



Evaluation of preference of brown marmorated stink bug, *Halyomorpha halys* (Stål) for different colour bell peppers and the role of plant protein

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Abstract

The brown marmorated stink bug (BMSB), *Halyomorpha halys*, (Stål) (Hemiptera: Pentatomidae) a highly polyphagous invasive pest from Asia, has become a major agricultural pest in the United States. Laboratory experiments were conducted to determine nymph and adult BMSB preference for bell peppers of different colours (red, yellow, green, and orange). Peppers were presented to BMSB using a Dual Choice Arena Test (DCAT) with and without a standard (cowpea pod). The experiments were video recorded using the Noldus ObserverXT video system. A 4-arm olfactometer was used to determine plant volatile involvement in observed preferences. The Pierce Bicinchoninic Acid Protein assay was used to measure total protein concentration of the peppers. Results on preference measured by a Preference Index (PI, range 0–2), showed that nymphs and adults preferred peppers ($PI \geq 1.1$) over cowpea pod. In a pairwise DCAT with all possible combinations, orange-coloured pepper was preferred over all the other peppers. Olfactometer test indicated that *H. halys* were more attracted to red and yellow peppers. Orange peppers showed the highest protein concentration whilst green peppers showed the least protein concentration.

Keywords *Halyomorpha halys* · BMSB · Peppers · Plant protein · Olfactometer · Preference

Introduction

Halyomorpha halys, (Stål) (Hemiptera: Pentatomidae) an invasive pest from Asia, was first reported in Allentown, Pennsylvania, United States, in 1996 (Hoebeke and Carter 2003; Leskey et al. 2012). Since then, it has spread steadily in the Mid-Atlantic region of the United States causing significant economic damage. This pest is highly polyphagous and has been reported to feed on over 100 host plants (Nielsen et al. 2008). Its host plants include field crops such as soybean (*Glycine max*), corn (*Zea mays*), cotton (*Gossypium hirsutum*), tree fruits such as pear (*Pyrus* spp.), apple

(*Malus pumila*), peach (*Prunus persica*), and ornamentals such as catalpa (*Catalpa* spp.), tree of heaven (*Ailanthus altissima*), princess tree (*Paulownia tomentosa*), and vegetable crops including okra (*Abelmoschus esculentus*), eggplant (*Solanum melongena*), tomato (*Solanum lycopersicum*), lima beans (*Phaseolus lunatus*) and pepper (*Capsicum annuum*) (Rice et al. 2014) and cowpea (Ewunkem et al. 2014). Multimillion-dollar losses were recorded in 2010 as a result of damage caused by the pest's feeding activity on orchard crops (Leskey et al. 2012). In 2010 alone, *H. halys* caused over \$37 million in damage to the mid-Atlantic apple production (United States Apple Association [USAA] 2011).

The brown marmorated stink bug currently poses a significant threat to the United States farmers. Based on climatic data, Zhu et al. (2012) predicted that *H. halys* would spread throughout the United States, implying that many more important crops, including bell peppers, will be at risk of BMSB damage. Bell pepper, *Capsicum annuum*, is a specialty crop and one of the most consumed vegetables in the world due to its nutritional value, flavor and colour (Omondi 2018). Bell peppers come in several colours, with green, yellow and red being predominant in market outlets

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and green the most produced and consumed (Frank et al. 2001). The different coloured peppers all start as green and change colour as they mature or ripen from green to yellow to orange and finally to a red colour (Roth 2010). However, there are some bell peppers that remain green even when ripened (Smith 1950; Shifriss and Pilovsky 1992; CDC 2002). Bell peppers differ from most other members of the genus *Capsicum* in that they generally do not have the “hot” taste associated with the Chili and Tabasco types (van Ruth and Roozen 1994). In addition, differences exist in the nutrient composition of bell peppers that are related to the colour of the peppers (Zhang and Hamazu 2003; Sun et al. 2007; Roth 2010). For example, carotenoids and flavonoids are important compounds associated with orange and red-coloured peppers (Arimboor et al. 2014); the green colour is attributed to chlorophyll (Marin et al. 2004) and the yellow-orange colour formed by the presence of α - and β -carotene, zeaxanthin, lutein, and β -cryptoxanthin (Howard 2001). The aim of this study was to assess the preference of *H. halys* for different bell pepper colours in relation to their protein content.

Materials and methods

Experimental insects

Adult brown marmorated stink bugs were collected from contiguous integrated pest management (IPM) experimental plots at the North Carolina A&T State University Research Farm in Greensboro, North Carolina (36.0586243° N, 79.7358932° W). One plot was planted with corn (Trucker’s favorite), soybean (Pioneer 95M82), cowpea (Mississippi Silver and Early Scarlet), and sunflower (Zohar F1). A close-by plot had mature princess trees and the third was a research plot planted with several varieties of cowpea. The insects were used to establish a laboratory culture specifically for this study. Insects were placed in 500 ml transparent polypropylene containers with a partial mesh screen lid (BioServ, Frenchtown, New Jersey, United States of America) containing moist cotton balls and transported to the IPM laboratory. The insects were transferred to screen cages (30.5 × 30.5 × 30.5 cm; BioQuip, Rancho Dominguez, California, United States of America) and provided carrots, green beans, tomatoes, grapes, corn, princess tree leaf under ambient laboratory conditions (26.0 ± 2.00C, 70% relative humidity, 16/8 light/day cycle) (Dingha and Jackai 2016). Egg masses were laid by adult females on the top or side of the cage and they were removed every other day and placed on paper towels in small plastic cups (2.5 cm diameter) and transferred into separate round clear plastic containers (11 × 8 cm) in which moistened cotton balls were added to increase humidity. After eggs hatched, first instar

nymphs remained aggregated in clusters until they molted into second instar nymphs which were transferred into larger (18.5 × 8 cm) round clear plastic containers where they were maintained till the fourth or fifth instar. They were then transferred into large bug cages (28 × 28 × 28 cm, Bug Dorm; BioQuip) for the rest of their development. During transfer of nymphs into plastic containers or cages, soft camel hair brush (Crayola® Wash Brush Series 310) or occasionally featherweight forceps were used to carefully handle the insects to avoid injury or death.

Experimental plants

Bell peppers of four different colours (green, yellow, orange and red) and one cowpea variety (Early Scarlet) were used in the preference bioassay. The different colours (green, yellow, orange and red) generally represent different stages of ripening, starting with green and progressing to yellow, orange, and finally red. Mature bell peppers (Redsun greenhouse vegetables tri-colour peppers and green pepper) were purchased from a local grocery store (Wal-Mart supermarket) in Greensboro, NC. The peppers were kept fresh in a refrigerator until ready for use. Early Scarlet (ES) cowpea variety was added to the study because it has been reported to be highly attractive to BMSB (Dingha and Jackai 2014, 2016; Nyaupane et al. 2017). It was used as a standard against which the different peppers were tested. During the summer, ES cowpea pods were harvested from the IPM experimental plot and in the fall and spring they were obtained from cowpea grown in the greenhouse without fertilizer or pesticide. Mature cowpea pods were harvested at the R6 stage (50% of pods with fully developed seeds) with a sharp knife from the pedicel, placed in brown paper bags and transported to the laboratory. All plants used in the tests were cleaned with a solution containing 0.5% bleach to remove any contaminants and air-dried prior to being used. We had established that the bleach solution did not affect feeding by the insect (Dingha and Jackai 2016).

Host preference experiments

Laboratory bioassays were conducted using Dual Choice Arena Test (DCAT) to determine the feeding preference of brown marmorated stink bug among bell peppers of four different colours (green, yellow, orange and red). These do not represent different pepper varieties but are different stages of ripening/maturity, starting as a green-coloured pepper to yellow to orange and finally to a red-coloured pepper. The choice arena was prepared using a glass pyrex® Petri dish (15 cm diameter × 2 cm depth glass) for adult BMSB and a plastic Petri dish (8.2 cm diameter × 1 cm depth) for third instar nymphs. Each arena was divided into 4 equal

quadrants and had a paper towel lining the bottom of the container.

Experiment I: dual-choice arena test (DCAT)

DCAT (with early scarlet (ES) cowpea variety as standard)

Disc portions (2.5 cm diameter) were obtained from the peppers using a metal cork borer, and equal lengths of ES pods (2.5 cm) were also obtained. Two discs of one pepper colour were placed in opposite quadrants and two cowpea pods in alternate quadrants. Four insects (either as adults or as third instar nymphs) were introduced in the center of each arena using camel brush (Crayola® Wash Brush Series 310). Insects were then allowed to freely choose between the two food substrates. The number of insects present on each food substrate was recorded at 30-min intervals for 3 h starting from the release time. There were 4 pepper colour treatments prepared as described above, each with ten replications.

DCAT (no cowpea standard)

In this experiment, there was no cowpea standard included. Instead, one of the peppers served as the standard for comparison. Two discs of each pepper colour were placed in alternate quadrants in the arena. There were 6 possible dual combinations of the four pepper colours, viz; red + yellow, red + green, red + orange, orange + yellow, orange + green, and yellow + green, and in each pair the first named colour served as the standard. As in the previous DCAT, four insects (either as adult or as 3rd instar nymphs) were released into the center of the arena and allowed to choose between the two pepper colours. The number of insects present on each food substrate was recorded at 30-min intervals for 3 h starting from the release time. Each treatment was replicated 10 times.

Experiment II: behavioral analysis—using the Noldus ObserverXT video

Behavioral video analysis test was conducted using the Noldus Observer XT 14.0 video system. The system was setup to record preference of *H. halys* for the different food substrates and observational data gathered was coded using the Noldus video set up. Choice arena bioassays (DCAT) were set up as in the previous experiments. Arenas of the different choice treatments were video recorded for 30 min each, and this was replicated 5 times. The saved video file

was played back, coded and the time each individual insect spent on each of the food substrates observed and recorded in the Noldus Observer XT 14.0 software.

Olfactometer bioassay

A four-arm olfactometer was used to determine the likely role of kairomonal compounds (volatiles) in mediating the observed preference of *H. halys*. The olfactometer (Sigma Scientific, Gainesville, FL) consisted of a central chamber (30 cm long by 30 cm wide by 5 cm high) with four “arms” or openings (7 cm long by 2 cm in diameter) at the four sides and a central opening where air flowing from the arms meet and mix. The openings of the olfactometer were connected through Teflon-glass tube connectors to four pumps on an air delivery system equipped with a vacuum pump. Purified air was drawn at a constant rate of 200 ml/min through each of the four arms and removed by suction via the vacuum pump through the central opening of the olfactometer at the rate of 1000 ml/min.

We weighed approximately 10 g of each pepper colour and placed one pepper colour in each arm of the olfactometer. The purified air supply system was left to run for 5 min to allow for the release of volatiles (odour) from each pepper to be stabilized in the main chamber. Four insects (either as adults or as third instar nymphs) were released at the bottom of the central chamber. The insects were allowed 15 min to make a choice among the four air fields, and those found in each arm were counted and continuous observation and data collection taken every 15 min over a 1-hour period on the number of insects present in each arm. Experiment was replicated 8 times.

Quantification of total protein

The Pierce Bicinchoninic Acid Protein Assay Kit (Thermo-Scientific, Waltham, MA) was used to determine total protein concentration of the four colour bell peppers following the manufacturer’s protocol as described previously (Worku et al. 2016; Karlton-Senaye et al. 2018). A series of dilutions of known concentration of bovine serum albumin, was prepared and used as standard. Twenty-five microliters of samples together with 200 µl of a working reagent (prepared from two reagents contained in the protein assay kit: Reagent A containing sodium carbonate, sodium bicarbonate, bicinchoninic acid and sodium tartrate in 0.1 M sodium hydroxide and Reagent B containing 4% cupric sulfate) was added to each well and mixed thoroughly on a plate shaker. The 96-well plate was covered and incubated at 37 °C for 30 min. The absorbance was measured at 562 nm on a microplate spectrophotometer (BioTek™ Epoch™). A standard curve

was plotted from the readings of the albumin standards to determine their protein concentrations.

Data analysis

Dual choice arena test (DCAT)

The index of comparison in the DCATs is the Preference Index (PI). PI for each color pepper relative to the standard was calculated as $PI = 2T/(T + S)$ (Kogan and Goeden 1970), where T is the number of insects at the test plant and S the number of insects at the standard. Comparisons between peppers were made on mean PI's for the peppers over the duration of the tests. For DCAT with all possible dual combinations of peppers, comparisons between peppers were made on mean PIs for the peppers over the duration of the tests. All DCAT data were analyzed using a one-way ANOVA followed by a Fisher's protected LSD test, $\alpha = 0.05$, for mean separation.

Olfactometer bioassay

In the olfactometer bioassay, data for the distribution of *H. halys* adults and nymphs in the olfactometer arms were analyzed by one-way ANOVA using PROC GLM procedure for repeated measures. Fisher's protected LSD test was used for separation of means at 0.05 level of significance. All analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC).

Total protein content assay

Data were analyzed by one-way ANOVA followed by a Fisher's protected LSD test used for separation of means at 0.05 level of significance for comparison.

Results

DCAT with early scarlet (ES) cowpea as standard

From the DCAT with Early Scarlet cowpea as standard, the Preference Index (PI) for adult BMSB was similar ($F = 1.39$, $df = 3, 20$, $P = 0.2734$) among the different peppers (Fig. 1a). In the case of *H. halys* nymphs' a significant difference was observed ($F = 22.18$, $df = 3, 20$, $P = 0.0001$) among the peppers with yellow pepper recording the lowest PI of 1.15 which was significantly different from the other peppers (Fig. 1b).

DCAT with no standard

For adult *H. halys*, using red bell pepper as the standard against which the other bell peppers were compared, orange bell pepper was highly preferred ($PI = 1.20$) ($F = 5.07$, $df = 2, 15$, $P = 0.0208$) (Table 1). When yellow bell pepper was used as the standard the difference was not significant ($F = 3.47$, $df = 2, 15$, $P = 0.0579$) among the peppers. The PI values for red bell pepper was 1.28, and for orange bell pepper which was preferred just as much as the standard, the $PI = 1.0$; as was the green bell pepper ($PI = 0.75$) (Table 1).

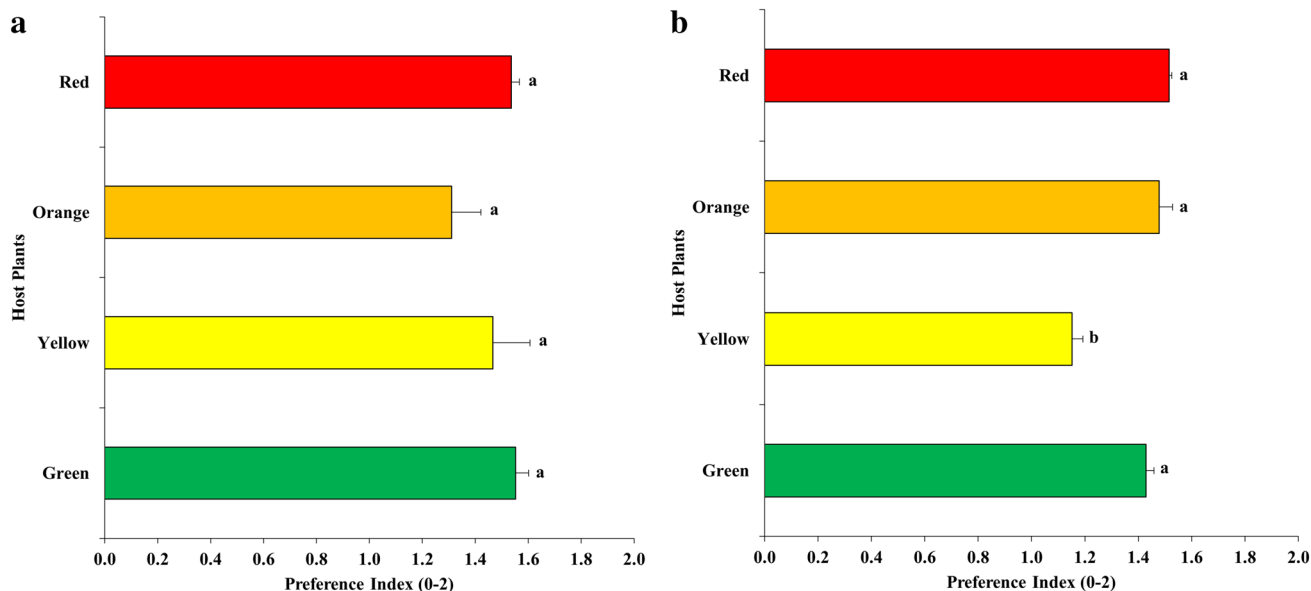


Fig. 1 Mean (\pm SE) Preference Index (PI) of *H. halys* adults (a) and nymphs (b) on different bell pepper colors over a 3-h period

Table 1 Mean (\pm SE) Preference Index (PI) of *H. halys* adults and nymphs on different color peppers over a 3-hour period

Color	Mean PI of adults*				Mean PI of nymphs*			
	Red as standard	Yellow as standard	Green as standard	Orange as standard	Red as standard	Yellow as standard	Green as standard	Orange as standard
Red	–	1.28 \pm 0.18 ^a	1.40 \pm 0.10 ^a	0.80 \pm 0.13 ^{ab}	–	0.99 \pm 0.04 ^a	0.83 \pm 0.03 ^b	0.79 \pm 0.08 ^c
Yellow	0.72 \pm 0.18 ^b	–	1.25 \pm 0.15 ^a	1.00 \pm 0.08 ^a	1.01 \pm 0.04 ^b	–	0.95 \pm 0.06 ^{ab}	1.20 \pm 0.04 ^a
Green	0.60 \pm 0.10 ^b	0.75 \pm 0.15 ^a	–	0.49 \pm 0.10 ^b	1.17 \pm 0.03 ^{ab}	1.05 \pm 0.06 ^a	–	0.98 \pm 0.04 ^b
Orange	1.20 \pm 0.13 ^a	1.00 \pm 0.08 ^a	1.51 \pm 0.10 ^a	–	1.21 \pm 0.08 ^a	0.80 \pm 0.04 ^b	1.02 \pm 0.04 ^a	–

*In each column, means having the same letter in common are not significantly different at the 5% level of significance as according to the Fisher's protected LSD test

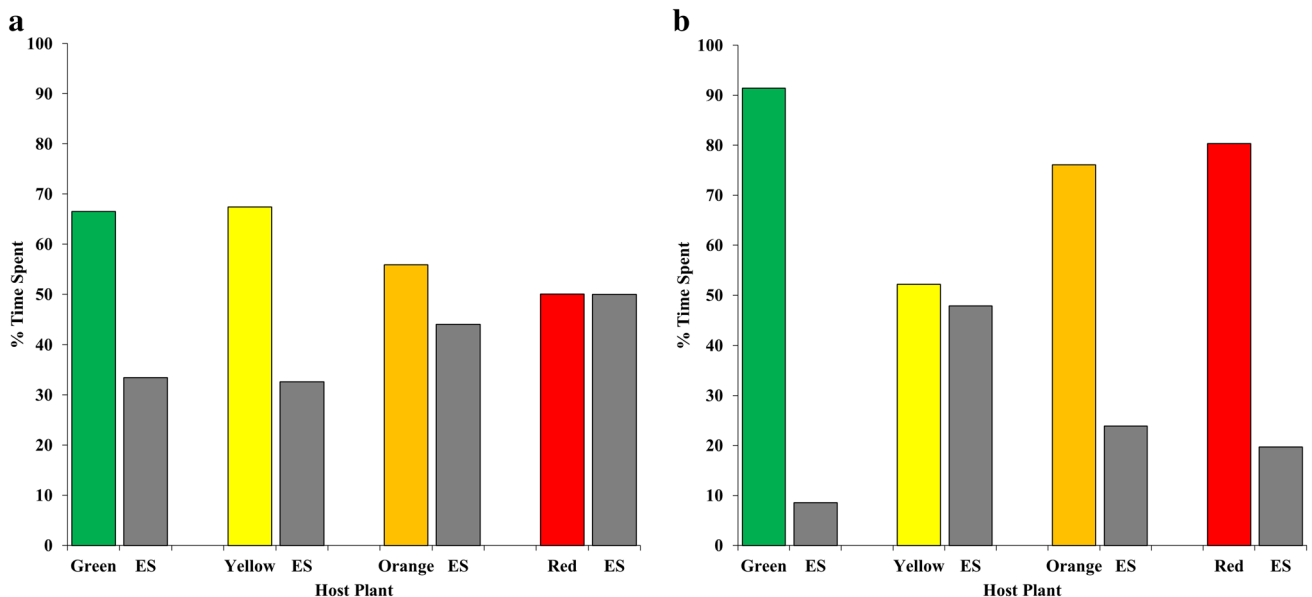
When green bell pepper was the standard differences among the peppers were not significant ($F = 1.11$, $df = 2, 15$, $P = 0.3557$) (Table 1). When orange bell pepper was used as the standard there was significant difference ($F = 5.56$, $df = 2, 15$, $P = 0.0156$) among the peppers (Table 1). BMSB preferred the standard over red and green peppers, which had PI values 0.80 and 0.49, respectively (Table 1).

For the nymphs, when red pepper was used as the standard orange (PI = 1.21) was the most preferred pepper ($F = 4.14$, $df = 2, 15$, $P = 0.0371$) (Table 1). The others were as follows; yellow as the standard red (PI = 0.99) and green (PI = 1.05) were the most preferred peppers ($F = 7.33$, $df = 2, 15$, $P = 0.0060$) (Table 1), with green pepper as the standard, orange pepper (PI = 1.02) was the most preferred ($F = 4.17$, $df = 2, 15$, $P = 0.0364$) (Table 1) and when orange pepper was used as the standard, yellow was the most preferred pepper ($F = 13.06$, $df = 2, 15$, $P = 0.0005$) (Table 1).

Behavioral analysis using the Noldus video

Data from 30 min video recording segments of the DCAT with Early Scarlet (ES) cowpea as standard show that adult *H. halys* spent most time on the different colour bell peppers than on the cowpea except for the red bell pepper where *H. halys* spent an equal amount of time (50%) as it did on the cowpea. Figure 2a shows that the time spent by *H. halys* on the yellow, green, orange and red bell peppers was 67% 66%, 55% and 50%, respectively. The third instar nymphs of *H. halys* spent more time on all the peppers than on the standard. Time spent on green, red, orange and yellow peppers was 92%, 82%, 76% and 52%, respectively (Fig. 2b).

In the DCAT without cowpea, all possible dual pepper combinations as shown in Fig. 3a, adult *H. halys* spent more time on orange when it was paired with all other peppers whilst green recorded the least when it was paired with all other colour peppers. For nymphs, *H. halys* spent 53% of the

**Fig. 2** Percentage time spent by BMSB adults (a) and nymphs (b) on ES cowpea vs. four different color peppers (DCAT) over 30-min period

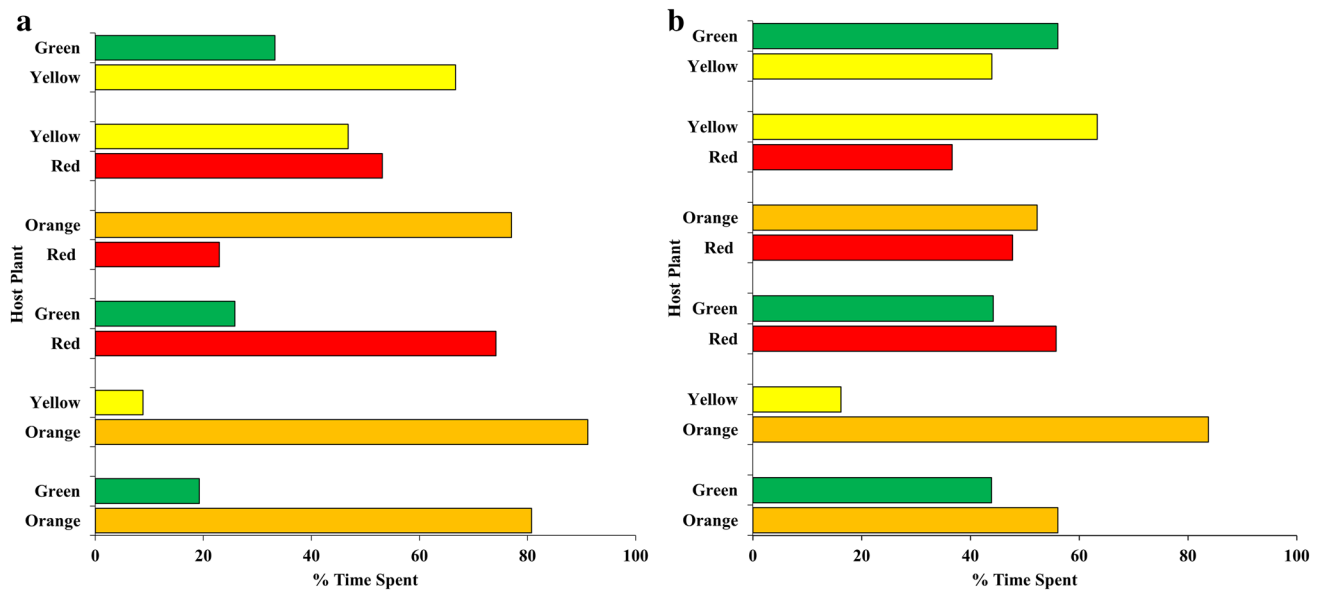


Fig. 3 Percentage time spent by BMSB adults (a) and nymphs (b) on four different color peppers (DCAT) when paired in all possible combinations over 30-min period

time on orange and 47% on green; 87% of the time on orange and 16% on yellow; 55% on orange and 45% on red an indication that *H. halys* nymphs spent more time on orange when it was paired with all other peppers (Fig. 3b).

Olfactometer test

When adult *H. halys* were presented with volatiles from the four colored bell peppers in the four-arm olfactometer, no statistical differences were observed in the distribution among the coloured bell peppers after 15 min ($F = 1.87$, $df = 3, 28$, $P = 0.1582$) and 60 min ($F = 2.42$, $df = 3, 28$, $P = 0.0875$) of exposure (Table 2). However, significant differences existed between *H. halys* distribution on the peppers at time 30 and 45 min. At 30 min, the red bell pepper was the most attractive and was significantly different ($F = 3.36$, $df = 3, 28$, $P = 0.0329$) from green (Table 2). At 45-min red and yellow were the most attractive and were significantly

different ($F = 3.18$, $df = 3, 28$, $P = 0.0394$) from green the least attractive (Table 2). There were significant differences ($F = 4.42$, $df = 3, 28$, $P = 0.0116$) in the distribution of nymphs among the coloured bell peppers. At 15 min, red and yellow were most attractive over green and orange (Table 2). At 30 and 45 min, there were no significant differences ($P > 0.05$); however, at 60 min there was a significant difference ($F = 4.42$, $df = 3, 28$, $P = 0.0178$) among peppers with red the most attractive (Table 2).

Total protein concentration

The total protein concentration and expression from the protein analysis of the four coloured bell peppers is shown in Fig. 4. There was significant difference ($F = 137.3$, $df = 6, 11$, $P < 0.0001$) among the peppers with respect to their protein concentration with the highest recorded in the orange

Table 2 Means (\pm SE) distribution of *H. halys* adults and nymphs on different color peppers in olfactometer at 15, 30, 45, and 60 min after release

Color	Number of adults*				Number of nymphs*			
	15 min	30 min	45 min	60 min	15 min	30 min	45 min	60 min
Red	1.38 \pm 0.42 ^a	1.63 \pm 0.46 ^a	1.63 \pm 0.46 ^a	1.50 \pm 0.42 ^a	0.88 \pm 0.12 ^a	1.13 \pm 0.30 ^a	1.13 \pm 0.30 ^a	1.63 \pm 0.32 ^a
Yellow	1.00 \pm 0.38 ^a	0.88 \pm 0.30 ^{ab}	1.25 \pm 0.49 ^a	1.25 \pm 0.49 ^a	0.88 \pm 0.30 ^a	0.88 \pm 0.30 ^a	1.00 \pm 0.33 ^a	1.13 \pm 0.30 ^{ab}
Green	0.38 \pm 0.18 ^a	0.25 \pm 0.16 ^b	0.13 \pm 0.12 ^b	0.25 \pm 0.16 ^a	0.13 \pm 0.12 ^b	0.38 \pm 0.26 ^a	0.38 \pm 0.26 ^a	0.50 \pm 0.27 ^b
Orange	0.75 \pm 0.16 ^{ab}	0.88 \pm 0.23 ^{ab}	0.75 \pm 0.25 ^{ab}	0.75 \pm 0.25 ^b	0.25 \pm 0.16 ^b	0.25 \pm 0.16 ^a	0.38 \pm 0.18 ^a	0.50 \pm 0.19 ^b

*In each column, means having the same letter in common are not significantly different at the 5% level of significance as according to the Fisher's protected LSD test

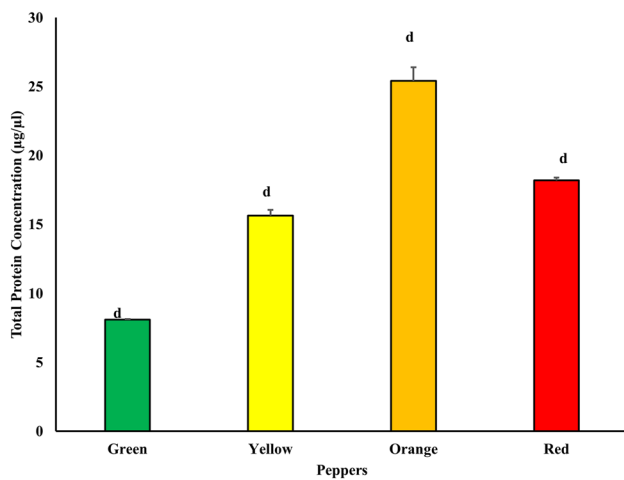


Fig. 4 Total protein concentration in red (RED), yellow (YEL), green (GRN) and orange (ORN) bell peppers

bell pepper (25.4 µg/µl) and the lowest (8.1 µg/µl) in the green bell pepper (Fig. 4).

Discussion

Host plant selection by insects is a complex phenomenon involving a variety of factors that culminate into acceptance and feeding on a selected host (Carrasco et al. 2015). This is true considering the complex environment in which insects find themselves, as well as the specialized diets that some require for growth and reproduction (Price et al. 2011). Both visual and chemical cues (kairomones) play prominent roles in host finding and acceptance. For example, both visual and chemical cues have been reported to play a role in the host plant location by the cabbage moth (*Mamestra brassicae*; Lepidoptera: Noctuidae) (Rojas and Wyatt 2002) and the citrus psyllid (*Diaphorina citri*; Hemiptera: Laviidae) (Wenninger et al. 2009). In the present study, olfactory cues appear to have played an important role in the preference of *H. halys* among the four different colours of bell peppers.

Halyomorpha halys appeared to respond to chemical cues from the different peppers as they searched for host plants in both the choice arenas and olfactometers. *H. halys* preferred the bell peppers over the cowpea in dual choice tests. Colour does not appear to be the basis for the choice because the cowpea is green, one of the pepper colours. This is confirmed in a field study by Zobel et al. (2016), green bell peppers were observed to be the preferred host for most of the season compared to other crops including, green beans, eggplant and tomato. The preference of *H. halys* for bell peppers compared to cowpea may be a result of biochemical, nutritional or the physical characteristics of the plant or a combination of these characteristics. Studies have

shown that nutritional profile value of cowpea is different from that of bell peppers (Frank et al. 2001; Gebhardt and Thomas 2002; Health Canada 2008; Nadeem et al. 2011; Goncalves et al. 2016). Cowpea contains higher amounts of secondary metabolic compounds such as tannin and phenolic compounds, which are deterrents to insect feeding compared to bell peppers (Deng et al. 2013; Ogunlade et al. 2013, 2014; Adjei-Fremah et al. 2015, 2018). These secondary metabolites have a role in plant resistance in reducing nutrient availability and the utilization. Secondary metabolites can also reduce overall nutrient content of the host plant (Bernays et al. 1989; Barbehenn and Constabel 2011; War et al. 2011, 2012).

The different colours seen in the bell peppers used in this study generally represent different stages of ripening, starting with green and progressing to yellow, orange, and finally red. During the ripening process, biochemical changes occur; at the green stage, the peppers are packed with chlorophyll pigment and 2-methoxy-3-isobutylpyrazine (also known as Grindstaff or bell pepper pyrazine), the substance that confers the characteristic aroma to green bell peppers (Roth 2010). As the fruit ripens, chlorophyll together with other photosynthetic pigments decompose slowly and yellow and orange pigments develop. The colouration in the yellow bell pepper is primarily due to the presence of violaxanthin, and lutein and beta-carotene in the orange bell peppers. The red colouration is due to the production of the carotenoids capsanthin and capsorubin, both of which are found in red peppers and synthesized after degradation of the yellow-orange lutein and beta-carotene (Perry et al. 2007; Roth 2010). Yellow, orange and red bell peppers contain a significantly higher amount of carotenoids compared to green bell peppers with red containing the highest concentration of carotenoids (Blanco-Rios et al. 2013; Sun et al. 2007). Carotenoids play important roles in insects and are involved in providing colouration to various portions of their bodies and eggs, mating signals, vision and diapause as well as serving as antioxidants (Heath et al. 2013). Beta-carotene, a major carotenoid and precursor to vitamin A, is required by many invertebrates for the biosynthesis of visual pigment chromophores (Stavenga and Arikawa 2006) and has been reported to be very attractive to insects (Heath et al. 2013; Benkeblia 2017). This could be the reason *H. halys* highly preferred the orange bell pepper to the other colour bell peppers. In addition, in a survey of *H. halys* abundance on several host trees in the United States, Martinson et al. (2015) reported fruit maturity to be a strong predictor of seasonal abundance. These authors observed that *H. halys* preferred not just fruiting trees but also trees with mature ripe fruits were preferred over those with immature fruits. This could explain the higher preference of *H. halys* for the ripening stages of the bell peppers compared to the unripe green stage.

Differences in protein concentration among the bell peppers may be indicative of differences in nutritional quality of the peppers. The orange bell pepper had the highest protein concentration followed by red; yellow and green had the least protein estimate. Insects are known to discriminate between available food choices to select the most suitable to satisfy their nutritional requirements (Nation 2008) and the choice by *H. halys* may be influenced by a weighted combination of these requirements. *H. halys* was observed to respond to volatiles emanating from the peppers in the olfactometer tests. As in most of the tests, the green bell pepper was the least attractive pepper. Yellow, orange and red bell peppers have a mild fruity smell compared to the strong characteristic smell described simply as “bell pepper pyrazine” of green bell peppers. The chemical 2-methoxy-3-isobutylpyrazine which is responsible for the strong green pepper aroma could be the reason for this observation possibly functioning as an allomone leading to repellent behavior for *H. halys* (Roth 2010; El-Ghorab et al. 2013) whilst the fruity smell of the other peppers produced kairomonal effects that attracted the insects.

In all the bioassays (DCAT and Noldus ObserverXT video), some differences were observed between the preferences of adult and nymph BMSB, which is expected for most insects. While adult BMSB showed some consistency in their preferences showing high attraction towards orange and least towards green, BMSB nymphs were mostly inconsistent in their preferences. Results from the olfactometer study indicate that the volatiles from the red-coloured peppers attracted the most nymphs while orange and green-coloured peppers were the least attractive. These behavioral differences between adult and nymphal preference may be attributed in part to the physical or morphological differences between the developmental stages of the insect. For example, the olfactory senses of several insects have been shown to develop with maturity (Yager 1996; Wheeler et al. 2011; Ache and Young 2005; de Bruyne and Baker 2008). Stink Bugs, *Nezara viridula* (Hemiptera: Pentatomidae) and *Piezodorus guildinii* (Hemiptera: Pentatomidae) showed differences in their pattern of soybean pod selection between nymphs and adults in a free-choice test where different phenological stages of soybean pods were provided (Molina and Trumper 2012). It is, therefore, no surprise that some differences were observed between adult and nymph *H. halys* preferences. Our observations may not be directed at single factor but a combination of factors coming to play in the preference of *H. halys* for the different colour peppers. Further studies could delve into the possible factors to bring out more information and a better understanding of the preferences of *H. halys* for bell peppers, especially to enable application of the findings.

Based on the results of this study and given the high preference of *H. halys* for ripened (yellow, orange, and red)

bell pepper over cowpea, bell peppers could be deployed as an inter-specific trap crop for other higher-value cash crops such as corn, soybean, and tomato. There is some indication that this is a viable option (Zobel et al. 2016) but clearly, further studies would be needed to evaluate this potential.

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