FINAL PROJECT REPORT Year 4 of 3 (with a one year no cost extension)

WTFRC Project Number CP-13-100A

Project Title: Chemical mediation of aggregation by brown marmorated stink bug

PI:	Peter Landolt	**Co-PI(2)**:	Jocelyn Millar
Organization:	USDA, ARS	**Organization:**	University of California
Telephone:	**(509) 454-6551**	**Telephone**:	(951) 827-5821
Email:	peter.landolt@ars.usda.gov	**Email**:	Jocelyn.millar@acr.edu
Address:	5230 Konnowac Pass Road	**Address**:	Department of Entomology
Address 2:		**Address 2**:	3401 Watkins Drive
City:	Wapato,	**City**:	Riverside
State/Zip:	WA 98951	**State/Zip**:	CA 92521

Cooperators: Tracey Leskey, USDA, ARS Kearneysville, WV
Helmuth Rogg and Todd Adams, Oregon State Dept. Agric., Salem Oregon

Other funding sources

Agency Name: USDA, CSRS, SCRI program.
Amount awarded: $16,500
Notes:

Total Project Funding: $120,000

Budget History: Combined Wapato and Riverside

Item	**2013**	**2014**	**2015**
Salaries			
Benefits			
Wages	$24,535	$24,776	$25,022
Benefits	6,328	6,415	6,513
Equipment			
Supplies	8,137	7,809	7,465
Travel	1,000	1,000	1,000
Plot Fees			
Miscellaneous			
Total	$40,000	$40,000	$40,000

RECAP OF ORIGINAL OBJECTIVES

The overall objectives of the project were to discover and develop chemical attractants and attractant synergists for brown marmorated stink bug (BMSB) based on their host-and mate-location behavior. The experimental objectives were to:

1. Determine sex attraction responses of female BMSB, including physiological and environmental regulators of that behavior.
2. Determine host plant preferences, and female and male BMSB attraction to host plant odor.
3. Determine host plant effects on BMSB sexual pheromone behavior.
4. Isolate and identify plant kairomones that mediate or enhance BMSB attraction behavior.
5. Determine interactions between male BMSB pheromones and host plant kairomones, to develop superior attractants.

SIGNIFICANT FINDINGS

1. Both attraction and repulsion of bugs by plant odors was demonstrated. This provides initial target plants and a bioassay to use in isolating and identifying plant kairomones.
2. Strong female BMSB attraction to males was demonstrated, providing opportunities to isolate a male-produced attractant, and a bioassay method to use for that purpose. This behavior was subsequently shown to be related to male aggregation within shelters provided to them.
3. A pheromone was recovered from solvent washes of jars housing male BMSB that is very attractive to females and is repellent to males.
4. An alarm pheromone response was demonstrated for BMSB. This work is being pursued to determine the functions of alarm pheromone, and to understand the various roles of complex BMSB body odor and signal chemistry.
5. Thigmotaxis was demonstrated. This behavior is important to study and understand the conditions under which BMSB aggregates, and then the roles of pheromones in that aggregation.
6. An electro-antennal detector (EAD) was modified for the BMSB antenna and its effectiveness was demonstrated using BMSB and published pheromone chemicals. This system was then used to determine which compounds in defensive secretions are detected by BMSB, and which compounds in samples of male volatiles are detected by female BMSB.
7. The volatile chemistry of BMSB males and females was characterized and compared, to provide a baseline from which to detect and determine chemical signaling.
8. The volatile chemistry of BMSB defensive/alarm secretions was characterized. A set of these chemicals were found to be repellent to paper wasps that are potential predators, affirming the defensive roles of these compounds.
9. A new combination of pheromone lure and trap was found to be significantly more effective in capturing BMSB, compared to the most-used commercial trapping system.

RESULTS AND DISCUSSION.

We were able to demonstrate several behaviors in BMSB that relate to attraction and aggregation. Knowing that these behaviors exist provides the opportunities to pursue isolation and identification of active semiochemicals, and the development of bioassays that are necessary to isolate the active chemicals involved. These behaviors include attraction and repulsion by plant odors, sex attraction, alarm and defense, and thigmotaxis (arrest in response to contact with surfaces).

Attraction and repulsion of bugs by several plant odors. A Y-tube olfactometer system (Landolt et al. 2000), was used to determine BMSB responses to plant odor (Figure 1). For each plant species, we tested female BMSB response to a bouquet of foliage (often with fruits) versus an empty chamber. A minimum of 60 bugs were tested one at a time per plant species, with the bouquet replaced for each ten females. For most plant species, there was not a significant response. Of particular note was the repellency of wild *Clematis*, which is a preferred late season plant for native stink bugs, and attractiveness of both potato and green beans to BMSB. We also determined that the bug responses are faster in a vertical orientation compared to a horizontal orientation. The strong difference between male and female stink bug response in this test (Figure 1) suggests that the behavior is host finding for purposes of oviposition site selection, rather than a search for adult food.

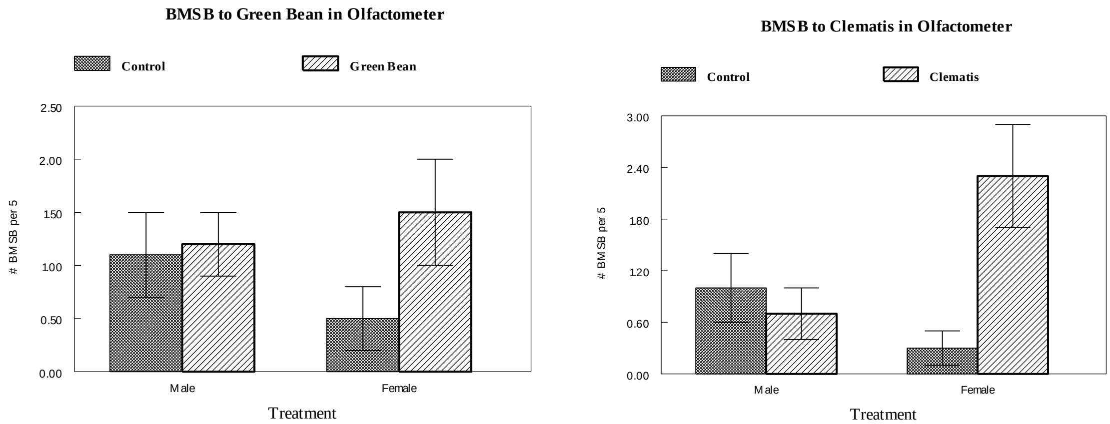


Figure 1. Examples of numbers of female BMSB responding to airflow from over plant material (green beans and clematis) in a choice olfactometer. N = 60, as 12 groups of 5. Control is airflow through an empty jar.

BMSB Thigmotaxis. BMSB appear to seek out and hide in tight places. We constructed 3D slatted shelters out of cardboard, and placed a 3 inch wide shelter in the corner of a 16 X 16 X 16 inch screened cage. Most stink bugs moved into these shelters and stayed in these shelters (Figure 2). This behavior may be an important aspect of one type of aggregation behavior and was the basis for our assay for sex attraction.

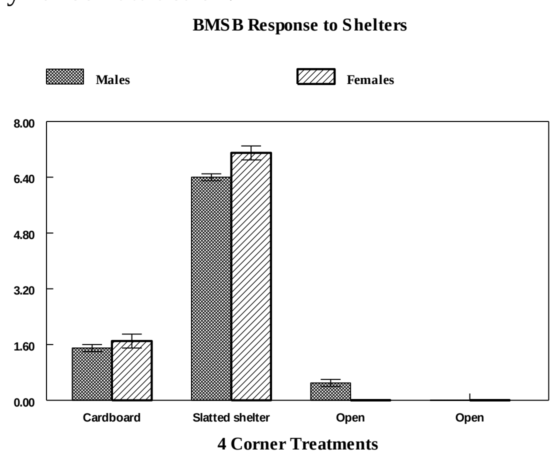
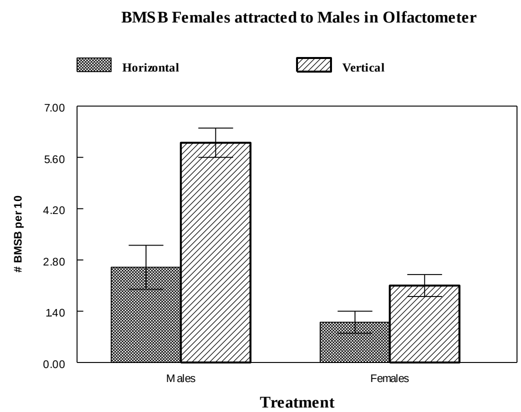


Figure 2 (left) Numbers of male and female BMSB moving into shelters made of cardboard and placed in upper corners of cages. Numbers are counts of those within or on shelters or in the remaining open upper corner of the cage.

Female BMSB attraction to males. Several experiments tested the hypotheses of female attraction to males and male attraction to females. Either male or female BMSB were placed in a cage with a shelter in which they entered and stayed. This shelter was in turn tested for attractiveness to other BMSB in an olfactometer assay. Each assay involved the testing of 60 stink bugs, one at a time, in serues of 6 batches of 10, with the treatments (stink bug shelters with residual odor) replaced for each set. Responses of females to males was strong, but not female response to females (Figure 3). A much stronger response was seen when the assays were conducted in the scotophase (night) under red light versus the photophase (day), and when the olfactometer was oriented vertically compared to horizontally (Figure 3). A further breakthrough was the successful "capturing" of the sex pheromone in solvent used to rinse glass jars housing males in shelters. This solvent was then very attractive to females (Figure 4), indicating the presence of the pheromone in the sample, which was then analyzed by GC-MS. Interestingly, the same samples were repellent to males (Figure 4).



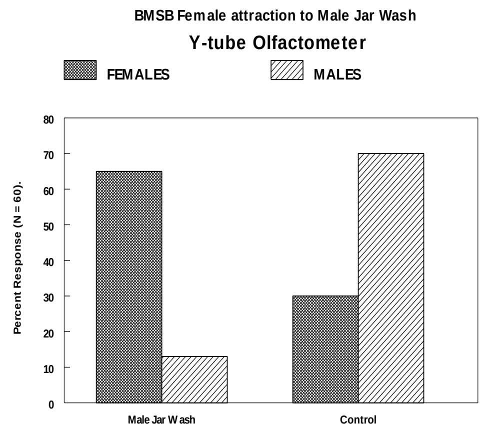


Figure 3 (left). Numbers of female BMSB orienting to airflow from over a male-occupied shelter versus a female-occupied shelter in a choice olfactometer. N = 60.
Figure 4. (right). Percentages of female and male BMSB responding to a solvent wash of a jar housing male BMSB, applied at a rate of one male-day equivalent. The control is a solvent wash of an empty jar.

BMSB Alarm Response. An alarm pheromone response was demonstrated for BMSB. In an arena type assay, stink bugs showed an escape reaction in a 20 second response to a puff of air from a chamber with a disturbed bug (Figure 5). This work was pursued to determine if there is a conspecific alarm-type response to BMSB defensive chemistry (an alarm pheromone), and to obtain overall a better understanding of the complex roles of BMSB body odor chemistry.

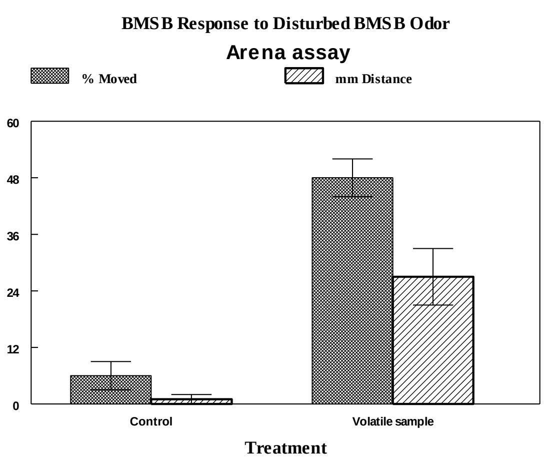


Figure 5. Numbers of male and female BMSB (combined) responding to airflow from a single disturbed BMSB. Movement was noted, as well as the distance moved in the 20 second long test. N = 100 as 20 groups of 5. Control is air from an empty jar.

Additionally, we showed repellency of *Polistes* paper wasps to the odor of disturbed BMSB and to individual chemicals that make up the defensive secretion of the bug.

Coupled Gas Chromatographic-Electroantennographic Detection (GC-EAD) Analysis. Coupled GC-EAD analysis was performed. The column effluent was split 1:1 in the oven to produce airstreams to both to the GC detector and into a humidified air stream directed toward the mounted antennae of brown marmorated stink bug.

One of the two antennae was separated from the head and it was positioned between two gold wire electrodes immersed in saline-filled (46mmol NaCl, 182mmol KCl, 3 mmol CaCl2, and 10mmol TrisHCl at pH 7.2) micropipettes in an acrylic holder. The output signal from the antenna was amplified (10×) by a customized high input impedance DC amplifier and converted to a digital signal (IDAC-232, Syntech) and recorded on a computer using a dedicated software (GC-EAD, Syntech). A total of ten antenna set-ups were prepared and each antennae preparation was tested on SPME headspace adsorption of a commercial stink bug lure (Sterling). Consistent and significant antennal responses were achieved for 5 different female pheromone chemicals, using male BMSB antennae. This development is important because there are no good precedents in the literature for the methods or even the ability to obtain electroantennal responses to semiochemicals from stink bugs. This accomplishment provides a powerful tool for us to isolate other semiochemicals such as plant kairomones or pheromones involved in BMSB aggregation behavior. This technique for example was critical to our rapid identification of a feeding attractant lure for spotted wing drosophila, using volatile chemicals from a wine/vinegar bait (Cha et al. 2012).
Consistent and measurable antennal responses were obtained to synthetic samples of pheromones reported in the literature. This GC-EAD system was then used to determine which chemicals in disturbed BMSB samples are detected by paper wasps, and which chemicals in male BMSB samples are detected by female BMSB, using behavioral assays developed.

The volatile chemistry of BMSB males and females was characterized and compared, to provide a baseline from which to detect and determine chemical signaling. Volatile collections from over female BMSB showed the presence of 6 compounds when the stink bugs were quiet, which increased to 20 compounds when they were disturbed. Undisturbed males released 4 compounds, while disturbed males released 20 chemicals. All of these chemicals are identified and a number of them have been evaluated in bioassays and field tests.

Field sampling of stink bugs. During 2015, a dozen traps were placed in residential properties, and about 60 additional field collections were made to assess the species makeup of stink bugs, to detect the presence and spread of BMSB, and to determine potential preferred host plants. Sampling was accomplished with a beating sheet and sweep net to sample foliage in non-agricultural habitats. These collections were principally in Yakima County, and yielded nearly 450 stink bugs, all which were identified to species. About 20 BMSB were collected in 2015 as a part of this study, in pheromone traps, on plants, on structures, and through WSU Extension of Yakima and the Master Gardener Program, in the cities of Yakima and Sunnyside. BMSB were found over a broad area of the west side of the city, but not in agricultural or rural areas. During 2016, the use of 20 pheromone baited traps in addition to plant sampling, observations on structures, and the Master Gardeners, yielded over 250 BMSB collected in Yakima, with the largest numbers west of downtown and with increased numbers and sites in the West Valley area out to Ahtanum and the Apple Tree Gulf Course.

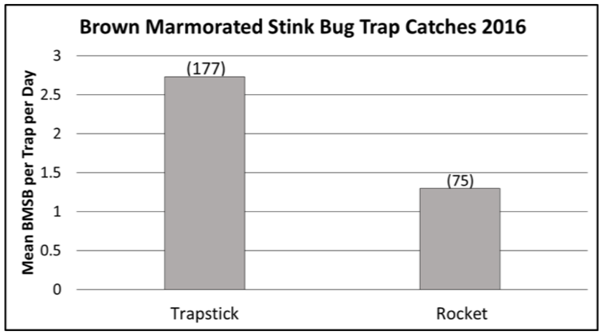


Figure 6. Mean numbers of brown marmorated stink bugs captured in Sterling International Inc. TrapStik versus Rescue (Rocket) traps. N = 18. Yakima County, WA.

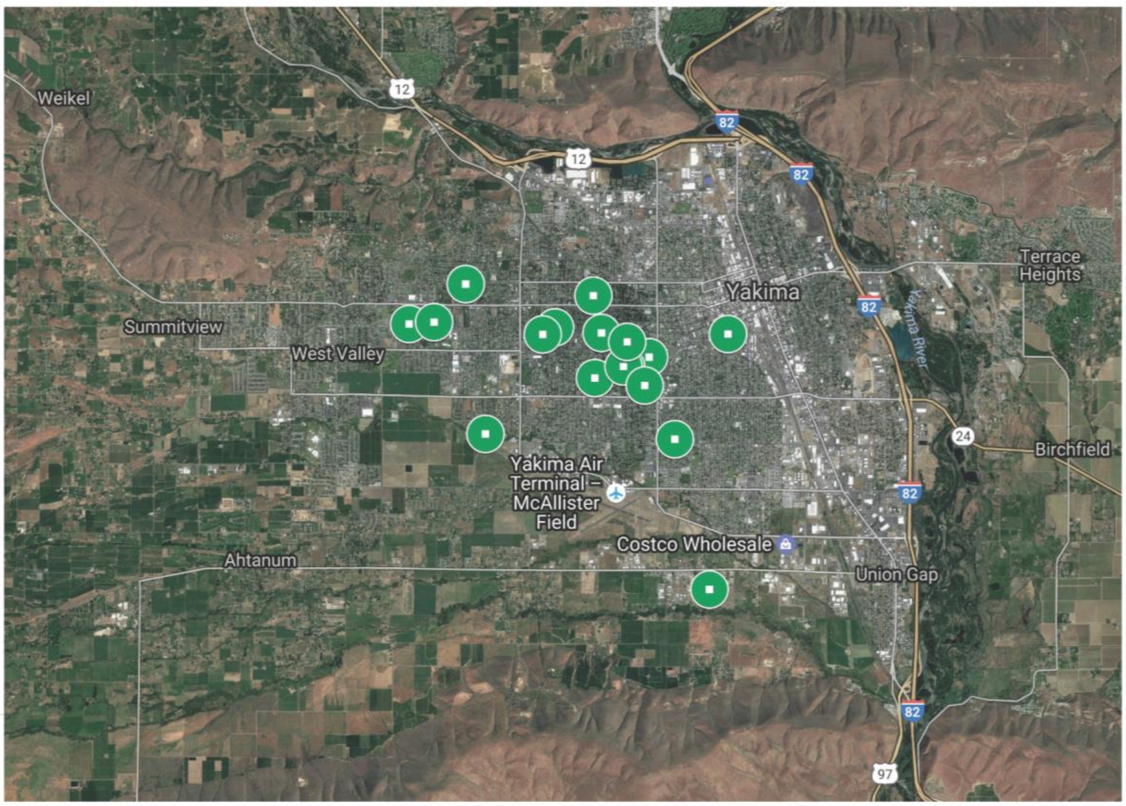


Figure 7. Positive sites in Yakima for BMSB, Sept./Oct 2016, using Sterling Inc. TrapStik and Rescue (Rocket) pheromone-baited traps.

EXECUTIVE SUMMARY

The adult brown marmorated stink bug is long-lived. The adult stage may persist for nearly a year and the bug displays a diversity of semiochemical-mediated behaviors over the course of that year that provide multiple opportunities for manipulation if they are determined and understood. By demonstrating different behaviors (aggregation, attraction, and alarm) and behavioral responses to odor signals in the laboratory (sex attraction, adult aggregation, alarm) we developed and provided assay methods needed for isolation and determination of the chemical signals that elicit these different behavioral responses.

We characterized the chemistry of BMSB secretions and odors, and related those to the behaviors studied. We determined BMSB volatile compounds that are specific to males and those specific to females. We determined the compounds emitted by BMSB when they are disturbed and showed that several of these chemicals repel predatory wasps, confirming the defensive nature of those compounds. We also showed that alarm pheromone stimulates BMSB dispersion. There is potential for these compounds to be used to disrupt BMSB aggregations and other activities. We characterized the chemistry of male-specific chemicals released when males are attractive to females and confirmed the attractiveness of those chemicals to BMSB.

At the end of the 2016 field season, some of this work came to a fruition with a significant improvement to a pheromone-based trapping system. A change in the combination of pheromone lure and trap design more than doubled trap catch compared to the commercial system most commonly in use. This result provides a simpler, cheaper, and more effective means of detection of BMSB in the field. We took advantage of this development to take a quick late season assessment of the prevalence of BMSB in Yakima. That assessment shows the widespread nature of the bug in the city.

Although the techniques that we used to assess local BMSB populations changed over the life of this project, the results of sampling and trapping show a clear and sharp upward trend in their numbers and distributions, although still largely restricted to urban and suburban landscapes. That trend is expected to problems where orchards and human habitations and neighborhoods are in proximity. This situation appears to differ from that of the native consperse stink bug which is problematic in apples where orchards border largely feral (old field, and Steppe) rather than urban/suburban landscapes.

Future directions. Our findings and experimental results, together with advances made by other laboratories, indicate 3 potential roles for applied chemical ecology to manage BMSB. 1) The determination of BMSB defensive chemistry having an alarm function suggests a possible application to disrupt aggregation behavior. 2) The movement of bugs to shelters and production of a female attractant while in those shelters suggests that females can be captured and removed by a suitable combination of male pheromone and shelter. Similar shelters have been evaluated for capture of overwintering bugs, and this might be expanded with chemical attractants, but also for use with non-overwintering bugs. 3). There are currently problems with stink bug pheromones attracting bugs that are not captured in traps; potentially worsening a situation in a crop. Strong advances in trap efficacy may be a solution to that problem, and improve the use of pheromone-baited trapping for monitoring in cropping systems.