrada hilaris* (Burmeister) [26–29], research on feeding behavior of these insects is critical. Currently, much of what is known with regards to periodicity, frequency and duration of feeding activity for these insects has been determined by intermittent or constant visual observation of feeding over 24 hour periods [30–34]. However, Shearer and Jones [35] demonstrated that electronically-monitored feeding can be used to quantify periodicity of select feeding behaviors in *N. viridula* very effectively [35]. Electronically monitored insect
feeding behavior (EMIF) originated as a methodology to study feeding in piercing-sucking insects [36]. In EMIF, the insect is placed into an electrical circuit that is completed only when the proboscis contacts the host plant. This provides data on the timing, duration and frequency of select feeding behaviors of these insects. In its most basic application, EMIF is similar to basic visual observations of feeding behavior as it records when the stylets are in contact with the food item and does not discriminate different feeding behaviors [35]. More precision can be obtained with electrical penetration graph (EPG) monitors, the dominant modern form of EMIF. EPG can measure changes in resistance and biopotential in the circuit that is completed by an insect feeding on an electrified host plant [37]. EPG produces high resolution data containing waveforms that can be precisely correlated with specific feeding behaviors such as penetration, salivation, fluid uptake, ingestion, and can yield important information about pathogen transmission [36,38,39].

EPG has primarily been a tool used for Hemiptera from suborders Sternorrhyncha and Auchenorrhyncha (previously Homoptera), such as aphids (Aphididae), planthoppers (Fulgoroidea), leafhoppers (Membracoidea), whiteflies (Aleyrodidae) and psylla (Psyllidae) [37]. Excluding leafhoppers, these insects tend to be true salivary sheath feeders, where the stylets are inserted into phloem or xylem without rupturing intermediate cells and a complete salivary sheath is formed [40–42]. Pentatomoidea are very versatile feeders and may use different feeding strategies depending on the plant tissue targeted [43,5,44]. To feed on vascular tissue, the strategy resembles salivary sheath feeding, but the stylets or salivary osmotic potential may be used to rupture mesophyll or phloem cells. When feeding on tissue such as endosperm in seeds, there is greater reliance on “lacerate-and-flush” strategy where mechanical and enzymatic breakdown of cells is used and salivary sheaths are incomplete. Halyomorpha halys has been observed to feed on leaves, stems, petioles, even through bark [45]. However, pentatomid feeding on fruits, nuts, pods, and seeds, tends to cause the greatest economic crop damage [1,2,46]. Thus, reproductive plant structures are a focal site for the plant/insect interaction for pentatomids, and EMIF systems that can work on fruits and vegetables are important to improve knowledge of their feeding behavior.

In this study, we examined the feeding activity of field-collected H. halys using an EMIF system that was designed to continuously monitor feeding behavior of Pentatomidae on cut fruits and vegetables with minimal handling of insects. Our system has high replication potential and can function in environmental chambers. The feeding behaviors we monitored were limited to stylet contact with the food and stylet penetration with the food. Hereafter, we use the word ‘probe’ and derivative forms to include all published definitions of the word, i.e., exploratory or sensory stylet contacts or labial dabbing, and stylet penetration of the food [37]. Our objectives were to 1) describe ‘probing’ activity patterns of H. halys in terms of frequency, duration, and total time spent ‘probing’ per day, 2) to examine how environmental conditions could affect ‘probing’ activity, and 3) examine how changes in environmental conditions affect diel periodicity of ‘probing’ activity over a season. These data are intended to improve our
understanding of feeding behavior for *H. halys*. This information will help optimize sampling and management, and will provide a foundation for further studies on how feeding behavior relates to crop damage from this pest.

**Materials and Methods**

The design of our EMIF activity monitor was modified from Shearer and Jones [35], with some refinements in configuration and instrumentation. A common concept between the aforementioned study, earlier studies [47–49], and the current study, is that the insect is confined to forage on a conductive metal screen attached to a power source. On the other side of the screen, a food item connected by wire to a recording instrument is placed within reach of the insect’s proboscis, but not close enough to touch the screen. When the insect commences ‘probing’ of the food item, the circuit becomes closed. When the circuit is closed, current flows from the power source, into the screen, through the tarsi and mouthparts of the insect, into the food item and the wire until the current is finally received and logged at the recording device. Conveyance of substrate current to the insect is reversed from typical EPG applications where the power source is connected to the host plant.

**Electronic feeding tables**

Configuration of our EMIF system differs from systems used in previous studies in that we used two self-contained ‘feeding tables’, each with eight individual feeding 'stations'. Feeding tables were essentially boxes that allowed compact layout of feeding stations so that feeding activity of eight individual insects per table could be monitored concurrently in environment chambers. Two feeding tables were constructed of wooden boxes (38.75 cm length, 25.5 cm width) and each contained eight holes (57 mm diameter), which accommodated tightly fitting screw caps lids (S-14506 ULINE, Pleasant Prairie, WI) and white polypropylene jars (120 ml; Figure 1). The centers of the fitted cup lids contained a smaller 50 mm diameter hole, leaving a threaded ring in the wood hole allowing the cups to be easily screwed into place from under the box to place *H. halys* into the feeding station (Figure 2). The entire top of each box was covered with a sheet of woven copper mesh (0.43 mm strand, 1 mm opening) that was stapled tightly to the box (Figure 1). Two portholes (½ in, 127 mm) were drilled into the long side of each box at the mid point between the first and second pair of holes to accommodate the electrode assemblies. The electrode assemblies consisted of two chains of ¼ in (64 mm) Loc-Line (LP-41401, Lockwood Products, Lake Oswego, OR) articulated plastic tubing segments, and these were joined by a Y fitting (LP-41408) and threaded into a port with a coupler (LP-41406). The tip of each electrode assembly was fitted with a nozzle (Loc-Line, LP-41403) and a 7.62 cm segment of solid copper wire (10 Ga, Artistic Wire, Coatesville, PA) soldered to a length of braided electrical wire (16 Ga.) was threaded through the port in the
Figure 1. Top view of a feeding table.
doi:10.1371/journal.pone.0113514.g001

Figure 2. Bottom view of a feeding table.
doi:10.1371/journal.pone.0113514.g002
feeding table and through each tube segment and nozzle until just the large-gauge copper wire was protruding from the nozzle. A drop of hot glue applied between this wire electrode and the nozzle tip prevented the wire from sliding into the nozzle. A central port (190.5 mm) was added to one of the short sides of each box (“front”), and through this opening all of the wires from the individual electrodes were routed (Figure 1, 2). An automotive 9-pin male/female quick-connect coupler (with one wire removed) was attached to the 8 electrode lead wires, with a unique color representing each electrode and station. The other end of the coupler was wired to a 3 m long bundle of 8 wires representing the different electrodes. Wire bundles were wrapped with electrical tape and cable sleeves. The wire bundles connected to each box (8 wires each) were wired directly to the analog input (AI) terminals of a single USB data acquisition instrument (USB-6210, National Instruments Corporation, Austin, TX), which was connected to a PC via USB port. A regulated DC power supply (Mastech HY3003D, Acifica, Inc., San Jose, CA) was set to deliver +5 VDC to a steel L bracket that was screwed into table surface to serve as a power connection to the copper screen through a wire (16 Ga) and alligator clip. This single common screen served as the sole power substrate for each station on each table.

The ground terminal on the DC power supply was wired to the ground terminal on the USB-6210. The AI SENSE terminal on the USB-6210 was wired to one of the analog channels and to the black (−) terminal on the power supply to create a non-referenced single-ended (NRSE) input configuration ([50]; Figure 3). In this configuration there were 16 feeding stations on the two feeding tables. However, there were only 15 functional feeding stations as one channel on the USB-6210 was sacrificed to connect with the AI SENSE terminal, which reduced ambient signal noise. The resistance of the circuit elements between the power source and the copper screen, and between the electrode and the USB-6210 were approximately 0.5 Ω. The green beans that were attached to the electrodes as the food source provided a mean resistance of $12.36 \times 10^6$ Ω ($\pm 0.57$ SEM) between the electrode and surface of the bean. Analog input impedance on the USB-6210 is greater than $10 \times 10^9$ Ω per channel [51], so the total input resistance ($R_i$) in each circuit was more than $10.012 \times 10^9$ Ω (excluding resistance added by the feeding insect). Resistance is high enough between the surface of tarsi and mouthparts in H. halys anesthetized by CO$_2$ that it cannot be measured with a multimeter ($\text{max} \approx 200 \times 10^6$ Ω; Aide Tek VC97, Syncont. Inc., Parlin NJ). Thus, when the insect touched the surface of the bean with its mouthparts, it would experience a current of less than $4.99 \times 10^{-10}$ A, or 0.0005 μA ($\Lambda = \text{V} / \Omega$; Ohm’s law). This current is equivalent to using 500 mV on a standard commercial $1 \times 10^9$ Ω DC-EPG system ($5 \times 10^{-10}$ A). The resistors represented by the insect and the bean on both sides of the gap in the circuit between the screen and the food precluded the possibility that false readings could occur. If the bare electrode (no bean) were placed within one millimeter of the screen, only 2–4 mV could be detected from the air in the space, thus static electricity would not generate signals above what would have been classified as noise in the analysis (see data analysis below).
Data acquisition

A data acquisition program was written in the LabVIEW 2012 Version 12.01 (National Instruments Corp.) development environment. Because of delayed discharge between channels, rapid sampling between terminals on the USB-6210 device (250 kS/s scan rate), and high impedance in the circuits, signal response on neighboring channels (“crosstalk”) was initially a problem. We addressed this by modifying the driver configuration for the USB-6210 by adding dummy channels to increase inter-channel sampling delay. This allowed the multiplexer in the USB-6210 to fully discharge (“settle”) between readings so that the signals on the individual channels would not interfere with each other. For the purposes of this experiment, we acquired voltage readings at one-second intervals along with a date and time stamp. All data were output into comma-separated-value format. A front panel for the data acquisition program was designed to allow the user to set the duration of the feeding trial, and to set a threshold voltage to indicate feeding. Two line graphs showed voltage readings from the electrode in real-time for each station (8 stations each) and allowed the user to determine the stations that displayed feeding activity. Additionally, LED lights, one per station, were triggered when the insect was feeding. The LED’s illuminated when channel voltage exceeded the threshold set by the user.
Food source

Fresh, organic green beans (*Phaseolus vulgaris* L.) were used as the food. Straight lengths of bean (approx. 7–10 cm) were cut from the stem, and the cut ends were wrapped tightly in Parafilm (Pechiney Plastic Packaging Company, Chicago IL). This kept beans fresh throughout each 48 h experimental period. Beans were pierced with a dissecting needle through the Parafilm in the center of the bean to pilot a straight hole for the electrode. A prepared bean was placed on each electrode and the assembly was positioned so that the maximum surface area of the bean was close to the copper screen without making contact with it (~2 mm gap).

Environmental chambers

Each feeding table was placed into a programmed environmental chamber (0.8 m³ interior vol.; CU-22, Percival Scientific, Inc., Perry IA). Monthly temperatures within each chamber represented a typically warm or cold average environment found in the Willamette Valley, OR relative to 30-year means (NOAA, Table 1). Environmental controls were adjusted every month for the duration of the study to most closely represent daily ambient conditions. There were nine daily temperature and three light intensity adjustments. Temperature steps were based on a sinusoidal representation of daily temperature cycles and were dependent on daily sunrise and sunset settings. Photoperiod timings were similarly adjusted on a monthly basis and were similar in the warm and cool environmental chambers (Table 1). Sunrise and sunset simulations were recreated by dawn and dusk light intensities (45 PPFD; photosynthetic photon flux) based on U.S. Naval Observatory Astronomical Applications Department projections for W123.16, N44.34 at 1 hr periods. Light intensity for the remainder of photophase was 85 PPFD. The coolest temperature was always represented at the last step before simulated sunrise and the warmest temperature was always represented at the step following the midpoint of photophase, thus simulating the thermal delay phenomenon. The other temperature steps represented gradual temperature increments between the highs and lows on the sine curve. Humidity was maintained at 70%.

Collection of insects

*Halyomorpha halys* were collected with beating trays from host plants in the Willamette Valley [52] and maintained overnight at 4.5°C before placement in feeding tables the following morning. We did not consider the insects to be starved after this period of 15–16 hrs without food because of the lack of activity exhibited by *H. halys* at this holding temperature.

Data analysis

A program was written in the open source statistical environment R [53] to retrieve all voltages and timestamps for sequences of voltages above the threshold
of 1.5 V DC for each station over the 48 h assay from the CSV output files representing each trial. Observationally, this was the voltage threshold determined to correlate with ‘probing’ behavior. If voltages above the threshold were separated by more than 15 s, they were considered disparate ‘probing’ bouts. Voltage data from were merged with environmental data, which was collected with a HOBO data logger (UA-002-64, Onset Computer Corp., Bourne MA) using R package “xts” [54]. Analyzed variables were the number of ‘probes’, the duration of individual ‘probes’, and the total time spent ‘probing’. Statistical analyses on these variables were standard linear methods including ANOVA and Fisher’s LSD with log transformations to improve data normality where necessary. The number of H. halys that probed at each temperature across the range of temperatures tested over the course of this study was fit with a polynomial model to produce a temperature-dependent ‘probing’ response curve. The polynomial was solved to find the x intercepts that indicated the lower and upper feeding threshold, and the optimal ‘probing’ temperature was determined visually. We also looked at diel periodicity, where data were pooled into consecutive 2-hr increments to improve visualization of trends. To test diel periodicity of ‘probing’ activity, means of variables that were pooled within scotophase and within photophase were compared using Welch’s t-test, assuming that variances were unequal given more samples in the photophase data [35]. In one case, testing of the proportion of H. halys feeding during scotophase and photophase was accomplished using a binomial exact test in lieu of the t-test. Because photoperiod varied between the hours of 06:00 and 07:00, as well as 20:00 and 21:00 for the different months (Table 1), these transitional periods were excluded from the pooled data from scotophase and photophase and the t-test. All analyses were conducted using R.

### Results

#### ‘Probing’ activity

We defined ‘probing’ in the broadest sense such that we included any behavior where the mouthparts contacted the food [41]. Of all of the 302 field-collected

---

**Table 1. Environmental conditions in the warm and cool environmental chambers for each month of the experiment.**

| Mo   | Warm cab.     | Cool cab.    | Photoperiod |
|------|---------------|--------------|-------------|
|      | High | Low | x | High | Low | x | Photo. | Scoto. | L(h) | D(h) |
| May  | 22   | 11  | 17 | 16   | 7   | 12 | 05:40 | 20:30 | 14.9 | 9.1  |
| June | 26   | 13  | 20 | 23   | 9   | 16 | 05:20 | 21:00 | 15.6 | 8.4  |
| July | 31   | 15  | 23 | 26   | 11  | 19 | 05:40 | 21:00 | 15.3 | 8.7  |
| Aug  | 28   | 13  | 21 | 23   | 9   | 16 | 06:10 | 20:20 | 14.2 | 9.8  |
| Sept | 25   | 11  | 18 | 18   | 7   | 13 | 06:50 | 19:20 | 12.5 | 11.5 |
| Oct  | 17   | 7   | 12 | 14   | 3   | 9  | 07:30 | 18:20 | 10.9 | 13.1 |

doi:10.1371/journal.pone.0113514.t001
adult *H. halys* placed in feeding tables in this experiment, 184 ‘probed’ at least once over the 48 h assay (61% response). Only responsive insects were included in the analyses. Individual adult *H. halys* ‘probe’ durations ranged from 1 sec to 184.6 min. The majority of the observed ‘probes’ were relatively short in duration; the arithmetic mean duration was 3.97 min (± 0.51, 95% CI; Figure 4a). Individual *H. halys* ‘probed’ 1–179 times per 24 h in the 48 h assay. The arithmetic mean was 9.77 ‘probes’ every 24 h (± 2.79, 95% CI; Figure 4b). Summing the total duration of ‘probes’ for individual insects indicated adults spent between 1 and 559.4 min (9.32 h) ‘probing’ per day, however the arithmetic mean duration was 38.8 min per 24 h (± 12.03, 95% CI; Figure 4c). ‘Probing’ activity data was highly skewed toward zero, and log transformation was used to improve normality for subsequent significance testing on these variables. Skew of pooled duration data toward zero meant that back-transformed means (geometric) shown in plots were considerably lower than arithmetic means reported above.

**Environmental effects on ‘probing’ activity**

There were significant differences in activity between insects depending on the temperature conditions in the environmental chambers (Table 1). *Halyomorpha halys* that were held in the warmer environmental chamber ‘probed’ for longer periods on average compared to insects held in the cooler chamber (F=4.81; d.f.=1,182; P=0.029; Figure 5a). However, insects ‘probed’ more frequently in the cooler chamber (F=6.43; d.f.=1,182; P=0.012; Figure 5b), so that overall, there was no difference in the mean ‘probing’ duration per insect per day (24 h) between the insects subject to the two environmental chambers (F=0.232; d.f.=1,182; P=0.631; Figure 5c).

The number of insects that ‘probed’ at each of the temperatures tested was fitted with a second order polynomial model (F=24.33; d.f.=2,21; P<0.001, \(r^2=0.67\); Figure 6). The lower and upper ‘probing’ thresholds predicted by the model were 4.89 and 27.97°C, respectively. A 95% confidence interval predicted that the minimal temperature at which ‘probing’ of *H. halys* could occur was at temperatures between 3.51 and 6.13°C, and that ‘probing’ would likewise cease at high temperatures between 26.53 and 29.60°C. The optimal ‘probing’ temperature was predicted to be approximately 16.5°C, and the 95% confidence interval predicted that the most conducive temperatures for feeding were between 16 and 17°C.

**Phenological effects on ‘probing’ activity**

The duration of ‘probes’ depended on the month in which the assay was conducted in both the warm (F=8.11; d.f.=5,84; P<0.001; Figure 7a), and the cool environmental chambers (F=2.38; d.f.=5,101; P=0.043; Figure 7b). Some of the longest mean ‘probe’ durations occurred in chambers programmed to represent early season conditions. However, there were fewer significant
differences between mean durations for the individual months in the cool environmental chamber (LSD test; $P<0.05$; Figure 7b). In the warm chamber, the shortest mean ‘probe’ duration was in August and September compared with May, June, and July. October mean ‘probe’ duration doesn’t appear to fit trends

Figure 4. Length of all discrete ‘probes’ by H. halys (a), number of ‘probes’ per insect (b), and the total time spent ‘probing’ per day (c).

doi:10.1371/journal.pone.0113514.g004

Figure 5. Means (± 95% CI) for environmental chambers; ‘probing’ duration (a), ‘probing’ frequency (b), and sum ‘probing’ by H. halys. *$P<0.05$, NS=not significant (c).

doi:10.1371/journal.pone.0113514.g005
suggesting it was a potential outlier. ‘Probe’ frequency was not correlated with the month of the bioassay in either environmental chamber ($P>0.05$). There were significant differences in total ‘probe’ duration per insect per day for months in the warm environmental chamber ($F=4.82$; d.f. = 5, 84; $P<0.001$; Figure 7c), but not in the cool chamber ($F=1.30$; d.f. = 5, 100; $P=0.272$; Figure 7d). Similar to the ‘probe’ duration data discussed above, adult *H. halys* collected in May, June and July tended to display longer durations per day than in August and September (LSD test; $P<0.05$; Figure 7c).

### Diel periodicity

In the warm chamber, the proportion of ‘probing’ *H. halys* steadily increased from the start of photophase to the peak of 0.44 within the hours of 16:00 and 17:00 (Figure 8a). The number of ‘probes’ declined as scotophase approached, and the lowest proportion of adults engaged in ‘probing’ (0.12) occurred during scotophase between the hours of 02:00 and 03:00. In the warm chamber, the proportion of adults that ‘probed’ during photophase was 0.94, and was significantly higher than the proportion that ‘probed’ during scotophase (0.43; Figure 8a).

In the warm chamber, a significantly higher proportion of the insects ‘probed’ during photophase (0.95) compared to scotophase (0.43; binomial test; $P<0.001$; Figure 9a). Similarly, in the cold chamber, the peak proportion of ‘probing’ *H.
halys (0.36) was recorded within the hours of 18:00 and 19:00 (Figure 8b). The observed maximum proportion of H. halys ‘probing’ was 0.45, which occurred between the hours of 16:00 and 17:00. Nonetheless, the lowest predicted observed proportion of ‘probing’ insects (0.14) occurred during scotophase between the hours of 00:00 and 01:00. The proportion of insects ‘probing’ during photophase (0.89) was again significantly higher than the proportion feeding (0.49) during scotophase (binomial test; \( P < 0.001 \); Figure 9b).

The mean number of ‘probes’ per insect that occurred within the 2 hr increments followed a similar pattern to the proportion of ‘probing’ insects in the warm and cool chambers. In the warm chamber, the frequency of ‘probing’ increased steadily after photophase began and reached a maximum (\( \bar{x} = 0.58 \) ‘probes’ per insect) slightly later between 18:00 and 19:00. Again, ‘probing’ frequency decreased after scotophase began, reached a minimum (\( \bar{x} = 0.11 \)) during the hours 02:00 and 03:00. In the cool chamber, ‘probing’ events per insect increased steadily over the day to its peak (\( \bar{x} = 2.26 \)) between the scotophase hours of 22:00 and 23:00. The observed low (\( \bar{x} = 0.38 \)) occurred between the hours 02:00 and 03:00, but the predicted low (\( \bar{x} = 0.78 \)) occurred between 04:00 and 05:00. On average, H. halys probed more frequently during photophase compared with scotophase in both the warm (\( t = 4.78; \ d.f. = 327; \ P < 0.001 \)) and cool chambers (\( t = 3.84; \ d.f. = 376; \ P < 0.001 \); Figure 9c–d).

In contrast to periodicity patterns discussed above, mean ‘probe’ duration peaked during scotophase in both warm and cool chambers (Figure 8e–f). In the warm chamber, the max observed mean (\( \bar{x} = 60.90 \) min) occurred during...
scotophase in the hours 00:00 and 01:00, and then mean ‘probe’ duration declined and remained relatively flat during the photophase hours (Figure 8e). A very similar pattern was found in ‘probe’ duration data from the cool chamber, but the magnitudes of the means were numerically lower (Figure 8f). Despite the elevated mean ‘probe’ duration between the hours of 00:00 and 04:00, there were also some low mean ‘probe’ durations during scotophase; consequently, there wasn’t a statistical difference in mean ‘probe’ durations between scotophase and photophase for the data from the warm ($t = 1.14; d.f. = 55; P = 0.259$) or the cool ($t = 0.21; d.f. = 103; P = 0.836$) incubator (Figure 9e–f).

Discussion

In this study we demonstrated the application of a simple EMIF system for assessing ‘probing’ activity, of large piercing-sucking hemipterans such as Pentatomidae. There have been several EMIF systems that exploit feeding through an electrified grid into a food item to complete an electrical circuit as a method to monitor feeding behavior of piercing-sucking insects [35,47–49]. Our design is specialized for working with cut fruits and vegetables, and besides green beans, apple slices have been used with success (NGW, unpublished). This versatility will allow for additional studies on the influence of different food sources. Other potential use of this EMIF system is for bioassays to evaluate effects of feeding stimulants, deterrents, or insecticide treatments on ‘probing’ activity. The EMIF
system is compact and can be used to monitor activity in small environmental chambers. Because the purpose of our system is to simply monitor presence or absence of proboscis contact with the food item, and not to capture high-resolution waveform data like EPG, the electromagnetic interference present in environmental control chambers is not a concern. Instead, our system allows laboratory studies to examine how temperature and photoperiod affect ‘probing’
behavior, and to efficiently replicate data for many insects. Another benefit of USB data acquisition devices is their channel capacity and relatively low cost compared to EPG systems. Although we utilized only 15 analog input channels in this study, there is potential to add many more channels by integrating more devices, or by using devices that have 80 or more analog input channels (e.g., NI USB-6255; National Instruments Corporation). This high capacity for replication is feasible because our system requires minimal handling of insects and no operator attention during the trial.

Shearer and Jones (1996) provided the precedent for our current study. They used a horizontal orientation of the feeding cup in their study, but here we have modified the orientation of the cup to a vertical axis. As they noted, it is important that excretions produced by the insects fall away from the screen to avoid false positives. The vertical orientation of the feeding station cup in our system is based on a rearing protocol for N. viridula where food is placed over netting covering the tops of buckets containing stink bugs [55]. The vertical cup allowed H. halys a choice between feeding or basking at the top of the cup, or seeking refuge in the bottom of the cup. Observation of H. halys feeding in natura suggest that underside of leaves and fruits and vegetables are often utilized, and it is clear that this insect often feeds in an upside-down orientation. Use of articulated arms for the electrodes allowed us to adjust positions of individual beans at each station. Subtle variation in bean morphology necessitates that the electrodes can be positioned individually to increase surface area of the bean that is available to the insect. We also reduced the circuitry by utilizing a common +VDC source by charging one large copper screen per table instead of 8 individual screens. By having this common voltage source and all of the stations beneath, we were able to substantially reduce the size of the instrument.

When examining the data distributions for the duration of ‘probing’, as well as the number of ‘probing’ events, the insect is often briefly touching the food with its mouthparts, but clearly there is not enough time for penetration or salivation. This leads to distributions being heavily skewed because of many recorded ‘probes’ of short duration, often lasting only one second. It is likely that the short ‘probes’ are actually not probes by the strict definition that the food is actually penetrated by the stylets [41], but rather represent “labial dabbing” a behavior that is characterized by the exploration phase of the feeding behavior sequence of Auchenorrhyncha, Sternorrhyncha, and Heteroptera [56]. Labial dabbing can be described as repeated touching of the food with the labial tip, and may be accompanied by a drop of sheath saliva from the stylets. Hemipteran mouthparts are heavily endowed with external sensilla that take a hair or peg-like, or multi-lobe form [44, 57]. These structures play a role in the sensory aspects of feeding, and labial sensilla may be particularly important for assessing food source acceptability during this exploring phase.

Assuming acceptability of the food after the exploratory phase, test ‘probing’ (wherein the stylets are inserted) is likely to follow [56]. Test ‘probing’ may end abruptly or proceed into exploratory feeding and excretion of watery saliva for digestion and finally ingestion. These latter behaviors are expected to be longer in
duration. Hemipterans are capable of salivating and ingesting multiple times without removing mouthparts by repositioning the tip of the stylets [58]. The salivary sheath is constructed from hardened protein-rich saliva that forms around the stylets, which may aid in sealing the wound against leakage, or lubricating or supporting stylets [8, 56, 44, 59, 60]. Counting of salivary deposits on the surface of a food item is often used as a method to gauge feeding activity of stink bugs [34, 61, 62]. However, it is difficult to distinguish whether the stylets actually penetrated the substrate or if ingestion took place from the presence of sheath saliva. This is because sheath saliva hardens as it oxidizes [56], and can transfer to the food during the exploratory phase of feeding, leaving what appear to be salivary sheaths that are not accompanied by a puncture [63].

While it is clear that a fair amount of labial dabbing and test ‘probing’ would occur in nature prior to salivation and ingestion, there is also potential that our experimental conditions led to increased ‘probes’ of short duration. One condition that may have led to an increased number of short stylet contacts was the use of field-collected insects. These insects were collected from a variety of hosts but never from green beans. Thus, not only were they unfamiliar with this standard stink bug laboratory food, but they were collected at various levels of satiation, nutritional status, and age. A second potential condition leading to short ‘probes’ was that the insects may be challenged in negotiating the screen to find a suitable feeding position. A third condition that may have led to short ‘probes’ is the execution of these experiments under variable and dynamic environmental conditions, including temperatures that were not conducive to feeding. This is supported by the result that cooler temperatures caused shorter duration ‘probes’. It is also important to recognize that the high number of short ‘probes’ may also be related to suboptimal ratio of voltage to input impedance. It is known that very small currents can have large behavioral implications in EPG [64].

The longer extremes in the mean ‘probe’ duration recorded for H. halys (up to 184 minutes) probably reflect establishment of salivary sheaths and perhaps multiple salivary deposits. Depieri and Panizzi (2011) examined ‘probing’ duration in four pentatomid species and found that mean ‘probe’ duration ranged from 71–133 min. However, their experiments were performed on starved laboratory insects under warm temperatures (25˚C) and only ‘probing’ with vertical head movements was recorded observationally. This suggests that exploratory labial dabbing was not a behavior that captured in that study. This may help explains why our mean ‘probe’ durations were lower. The few other studies on ‘probe’ duration of pentatomids have noted mean ‘probing’ durations lasting from 70–90 minutes, although these experiments are typically based on periodic observational data and again, do not include labial dabbing behavior [34, 65]. A problem with periodic visual observational data is observing the brief dabbing events, and the difficulty in distinguishing discrete ‘probes’. Clearly, these problems can lead to overestimation of probe durations. The amount of damage on soybean from stink bug has been correlated with feeding time [31], presumably because longer feeding correlates with salivation and digestion of plant material. Future studies should characterize and link EMIF activity data with crop damage.
and formation or lack of salivary sheaths, as this could help with diagnosis of
damage resulting from *H. halys*. Behavioral studies, waveform characterization
from EPG data, and histological studies on the food item will be necessary to
quantify and distinguish different feeding behaviors of *H. halys*. Diverse feeding
strategies including stylet sheath feeding, cell-rupture feeding or “lacerate-and-
flush” are used by pentatomids depending on the type of plant tissue that is
targeted [5,43,44,56]. The type of basic EMIF data used in this study has very
limited capacity to distinguish specific feeding strategies used by *H. halys*, and
EPG will have an important role to play for determining specific feeding strategies
of this pest.

The effect of environment on feeding behavior has not been widely examined in
in EPG or EMIF studies, partly because it is difficult to adapt EPG systems to
controlled environment chambers as experiments must be conducted in a Faraday
cage. However, at least one study has performed EPG outdoors [60]. Similar to
that study, we found temperature had important effects on *H. halys* ‘probing’
behavior. In the cooler environmental chamber, *H. halys* probed more frequently,
representing more labial dabbing than the insects that were kept in the warmer
environmental chamber. Because they were so active, the disparities observed in
mean ‘probing’ durations may be linked to a temperature-dependent physiologi-
cal component of feeding, rather than a metabolic limitation from temperature.
The higher frequency of ‘probing’ and the shorter mean ‘probe’ duration balanced
out under cool temperatures and resulted in similar total ‘probing’ time per day
between the two temperature treatments. However, the current study cannot
address whether ‘probing’ duration reflects nutritional value for *H. halys*.

Hemipteran watery saliva, which is secreted into the feeding site, contains a
cocktail of digestive enzymes, including amylase, proteinases, and pectinase,
which dissolve cell walls and contents [8,56,66–68]. The resulting slurry is then
imbibed by the insect, and digestion continues in the gut. However, enzymatic
reactions are temperature dependent, and are more efficient at warmer
temperatures [69,70]. Thus, the amount of nutrients acquired could be less for
the cooler insects, and potentially left the insects unsatisfied. This might help
explain why our insects probed more frequently in the cooler chamber.

It is clear from our study that temperature was a key variable in explaining
when individual stinkbugs tended to actively ‘probe’. Interestingly, the lower
threshold for ‘probing’ activity is far below the minimum temperature threshold
for development, which is located between 12 and 15˚C [71–73]. Thus, ‘probing’
by *H. halys* may occur even when development may not. Although developmental
temperature thresholds apply primarily to molting in immature insects, in adult
insects they may relate to sexual maturation or other processes. Our data predict
that the optimal ‘probing’ temperature range is just above the minimum
temperature threshold for development. The predicted upper temperature
threshold for ‘probing’ may appear surprisingly low, as *H. halys* can be observed
feeding on very hot days. However, it must be noted that temperatures
experienced by *H. halys* feeding within a canopy can potentially be much cooler
than air temperatures due to interaction of factors such as evapotranspiration,
radiation absorption, and other processes [74]. Furthermore, *H. halys* is not an extraordinarily heat-tolerant insect. The upper threshold for development of *H. halys* has been determined to be 35.76 and 36.5 °C [71, 72], which is at or above the upper ‘probing’ threshold predicted by our data.

There were phenological differences in feeding activity between the different months when the assays were conducted. These differences may have reflected different environmental conditions, and they also coincide with what is known about the phenology of *H. halys* in temperate regions of the northern hemisphere [71, 75, 76]. In the spring months, *H. halys* adults emerge from overwintering sites where they have aggregated during winter. They then move to host plants and feed for the first time in months before beginning sexual maturation. Coinciding with this period are some of the longest photoperiods and warmest temperatures of the season. The combination of nutritional needs, warm temperatures, and a high degree of phenological synchrony among *H. halys* at this time could be the reason that there was higher feeding activity in May and June than in other months. In Oregon, there is potential for two generations of *H. halys* per year (Shearer and Wiman, unpublished), and after mid-summer the population collected in beat samples collected from host plants is comprised of a mix of residual overwintered adults, summer adults, and nymphs. In the fall, the stink bug largely cease feeding, and begin to move to winter aggregation sites as they enter diapause. Our data seemed to reflect this phenology, as the lowest adult feeding activity was found in August and September when winter aggregation behavior is occurring. Future studies will examine feeding by nymphs during the latter summer months, as they may potentially be a major source for late-season crop damage.

Using an EMIF system similar to the present study, Shearer and Jones (1996) found that *N. viridula* had the longest ‘probing’ periods during scotophase [35]. Similarly, Krupke et al. (2006) observed that the pentatomid *Euschistus conspersus* Uhler was more frequently observed feeding during scotophase [77]. While we did find that a substantial amount of feeding activity of *H. halys* occurred during scotophase, including some of the longest mean ‘probe’ durations, this activity was lower or no different from the ‘probing’ activity levels during photophase over all. Our data suggest that feeding periodicity of Pentatomidae could be more dependent on temperature than on photoperiod. Shearer and Jones (1996) conducted their study using laboratory insects under constant temperature (21 °C), while the Krupke et al. study had natural photoperiod and temperature with field-collected insects in the greenhouse, but observations were only conducted during warm months (late August and early September). The Willamette Valley OR, has relatively cool nights and such fluctuations of temperatures were reflected by our controlled temperature cabinet simulations as it occurs prior to dawn (photophase). We believe that field collected-insects were therefore preconditioned to very similar daily temperature fluctuations. Mean ‘probe’ duration during scotophase declined in our study as temperatures dropped in approach to the daily low. On the few summer nights where temperatures do not drop in the Willamette Valley, and in warmer climates, ‘probing’ duration would likely remain high throughout scotophase. However, the
relative importance of temperature and photoperiod as drivers of feeding behavior needs additional investigation.

There are several factors that determine crop damage from stink bugs including density of the bugs and feeding intensity. Our study focused only on the latter and we found that ‘probing’ activity of H. halys adults was highest in the early to mid-season. This is a time when many crops affected by H. halys are still developing, and fruit crops may be particularly susceptible to damage such as cat facing that becomes apparent at harvest. Some of the most severe crop damage usually begins to occur in the late season when stink bug densities are relatively higher and there is mass immigration into crops \cite{20,78}. However, ‘probing’ activity of adult H. halys is predicted to be lower at these times, and thus the insect is less damaging on a per-capita basis. Pentatomid nymphs may have increased feeding activity compared with adults \cite{34} and nymphs are abundant in the late season. Thus, it will be important in future studies to evaluate feeding behavior of H. halys nymphs in order to determine their relative potential to cause damage.

**Acknowledgments**

Laboratory and field assistance from Zoë Milburn and Bryan Smith was much appreciated for this project. The critical comments of two anonymous reviewers helped us substantially improve the manuscript.

**Author Contributions**

Conceived and designed the experiments: NW VW PS. Performed the experiments: NW. Analyzed the data: NW. Contributed reagents/materials/analysis tools: NW. Contributed to the writing of the manuscript: NW VW PS SR. Designed and built the EMIF system: NW. Wrote the data acquisition and analysis programs: NW. Wrote the initial draft: NW.

**References**

1. Panizzi A, Mcpherson J, James D, Javahery M, Mcpherson R (2000) Stink bugs (Pentatomidae). Schaefer CW, Panizzi AR, editors CRC Press, New York.

2. McPherson JE, McPherson R (2000) Stink bugs of economic importance in America north of Mexico. CRC Press, New York.

3. Esquivel JF, Medrano EG, Bell AA (2010) Southern green stink bugs (Hemiptera: Pentatomidae) as vectors of pathogens affecting cotton bolls – A brief review. Southwest Entomol 35: 457–461. doi:10.3958/059.035.0332.

4. Medrano EG, Esquivel J, Bell A, Greene J, Roberts P, et al. (2009) Potential for Nezara viridula (Hemiptera: Pentatomidae) to transmit bacterial and fungal pathogens into cotton bolls. Curr Microbiol 59: 405–412. doi:10.1007/s00284-009-9452-5.

5. Mitchell PL (2004) Heteroptera as vectors of plant pathogens. Neotrop Entomol 33: 519–545. doi:10.1590/S1519-566x2004000500001.

6. Hori K (2000) Possible causes of disease symptoms resulting from the feeding of phytophagous heteroptera. In: Schaefer C, Panizzi AR, editors. Heteroptera of Economic Importance. CRC Press. pp.
10–35. Available: http://www.crcnetbase.com/doi/abs/10.1201/9781420041859.ch2. Accessed 18 February 2014.

7. Miles PW (1969) Interaction of plant phenols and salivary phenolases in the relationship between plants and Hemiptera. Entomol Exp Appl 12: 736–744. doi:10.1111/j.1570-7458.1969.tb02567.x.

8. Peiffer M, Felton GW (2014) Insights into the saliva of the brown marmorated stink bug, Halyomorpha halys (Hemiptera: Pentatomidae). PLoS ONE 9: e89483. doi:10.1371/journal.pone.0089483.

9. Woodside AM (1950) Cat-facing and dimpling of peaches. Bull Va Agric Exp Stn 435: 18 pp.

10. Medrano EG, Esquivel JF, Bell AA, Greene JK, Roberts PM, et al. (2011) Analysis of microscopic injuries caused by southern green stink bug (Hemiptera: Pentatomidae) feeding on cotton bolls. Southwest Entomol 36: 233–245.

11. Apriyanto D, Sedlacek JD, Townsend LH (1989) Feeding activity of Euschistus servus and E. variolarius (Heteroptera: Pentatomidae) and damage to an early growth stage of corn. J Kans Entomol Soc 62: 392–399.

12. Hedstrom C (2014) The effects of kernel feeding by brown marmorated stink bug, Halyomorpha halys (Hemiptera: Pentatomidae) on commercial hazelnuts, Corylus avellana [MS Thesis]. Corvallis, OR: Oregon State University.

13. Boethel DJ, Russin JS, Wier AT, Layton MB, Mink JS, et al. (2000) Delayed maturity associated with southern green stink bug (Heteroptera: Pentatomidae) injury at various soybean phenological stages. J Econ Entomol 93: 707–712.

14. Lye B-H, Story RN, Wright VL (1988) Southern green stink bug (Hemiptera: Pentatomidae) damage to fresh market tomatoes. J Econ Entomol 81: 189–194.

15. CABI (2014) Halyomorpha halys (brown marmorated stink bug). Invasive Species Compendium. Wallingford, UK: CAB International. Available: www.cabi.org/isc.

16. Lee D-H, Short BD, Joseph SV, Bergh JC, Leskey TC (2013) Review of the biology, ecology, and management of Halyomorpha halys; (Hemiptera: Pentatomidae) in China, Japan, and the Republic of Korea. Environ Entomol 42: 627–641. doi:10.1603/EN13006.

17. Zhu G, Bu W, Gao Y, Liu G (2012) Potential Geographic Distribution of Brown Marmorated Stink Bug Invasion (Halyomorpha halys). PloS ONE 7: e31246.

18. Inkley DB (2012) Characteristics of home invasion by the brown marmorated stink bug (Hemiptera: Pentatomidae). J Entomol Sci 47: 125–130.

19. Watanabe M, Arakawa R, Shinagawa Y, Okazawa T (1994) Anti-invading methods against the brown marmorated stink bug, Halyomorpha mista, in houses. Jpn J Sanit Zool 45: 311–317.

20. Leskey TC, Short BD, Butler BR, Wright SE (2012) Impact of the Invasive Brown Marmorated Stink Bug, Halyomorpha halys (Stål), in Mid-Atlantic Tree Fruit Orchards in the United States: Case Studies of Commercial Management. Psyche (Stuttg) 2012: 14 pp.

21. Leskey TC, Hamilton GC, Nielsen AL, Polk DF, Rodriguez-Saona C, et al. (2012) Pest Status of the Brown Marmorated Stink Bug, Halyomorpha halys (Stål) in the USA. Outlook Pest Manage 23: 218–226. doi:10.1564/23oct07.

22. Shearer PW, Wiman NG (2014) Brown marmorated stink bug - a pending threat to Pacific Northwest agriculture. In: Hollingsworth C, editor. Pacific Northwest Insect Management Handbook [online]. Oregon State University, Corvallis OR. Available: http://pnwhandbooks.org.

23. Todd JW (1989) Ecology and behavior of Nezara Viridula. Annu Rev Entomol 34: 273–292. doi:10.1146/annurev.en.34.010189.001421.

24. Ruberson JR, Takasu K, Buntin GD, Eger JE Jr, Gardner WA, et al. (2013) From Asian curiosity to eruptive American pest: Megacopta cribraria (Hemiptera: Plataspidae) and prospects for its biological control. Appl Entomol Zool 48: 3–13. doi:10.1007/s13355-012-0146-2.

25. Suiter DR, Eger JE, Gardner WA, Kemerait RC, All JN, et al. (2010) Discovery and Distribution of Megacopta cribraria (Hemiptera: Heteroptera: Plataspidae) in Northeast Georgia. J Integr Pest Manag 1: F1–F4. doi:10.1603/IPM10009.

26. Huang T-I, Reed DA, Perring TM, Palumbo JC (2014) Feeding damage by Bagrada hilaris (Hemiptera: Pentatomidae) and impact on growth and chlorophyll content of Brassicaceous plant species. Arthropod-Plant Interact 8: 89–100. doi:10.1007/s11829-014-9289-0.
27. Huang T-I, Reed DA, Perring TM, Palumbo JC (2013) Diel Activity and Behavior of Bagrada hilaris (Hemiptera: Pentatomidae) on Desert Cole Crops. J Econ Entomol 106: 1726–1738. doi:10.1603/EC13048.

28. Bundy CS, Grasswitz TR, Sutherland C (2012) First report of the invasive stink bug Bagrada hilaris (Burmeister) (Heteroptera: Pentatomidae) from New Mexico, with notes on its biology. Southwest Entomol 37: 411–414. doi:10.3988/059.037.0317.

29. Palumbo JC, Natwick ET (2010) The Bagrada Bug (Hemiptera: Pentatomidae): A New Invasive Pest of Cole Crops in Arizona and California. Plant Health Prog. Available: http://www.plantmanagementnetwork.org/pub/php/brief/2010/bagrada/. Accessed 24 October 2014.

30. Corpuz LR (1969) The biology, host range, and natural enemies of Nezara viridula L. (Pentatomidae: Hemiptera). Phillip Entomol 1: 225–231.

31. Deperi RA, Panizzi AR (2011) Duration of feeding and superficial and in-depth damage to soybean seed by selected species of stink bugs (Heteroptera: Pentatomidae). Neotrop Entomol 40: 197–203. doi:10.1590/S1519-566x2011000200007.

32. Kester KM, Smith CM (1984) Effects of diet on growth, fecundity and duration of tethered flight of Nezara viridula. Entomol Exp Appl 35: 75–81. doi:10.1111/j.1570-7458.1984.tb03363.x.

33. Lockwood JA, Story RN (1986) The diurnal ethology of the southern green stink bug, Nezara viridula (L.), in cowpeas. J Ga Entomol Soc 21: 175–184.

34. Simmons AM, Yeargan KV (1988) Feeding frequency and feeding duration of the green stink bug (Hemiptera: Pentatomidae) on Soybean. J Econ Entomol 81: 812–815.

35. Shearer PW, Jones VP (1996) Diel feeding pattern of adult female southern green stink bug (Hemiptera: Pentatomidae). Env Entomol 25: 599–602.

36. McLean DL, Kinsey MG (1964) A technique for electronically recording aphid feeding and salivation. Nature 202: 1358–1359. doi:10.1038/2021358a0.

37. Walker GP, Backus EA (2000) Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior: proceedings of the symposium at the XIX international congress of entomology, Beijing, China. [S.I.]: Entomological Society of America.

38. Tjallingii WF (1978) Electronic recording of penetration behaviour by aphids. Entomol Exp Appl 24: 721–730. doi:10.1111/j.1570-7458.1978.tb02836.x.

39. Tjallingii WF (1988) Electrical recording of stylet penetration activities. In: Minks AK., Harrewijn P, editors. Aphids. Their biology, natural enemies and control. Amsterdam: Elsevier Science. pp. 95–108.

40. Backus EA, Bennett WH (2009) The AC–DC correlation monitor: new EPG design with flexible input resistors to detect both R and emf components for any piercing–sucking hemipteran. J Insect Physiol 55: 869–884. doi:10.1016/j.jinsphys.2009.05.007.

41. Backus EA (2000) Our own jabberwocky: clarifying the terminology of certain piercing-sucking behaviors of homopterans. In: Walker GP, Backus EA, editors. Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior. Lanham, MD: Thomas Say Publications in Entomology, Entomological Society of America. pp. 1–13.

42. Backus EA, Serrano MS, Ranger CM (2005) Mechanisms of Hopperburn: An Overview of Insect Taxonomy, Behavior, and Physiology*. Annu Rev Entomol 50: 125–151. doi:10.1146/annurev.ento.49.061802.123310.

43. Cobben RH (1978) Evolutionary trends in Heteroptera. II. Mouthpart structures and feeding strategies. Mededelingen Landbouwhogeschool Wageningen. Available: http://agris.fao.org/agris-search/search.do?recordID=US201301285130. Accessed 24 October 2014.

44. Backus EA (1998) Sensory systems and behaviours which mediate hemipteran plant-feeding: A taxonomic overview. J Insect Physiol 34: 151–165. doi:10.1016/S0022-1910(88)90045-5.

45. Martinson HM, Raupp MJ, Shrewsbury PM (2013) Invasive stink bug wounds trees, liberates sugars, and facilitates native Hymenoptera. Ann Entomol Soc Am 106: 47–52.

46. Panizzi AR (1997) Wild hosts of pentatomids: ecological significance and role in their pest status on crops. Annu Rev Entomol 42: 99–122. doi:10.1146/annurev.ento.42.1.99.

47. Kashin P, Wakeley HG (1965) An insect “bitometer.” Nature 208: 462–464. doi:10.1038/208462a0.
48. Kashin P (1966) Electronic recording of the mosquito bite. J Insect Physiol 12: 281–286. doi:10.1016/0022-1910(66)90143-0.

49. Sevacherian V (1975) Activity and probing behavior of *Lygus hesperus* in the laboratory. Ann Entomol Soc Am 68: 557–558.

50. National Instruments (2009) DAQ M series NI USB-621x user manual bus powered M series USB devices. Publ 371931F-01. Available: http://www.ni.com/pdf/manuals/371931f.pdf. Accessed 2014 Jul 23.

51. National Instruments (2009) NI USB-621x Specifications. Publ 371932F-01. Available: http://www.ni.com/pdf/manuals/371932f.pdf. Accessed 2014 Jul 22.

52. Wiman NG, Walton VM, Shearer PW, Rondon SI, Lee JC (2014) Factors affecting flight capacity of brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Pentatomidae). J Pest Sci. doi:DOI:10.1007/s10340-014-0582-6.

53. R Core Team (2013) R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Available: http://www.R-project.org/.

54. Ryan JA, Ulrich JM (2012) xts: eXtensible Time Series. Available: http://CRAN.R-project.org/package=xts.

55. Williams L, Rodriguez-Saona C, Paré PW, Crafts-Brandner SJ (2005) The piercing-sucking herbivores *Lygus hesperus* and *Nezara viridula* induce volatile emissions in plants. Arch Insect Biochem Physiol 58: 84–96. doi:10.1002/arch.20035.

56. Backus EA (1985) Anatomical and sensory mechanisms of leafhopper and planthopper feeding behavior. In: Nault L, Rodriguez JG, editors. The leafhoppers and planthoppers. John Wiley and Sons. pp. 163–194.

57. Leopold RA, Freeman TP, Buckner JS, Nelson DR (2003) Mouthpart morphology and stylole penetration of host plants by the glassy-winged sharpshooter, *Homalodisca coagulata*, (Homoptera: Cicadellidae). Arthropod Struct Dev 32: 189–199. doi:10.1016/S1467-8039(03)00047-1.

58. Backus EA, Habibi J, Yan F, Ellersiek M (2005) Stylole penetration by adult *Homalodisca coagulata* on grape: electrical penetration graph waveform characterization, tissue correlation, and possible implications for transmission of *Xylella fastidiosa*. Ann Entomol Soc Am 98: 787–813. doi:10.1603/0013-8746(2005)098[0787:SPBAHC]2.0.CO;2.

59. Chapman RF (1998) The insects: structure and function. Cambridge university press.

60. Son Y, Backus EA, Groves RL, Johnson MW (2012) Pattern of stylole penetration activity by *Homalodisca vitripennis* (Hemiptera: Cicadellidae) adults in relation to environmental temperature and light conditions. Environ Entomol 41: 1215–1230. doi:10.1603/EN11319.

61. Bowling CC (1979) The stylole sheath as an indicator of feeding activity of the rice stink bug. J Econ Entomol 72: 259–260.

62. Bowling CC (1980) The stylole sheath as an indicator of feeding activity by the southern green stink bug on soybeans. J Econ Entomol 73: 1–3.

63. Hollay ME, Smith CM, Robinson JF (1987) Structure and formation of feeding sheaths of rice stink bug (Heteroptera: Pentatomidae) on rice grains and their association with fungi. Ann Entomol Soc Am 80: 212–216.

64. Cervantes F, Backus EA (2014) Changes in behavioral responses of *Lygus lineolaris* (Hemiptera: Miridae) from various applied signal voltages during EPG recordings. Proc Hemipteran-Plant Interact Symp: 40.

65. Panizzi AR (1995) Feeding frequency, duration and preference of the southern green stink bug (Heteroptera: Pentatomidae) as affected by stage of development, age, and physiological condition. Ann Soc Entomol Bras 24: 437–444.

66. Madhusudhan VV, Taylor GS, Miles PW (1994) The detection of salivary enzymes of phytophagous Hemiptera: a compilation of methods. Ann Appl Biol 124: 405–412. doi:10.1111/j.1744-7348.1994.tb04146.x.

67. Baptist BA (1941) The morphology and physiology of the salivary glands of Hemiptera-Heteroptera. Q J Microsc Sci s2–83: 91–139.

68. Hori K (1975) Digestive carbohydrases in the salivary gland and midgut of several phytophagous bugs. Comp Biochem Physiol Part B Comp Biochem 50: 145–151. doi:10.1016/0305-0491(75)90314-4.
69. Chesley LC (1934) The influence of temperature upon the amylases of cold- and warm-blooded animals. Biol Bull 66: 330–338.

70. Cohen AC (1993) Organization of digestion and preliminary characterization of salivary trypsin-like enzymes in a predaceous heteropteran, Zelus renardii. J Insect Physiol 39: 823–829. doi:10.1016/0022-1910(93)90114-7.

71. Haye T, Abdallah S, Gariepy T, Wyniger D (2014) Phenology, life table analysis and temperature requirements of the invasive brown marmorated stink bug, Halyomorpha halys, in Europe. J Pest Sci 87: 1–12. doi:10.1007/s10340-014-0560-z.

72. Nielsen AL, Hamilton GC, Matadha D (2008) Developmental rate estimation and life table analysis for Halyomorpha halys (Hemiptera: Pentatomidae). Env Entomol 37: 348–355.

73. Coop L (n.d.) IPPC synthesis analysis, brown marmorated stink bug degree-day model. Integrated Plant Protection Center. Available: http://uspest.org/wea/model_BMSB_v1.pdf. Accessed 20 March 2014.

74. Jones HG (2013) Plants and microclimate: A quantitative approach to environmental plant physiology. New York: Cambridge University Press. 427 p.

75. Nielsen AL, Hamilton GC (2009) Seasonal occurrence and impact of Halyomorpha halys (Hemiptera: Pentatomidae) in tree fruit. J Econ Entomol 102: 1133–1140.

76. Nielsen AL, Hamilton GC, Shearer PW (2011) Seasonal phenology and monitoring of the non-native Halyomorpha halys (Hemiptera: Pentatomidae) in soybean. Environ Entomol 40: 231–238. doi:10.1603/EN10187.

77. Krupke CH, Jones VP, Brunner JF (2006) Diel Periodicity of Euschistus conspersus (Heteroptera: Pentatomidae) Aggregation, Mating, and Feeding. Ann Entomol Soc Am 99: 169–174. doi:10.1603/0013-8746(2006)99[0169:DPOECH][2.0.CO;2].

78. Brown MW (2003) Characterization of stink bug (Heteroptera: Pentatomidae) damage to mid- and late-season apples. J Agric Urban Entomol 20: 193–202.