Azarian Journal of Agriculture

Azarian J. Agric. VOL (8) ISSUE 1, 2021: 23-28

http://dx.doi.org/10.29252/azarinj.049

www.azarianjournals.ir

Azarian Journals

Research article **ISSN:2383-4420**

Laboratory assessment of host selection of Brown Marmorated Stink Bug (BMSB) on two selected cowpea varieties

Sushil Nyaupane[1], Beatrice Nuck Dingha[2*], Louis Ernest Jackai[2]

Article Info

Accepted:
29 March 2021

Keywords:
BMSB, Host plant selection, invasive, Noldus, olfactometer

ABSTRACT

The brown marmorated stink bug (BMSB) is an important invasive stink bug species which is native to Asia and spread to America causing serious losses to agricultural crops. Laboratory experiments were conducted to assess the preference of BMSB for three phenological growth stages (leaf, flower and pod) of two cowpea cultivars (Early Scarlet and Mississippi Silver). These two cowpea cultivars were selected based on a previous multiple choice and no choice experiments in which they were highly preferred by the BMSB compared to the other host plants which included soybean, corn, sunflower and princess tree leaves. To assess host plant selection by the BMSB, we used a four-arm olfactometer to determine plant volatile involvement in the observed preferences and in the decision making of the insects. The Noldus Observer XT video system was used to record the insect's activities based on visual cues. Results from these experiments show that BMSBs were attracted to both the vegetative and reproductive structures of Mississippi Silver, an indication that this cowpea variety could be used as a trap crop, although it should be verified through field experiment.

INTRODUCTION

The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Hemiptera: Pentatomidae) is an invasive stink bug native to East Asia (Yu and Zhang 2007). It is highly polyphagous in nature and has been reported to feed on more than 300 host plant species comprising field crops, valuable ornamentals, agricultural fruits, nuts and vegetable crops (Kriticos et al. 2017). More specifically, BMSB feeds on fruits, for instance, apple, peach, pear and grape, vegetables such as tomato, pepper, bean, eggplant and cucurbits and field crops, including, soybean, sunflower, corn, wheat, etc. (Day et al. 2011; Rice et al. 2014). In 2010, it was a major agricultural pest in the mid-Atlantic region in the United States of America, causing severe losses to the agriculture industry (Rice et al. 2014). The BMSB was first reported in Allentown, Pennsylvania, USA, in 1996 and later spread throughout the country. As of September 2020, BMSB has been detected in 46 states in the USA and in 4 Canadian Provinces in North America. In the USA, BMSB is recognized as a severe agricultural pest in 10 states, and as a nuisance in 21 states. New York, Pennsylvania, New Jersey, Delaware, Maryland, Virginia, West Virginia, North Carolina, Tennessee and Michigan are among the states where BMSB is causing serious damage to the agricultural sector (Stopbmsb.org 2021).

The BMSB is not only a problem in Asia and in the USA but it is rapidly becoming a global pest. It was first reported in Europe in Zurich, Switzerland in 2007 and later in other European countries (Dingha et al. 2020). It was reported in Liechtenstein (Rice et al. 2014; Seat 2015), France (Rice et al. 2014; Callot and Brua 2013) Germany (Rice et al. 2014; Heckmann 2012), Italy (Rice et al. 2014; EPPO 2013), New Zealand (Rice et al. 2014; Harris 2010), Greece (Milonas and Partsinevelos 2014), Hungary (Vetek et al. 2014), Australia (Rice et al. 2014), Canada (Rice et al. 2014; Fogain and Graff 2011), Serbia, Romania, Spain, Bulgaria (Kistner 2017) , Russia, Malta (Tassini and Mifsud 2019) and Slovakia (Hemala and Kment 2017). It was also found in Kazakhstan in central Asia (Esenbekova 2017). This shows that BMSB has the capacity to cause problems on agriculture globally (Valentin et al. 2017). Climatic

[1] Faculty of Agriculture, Far Western University, Tikapur, Kailali, Nepal.
[2]Department of Natural Resources and Environmental Design, North Carolina A&T State University, Greensboro, USA.
* E-mail: bndingha@ncat.edu

models suggest that this pest can spread to southern hemisphere and cause problem to agricultural crops (Kriticos et al. 2017).

Polyphagous insects may pose serious threat to the agriculture industry because they could switch host plant based on their biological and physiological requirements and also the external environmental conditions. Their preference and selection for different host plants and the resources therein, varies with respect to phenology or various growth stages of the host plant (Nielsen et al. 2011). Plants have several traits that may be different even between two cultivars. Traits such as colour, shape, texture, odour, would enable insects make a choice whether to land on a plant or use the plant for oviposition and development. Terpenoids, aromatic compounds, and volatiles are primarily different for different plants and based on these compounds, insects can choose which plants to use (Carrasco et al. 2015). Herbivorous insects such as the BMSB may choose their host plants for either feeding or oviposition using olfactory and /or visual cues. Under field conditions with multiple plant species available to choose from and the fact that these plants are at different phenological growth stages these cues play a crucial role for BMSB to make a choice (Rice et al. 2014). For example, in soybean field, BMSB preferred feeding on the pods compared to other growth stages (Nielsen et al. 2011). Generally, stink bugs prefer fruiting structures compared to vegetative structures. Additionally, in the field BMSB were reported to prefer younger seeds and fruits than matured pods (Dingha et al. 2020). They are attracted to specific host plants and specific growth stages to ensure fitness of their young and their own growth and development. BMSB was reported to show high preference for Mississippi Silver and Early Scarlet cowpea varieties compared to other crop host plants including soybean, corn, sunflower and princess tree leaves (Dingha et al. 2020). The objective of this experiment was to evaluate selective preference of BMSB for the different growth stages of two cowpea cultivars (Early Scarlet and Mississippi Silver) based on the use of olfactory and visual cues. The outcome could contribute to the management of the BMSB by using the most preferred cowpea variety and phonological stage as a trap crop. This ecological control strategy will have little or no impact to the environment and human health (Mensah-Bonsu et al. 2020; Nyaupane et al. 2021).

MATERIALS AND METHODS

Experimental Insects

The insects used in the experiments were obtained from a culture of BMSB reared at the Integrated Pest Management (IPM) Laboratory at North Carolina A&T State University, Greensboro, North Carolina. The insects were reared by setting optimum conditions (26 ± 2^0 C, 70% RH and 16:8 light/day) in the laboratory. Cages were supplied with organic corn, carrot, tomatoes, grapes and beans purchased from grocery stores and Princess tree leaves obtained from the North Carolina A&T State University research farm (36.0586243^0 N and 79.7358932^0 W) located in Greensboro, North Carolina (Dingha and Jackai 2017). The crops grown at the research farm at the time BMSBs were collected included: soybean (Pioneer 95M82), corn (Trucker's favorite), sunflower (Zohar F1) and cowpea (Early Scarlet and Mississippi Silver) with Princess tree planted along the edges of the field. No pesticides were applied to the field prior to insect collection. Adult BMSBs were hand collected from the crops planted on the research farm and put in 500 ml transparent polypropylene containers with a partial mesh screen lid (BioServ, Frenchtown, New Jersey, USA) and moist cotton balls placed inside to maintain the relative humidity. Insects collected were brought to the laboratory and transferred to BugDorm cages (30.5×30.5×30.5 cm; BioQuip, California, USA) as described by Dingha and Jackai 2017.

Experimental greenhouse plants

Cowpea *Vigna unguiculata* was used for the experiments. Two varieties (Early Scarlet and Mississippi Silver) were planted in pots in the greenhouse at North Carolina A&T State University in September, 2016. The plants were grown without pesticides and fertilizers application. Freshly collected green leaves, flowers and mature pods of R6 stage (50% of pods with fully developed seeds) were collected, wrapped in paper bags and taken to the laboratory. The plant parts were cleaned with moist paper towel.

Four-arm olfactometer experiment

A four-arm olfactometer was used to test the preference of BMSBs for the different plant parts of the two cowpea varieties (Early Scarlet and Mississippi Silver). The four-arm olfactometer (Sigma Scientific, Gainesville, Florida) was made up of a central square chamber (30 cm long × 30 cm wide × 5 cm high) with four arms or openings (7cm long ×2 cm diameter) on the four sides and a central opening (Figure 1). Different plant parts (leaves, flowers and pods (3 each)) were placed in one of the three arms of the olfactometer and the fourth arm was left empty and served as the control. At the start of each run, the olfactometer was turned on and pressure maintained at the rate of 200 ml/min. to push the volatile of the substrate uniformly over the center and sucking out the volatile via a vacuum pump at the rate of 1000 ml/ min. Ten starved adult BMSBs were placed in the center of the olfactometer and the system was allowed to run for one hour. During this time, BMSBs made their choice towards the different food substrate in the different arm based on olfactory cues. The number of BMSBs present in each arm was recorded after one-hour. This setup was repeated 10 times for a total of 100 adult insects used.

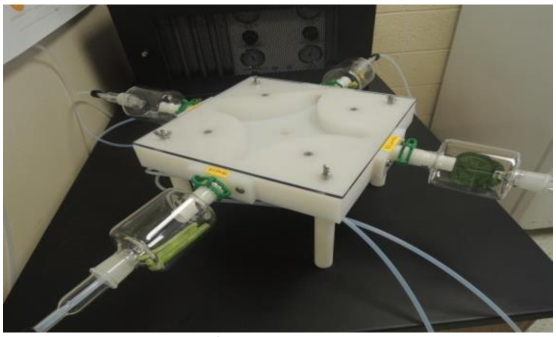

Figure 1. Four-arm olfactometer experiment

The distribution of adult BMSBs to the different food substrate in the three arms and the control was analyzed using one-way analysis of variance (ANOVA) with SAS software (version 9.3; SAS Institute, Cary, USA) after the data was square root transformed $(x+0.5)^{1/2}$. Means were separated using least significant difference test (LSD) at P≤0.05 when there was a significant treatment effects.

Noldus Observer XT video experiment

Since both the vegetative and reproductive parts (leaves, flowers and pods) of Mississippi Silver cowpea were attractive to BMSB, we set up experiments to assess the role of visual and olfactory cues. The Noldus Observer XT was used to record the preference of BMSB for Mississippi Silver cowpea in the laboratory (Figure 2). Bioassays were conducted using Choice Arena Test (CAT). The choice arena was prepared using a glass pyrex® Petri dish (15 cm diameter × 2 cm depth glass). Each arena was divided into six equal triangles from the center and had paper towel lining the bottom of the container. Mississippi Silver cowpea leaf, flower and pod were placed in opposite triangles. Four starved adult BMSBs were released at the center of the arena. The Noldus observer XT system was turned on and the preference of BMSB for the different food substrates recorded for 12 hours. The observational data gathered (feeding on leaf, flower and pod, crawling on container, static on container, crawling on leaf, flower and pod and static on leaf, flower and pod) was coded using the Noldus video setup. The experiment was replicated three times. Data collected was analyzed using one-way analysis of variance (ANOVA) with SAS software (version, 9.3; SAS Institute, Cary, USA).

RESULTS AND DISCUSSION

Results from the four-arm olfactometer experiment indicate that Early Scarlet cowpea pods attracted significantly more BMSB compared to the leaves and the control whereas, insect distribution on flowers and pods were similar (Table 1). From the four-arm olfactometer our results show that the distribution of BMSB among the leaf, flower and pod of Mississippi Silver cowpea was not significantly different (Table 2). Results from the Noldus observer XT video experiment show that BMSB spend most time feeding on the various food substrates (leaf, flower and pod), however, this was not significantly different (Table 3).

The brown marmorated stink bug is a polyphagous insect species which depends on multitude of plant resources as food source. They are able to choose specific host plants based on the

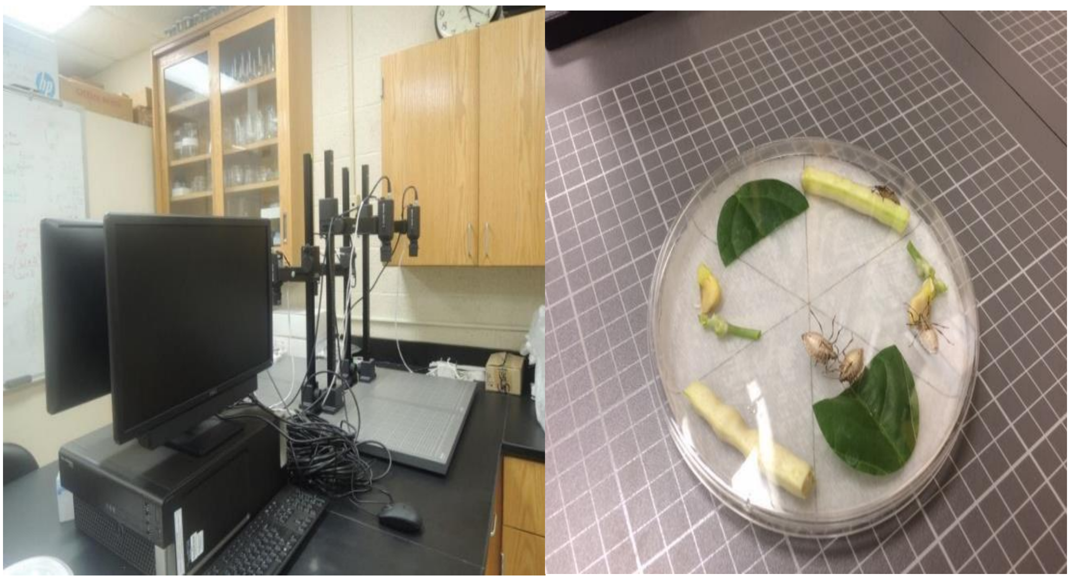

Figure 2. Noldus Observer XT video experiment

availability of resources on the plant to feed and/or oviposit. Over the course of evolution, phytophagous insects have developed capacity to detect organic volatile compounds produced by the plants in order to locate specific crops they want to feed on. It is important to determine if visual cue play a role in host plant preference or selection (Carrasco et al. 2015). In this study, we investigate the role of olfactory and visual cues using the four-arm olfactometer and Noldus observer XT video respectively in BMSB host plant selection.

Table 1. Mean (±SE) distribution of adult BMSBs for Early Scarlet cowpea after 1-hour period on four-arm olfactometer

Treatments	Observed insects
ES cowpea leaves	1.50±0.42[bc]
ES cowpea flowers	2.10±0.37[ab]
ES cowpea pods	2.90±0.60[a]
Control	0.70±0.21[c]
F	4.50
P	0.0088
CV,%	32.35

Means followed by the same lower case letter do not differ according to Tukey test (P≤0.05). The original data were square root (x+0.5)1/2 transformed for analysis. CV: coefficient of variation

Table 2. Mean (±SE) distribution of adult BMSBs for Mississippi Silver cowpea after 1-hour period on four-arm olfactometer

Treatments	Observed insects
MS cowpea leaves	1.50±0.34[ab]
MS cowpea flowers	2.10±0.45[a]
MS cowpea pods	2.60±0.61[a]
Control	0.80±0.20[b]
F	3.34
P	0.0299
CV,%	30.75

Means followed by the same lower case letter do not differ according to Tukey test (P≤0.05). The original data were square root $(x+0.5)^{1/2}$ transformed for analysis. CV: coefficient of variation

Based on the four-arm olfactometer experiment, BMSB preferred pods of Early Scarlet cowpea compared to the leaves whereas, BMSB preference for Mississippi Silver cowpea leaves, flowers and pods were similar. Previous experiment also indicated that BMSB's preference for Mississippi Silver leaf was highest after 2 hours when insects were released in a Dual Choice Arena Test (DCAT), compared to leaves of Princess tree, corn, sunflower, soybean and Early Scarlet cowpea, although this preference was not significantly different (Dingha et al. 2020). It was further confirmed by the Noldus Observer XT experiment. Data obtained from the Noldus observer XT video recording, shows that the preference of BMSB to leaf, flower and pod of Mississippi Silver was not significantly different. Results obtained from both the four-arm olfactometer and Noldus observer XT video experiments for Mississippi Silver cowpea were similar and it could suggest that without the use of visual cues, insects may be capable of making a choice based on volatiles produced by the host plants. The preference of BMSB to both the vegetative and reproductive structures of Mississippi Silver could be due to higher nutrients present in these structures. It has been reported that cowpea leaves also contain a good proportion of proteins, amino acids, minerals and vitamins (Owade et al. 2020) and may obviously have much more leaf volatiles to attract BMSBs. Mississippi Silver could be a good trap crop for the BMSB since the vegetative (leaf) stage was also highly preferred; this would enable the insects to be retained on the trap plant till podding stage or maturity.

Table 3. Mean (±SE) time period spent by BMSB for each behavior on Mississippi Silver cowpea at Noldus observer XT video experiment

Behaviors of BMSB	spent time period (min.)
Crawling at container	50.88±15.90[abcd]
Static at container	149.62±59.75[a]
Crawling at leaf	2.38±1.57[e]
Crawling at flower	16.00±10.77[cde]
Crawling at pod	38.85±9.11[bcde]
Static at leaf	12.85±12.85[de]
Static at flower	14.04±13.49[de]
Static at pod	75.21±49.23[abc]
Feeding on leaf	110.82±10.07[ab]
Feeding on flower	114.00±25.68[ab]
Feeding on pod	135.04±16.46[a]
F	5.41
P	0.0005
CV,%	43.04

Means followed by the same lower case letter do not differ according to Tukey test (P≤0.05). The original data were square root transformed for analysis. CV: coefficient of variation

CONCLUSIONS

The results obtained from both the four-arm olfactometer and Noldus observer XT video experiments suggest that Mississippi Silver cowpea attracts BMSB at the vegetative (leaf) growth stage which could retain the insect population until the flowering and podding stages. Our research also inferred that BMSBs are capable of choosing their host plants based on the volatiles produced by the different plant parts without necessary using visual cues. Since BMSBs were attracted to the vegetative stage (leaf) of Mississippi Silver, further research to analyze the nutrient and plant volatile profile of leaves will provide more insight to BMSB preference. Based on our findings, Mississippi Silver could be deployed as a trap crop. However, further validation on the field is needed.

ACKNOWLEDGMENTS

Authors would like to thank members of the Integrated Pest Management Laboratory at North Carolina A&T State University for their assistance. This research was funded by the United States Department of Agriculture and the National Institute of Food and Agriculture (USDA-NIFA) Evans Allen Program, Grant No. NC. X-287-5-15-170-1.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

ORCID

Sushil Nyaupane: https://orcid.org/0000-0003-4818-6513 and https://orcid.org/0000-0003-4818-6513

REFERENCES

Callot H. Brua C. (2013) *Halyomorpha halys* (Stål, 1855), the marmorated stink bug, new species for the fauna of France (Heteroptera Pentatomidae). L'Entomologiste, 69(2): 69-71.

Carrasco D. Larsson M. C. Anderson P. (2015) Insect host plant selection in complex environments. Current Opinion in Insect Science, 8: 1-7.

Day E. R. McCoy T. Miller D. Kuhar T. P. Pfeiffer D. G. (2011) Brown marmorated stink bug. Virginia Coop. Ext. Publ, 2902-1100.

Dingha B. N. Jackai L. E. (2017) Laboratory rearing of the brown marmorated stink bug (Hemiptera: Pentatomidae) and the impact of single and combination of food substrates on development and survival. The Canadian Entomologist, 149(1): 104-117.

Dingha B. N. Nyaupane S. Jackai L. E. (2020) Laboratory Assessment of Host Plant Selection of the Brown Marmorated Stink Bug (*Halyomorpha halys*). American Journal of Entomology, 4(2): 26-34.

EPPO (2013) European and Mediterranean Plant Protection Organization Reporting Service No 5. Pest & Disease spp., 10–11.

Esenbekova P.A. (2017) First record of *Halyomorpha halys* (Stål, 1855) (Heteroptera, Pentatomidae) from Kazakhstan. Eurasian Entomol. J, 16(1):23–24.

Fogain R. Graff S. (2011) First records of the invasive pest, *Halyomorpha halys* (Hemiptera: Pentatomidae), in Ontario and Quebec. Journal of the Entomological Society of Ontario, *142*: 45-48.

Harris A.C. (2010) *Halyomorpha halys* (Hemiptera: Pentatomidae) and Protaetia brevitarsis (Coleoptera: Scarabeidae: Cetoniinanae) intercepted in Dunedin. The Weta, 40: 42–44.

Heckmann R. (2012) Erster nachweis von *Halyomorpha halys* (Stål, 1855) (Heteroptera: Pentatomidae) für Deutschland. Heteropteron, 36: 17–18.

Hemala V. Kment P. (2017) First record of *Halyomorpha halys* and mass occurrence of *Nezara viridula* in Slovakia (Hemiptera: Heteroptera: Pentatomidae). Plant Protection Science, 53(4): 247-253.

Kistner E. J. (2017) Climate change impacts on the potential distribution and abundance of the brown marmorated stink bug (Hemiptera: Pentatomidae) with special reference to North America and Europe. Environmental entomology, 46(6): 1212-1224.

Kriticos D. J. Kean J. M. Phillips C. B. Senay S. D. Acosta H. Haye T. (2017) The potential global distribution of the brown marmorated stink bug, *Halyomorpha halys*, a critical threat to plant biosecurity. Journal of Pest Science, 90(4): 1033-1043.

Mensah-Bonsu M. Dingha B. N. Jackai L. E. Adjei-Fremah S. Worku, M. (2020) Evaluation of preference of brown marmorated stink bug, Halyomorpha halys (Stål) for different colour bell peppers and the role of plant protein. Arthropod-Plant Interactions, 14(3):363-372.

Milonas P. G. Partsinevelos G. K. (2014) First report of brown marmorated stink bug *Halyomorpha halys* Stål (Hemiptera: Pentatomidae) in Greece. EPPO Bulletin, 44(2): 183-186.

Nielsen A. L. Hamilton G. C. Shearer P. W. (2011) Seasonal phenology and monitoring of the non-native *Halyomorpha halys* (Hemiptera: Pentatomidae) in soybean. Environmental Entomology, 40(2): 231-238.

Nyaupane S. Tiwari S. Thapa R. B Jaishi S. (2021) Testing of bio-rational and synthetic pesticides to manage cabbage aphid (*Brevicoryne brassicae* L.) in cabbage field at Rampur, Chitwan, Nepal. Journal of Agriculture and Natural Resources, 4(2): 29-39.

Owade J. O. Abong' G. Okoth M. Mwang'ombe A. W. (2020) A review of the contribution of cowpea leaves to food and nutrition security in

East Africa. Food science & nutrition, 8(1): 36-47.

Rice K. B. Bergh C. J. Bergmann E. J. Biddinger D. J. Dieckhoff C. Dively G. ... Herbert A. (2014) Biology, ecology, and management of brown marmorated stink bug (Hemiptera: Pentatomidae). Journal of Integrated Pest Management, 5(3): A1-A13.

Šeat J. (2015) *Halyomorpha halys* (Stål, 1855) (Heteroptera: Pentatomidae) a new invasive species in Serbia. Acta entomologica serbica, 20: 167-171.

Stopbmsb.org (2021) Management of brown marmorated stink bug in US specialty crops. http://www.stopbmsb.org/where-is-bmsb/ (accessed 19 February, 2021).

Tassini C. Mifsud D. (2019) The brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Heteroptera: Pentatomidae) in Malta. EPPO Bulletin, 49(1): 132-136.

Valentin R. E. Nielsen A. L. Wiman N. G. Lee D. H. Fonseca D. M. (2017) Global invasion network of the brown marmorated stink bug, *Halyomorpha halys.* Scientific reports, 7(1): 1-12.

Vetek G. Papp V. Haltrich A. Redei D. (2014) First record of the brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Heteroptera: Pentatomidae), in Hungary, with description of the genitalia of both sexes. Zootaxa, 3780(1): 194-200.

Yu G. Y. Zhang J. M. (2007) The brown marmorated stink bug, *Halyomorpha halys* (Heteroptera: Pentatomidae) in PR China. In International Workshop on Biological Control of Invasive Species of Forests, Beijing, China, 58-62.