**FINAL PROJECT REPORT**


**Project Title**: Improving tools for early detection of brown marmorated stink bug
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**Percentage time per crop:** Apple: 65% Pear: 10% Cherry: 20% Stone Fruit:5%

**Other funding Sources**

**Agency Name:** USDA SCRI awarded to Washington State University, Brunner
**Amt. awarded:** $67,693 over three years (2012-2014)
**Notes:** This SCRI grant provides funds to Washington State University to assess distribution of BMSB in WA and to evaluate pheromone technology. Some of the funding (≈ $40,000) from the WSU portion of the SCRI BMSB budget will be used to support the activities proposed here.

**Agency Name:** USDA SCRI awarded to Oregon State University, Shearer
**Amt. awarded:** $146,995 over three years (2012-2014)
**Notes:** This SCRI grant provides funds to Oregon State University to develop management strategies for BMSB across several crops. The funds requested here are not provided in the SCRI funding.

**Agency Name:** USDA SCRI awarded to USDA-ARS, Leskey
**Amt. awarded:** $559,072 over three years (2012-2014)

**Total Project Funding:** **Year 1**: $110,927 **Year 2:** $39,863

**Budget 1 History**

| Item | 2013 | 2014 |
|---|---|---|
| **Salaries** | 14,080 | 0 |
| **Benefits** | 5,562 | 0 |
| **Wages**[1] (temporary labor) | 11,520 | 7,200 |
| **Benefits** [1] | 1,118 | 698 |
| **Equipment** | 0 | 0 |
| **Supplies**[2] | 500 | 1,000 |
| **Travel**[3] | 5,032 | 1,680 |
| **Plot Fees** | 0 | 0 |
| **Miscellaneous** | 0 | 0 |
| **Total** | 37,812 | 10,578 |

**Budget 2 History**

| Item | 2013 | 2014 |
|---|---|---|
| **Salaries** | 0 | 0 |
| **Benefits** | 0 | 0 |
| **Wages**[1] (temporary labor) | 11,250 | 7,830 |
| **Benefits (10%)** [1] | 1,125 | 4,385 |
| **Equipment** | 9,800 | 0 |
| **Supplies**[2] | 3,000 | 5,500 |
| **Travel**[3] | 1,000 | 1,000 |
| **Plot Fees** | 0 | 0 |
| **Miscellaneous** | 0 | 0 |
| **Total** | 26,175 | 18,715 |

**Budget 3 History**

| Item | 2013 | 2014 |
|---|---|---|
| **Salaries** | 20,822 | 0 |
| **Benefits (35%)** | 7,288 | 0 |
| **Wages**[1] | 13,565 | 7,009 |
| **Benefits (8%)** [1] | 1,085 | 561 |
| **Equipment** | 0 | 0 |
| **Supplies**[2] | 4,180 | 2,000 |
| **Travel**[3] | 0 | 1,000 |
| **Plot Fees** | 0 | 0 |
| **Miscellaneous** | 0 | 0 |
| **Total** | 46,940 | 10,570 |

**OBJECTIVES**:

1. Compare a new BMSB light trap with standard pheromone-baited pyramid trap.
2. Determine the pheromone release rate that optimizes attraction to and capture of BMSB in traps.
3. Determine host-plant odors (kairomones) that enhance attraction/capture of BMSB in pheromone-baited and/or light traps.

**SIGNIFICANT FINDINGS**

**Year one**

1. BMSB were detected in sites considered to have low populations and in three sites considered to have medium populations. At three of the four positive catch sites, BMSB were captured in pheromone-baited traps, NOT in light traps.
2. The release rate of USDA#20 pheromone from a commercially produced lure was low and appeared to be depleted, or stopped releasing, after about one week.
3. The release rate of MDT (a synergist for USDA#20 pheromone) from a commercial were releasing after 21 days.
4. Two compounds known from English holly, which were also present in tree of heaven, hexyl formate (hex) and cis-3-hexen-1-ol (cis3), showed positive response by BMSB in Y-tube bioassays.
5. The kairomones hex and cis3 alone did not capture of BMSB late in 2013. However, when they were combined with MDT there was significant increase in BMSB capture.

**Year two**

1. BMSB were detected at three out of the nine sites with light or light+pheromone traps. The light+pheromone traps captured as many or more bugs than the light only trap. However, traps placed in the same general area as the light traps that were baited with a pheromone only consistently capture more BMSB (64%-93).
2. The release rate from a two-component commercial lure, Sterling Rescue, differed by component. While the release rates of each component (packet) differed they both lasted past the four weeks advertised by the company.
3. Eight potential chemical attractants evaluated for release rates in the laboratory showed short longevity, <20 days, to very extended longevity, >60 days.
4. Using a new olfactometer design one chemical, heptanol, showed promise as an attractant for BMSB. However, when heptanol and four other chemicals, including cis3 evaluated in 2013, all suppressed BMSB capture in traps when they were combined with USDA#20 and MDT.

## RESULTS & DISCUSSION

### OBJECTIVE 1

In 2013, light and pheromone traps were set up in nine locations, five in WA and four in OR. Two light and two pheromone-baited traps were established at each location (Fig 1), with light and pheromone-baited traps alternating. Two locations were in Hood River, OR and two were in the Willamette Valley (Aurora and Talent, OR). There were five locations in WA, one in Vancouver, one in Pringle and one in Underwood and two in the Yakima area near to where BMSB was detected in 2012, Wiley, WA and the Apple Tree Golf Course. Based on results in 2013 we changed our approach and paired a light+pheromone trap and pheromone only trap at each location.

Fig. 1. Arrangement of light and pheromone traps at one location.

Since our objective was to determine what traps might be best at detecting low levels of BMSB we focused on areas where we anticipated housed low to moderate populations. The anticipated BMSB population at each location based on previous detections was rated by the project participants and is shown in Table 1. The Vancouver location was at the WSU extension center on 78$^{th}$ street, which was known to have BMSB but not in high numbers. Traps were placed in an open field but near to habitats that would likely house BMSB. This location was not in the epicenter of BMSB in the area but it, along with the Aurora, OR site, was thought to be the most likely location to easily capture BMSB adults. Most other locations were considered to have low to very low BMSB populations. The low BMSB sites were known to have existing populations in the area but not necessarily at the location where traps were placed. The very low BMSB sites were where only one or two bugs had been previously detected.

In 2013, BMSB were captured at five of the nine sites (Table 1). The sites anticipated to have medium levels of BMSB captured the most bugs, with one site showing high captures. A few BMSB were captured at the Underwood site, but none were captured at the other five sites. The only site to capture BMSB in the light traps was Aurora and at this site 88% of bugs were captured in the pheromone-baited traps.

Table 1. The location, estimated population level and captures in pheromone and light traps in 2013.

| Location | BMSB Population | Pheromone trap | Light trap |
|---|---|---|---|
| Aurora, OR | Medium-low | 216 | 29 |
| Vancouver, WA | Medium | 18 | 0 |
| Prindle, WA | Medium | 13 | 0 |
| Underwood, WA | Low | 4 | 0 |
| Hood River 1 | Low | 0 | 0 |
| Hood River 2 | Low | 0 | 0 |
| Talent, OR | Very Low | 0 | 0 |
| Yakima 1 | Very Low | 0 | 0 |
| Yakima 2 | Very Low | 0 | 0 |

2013 results suggested that light traps might be drawing BMSB to the area of the traps at night but once in the area the bugs entered the pheromone-baited trap instead of the light trap. Based on these results, in 2014 we paired two traps at each locations, both with lights but one with pheromones lure (USDA#20 and MDT) (Fig. 2). There were also two other traps placed at a distance from the light and light+pheromone traps, one baited with pheromone lures and one with no lures.

Fig 2. Light trap with pheromone lure.

In 2014 there were nine locations, three in OR and six in WA (Table 2). In most locations no BMSB were captured. In Underwood where four BMSB were captured in 2013, none were captured in 2014. At sites were BMSB were captured the light+pheromone captured more than in the light-only trap, however, at these same locations more BMSB were captured in traps with a pheromone only. While these data indicate that adding pheromones to light traps enhances BMSB capture it does not appear to be an improvement over traps baited with only pheromones.

Table 2. BMSB captured in traps baited with light only, light+pheromone, pheromone only and a black trap with no pheromone, 2014.

| Location | Light-NO pheromone | Light + pheromone | No lure | Pheromone |
|---|---|---|---|---|
| Pindle, WA | 0 | 0 | | 2 |
| Underwood #1, WA | 0 | 0 | 0 | 0 |
| Underwood #2, WA | 0 | 0 | 0 | 0 |
| Yakima, WA | 0 | 0 | 0 | 0 |
| Talent, OR | 0 | 0 | 0 | 0 |
| Oak Creek, OR | 0 | 2 | 0 | 0 |
| Vancouver 2, WA | 2 | 3 | | 56 |
| Hood River, OR | 3 | 23 | 0 | 63 |
| Vancouver 1, WA | 5 | 172 | 0 | 310 |

Conclusions from two years of BMSB trapping indicates there is added value to adding pheromones to pyramid traps with lights, however, there was no evidence that a trap with lights and pheromones was better at detecting / capturing BMSB in locations where populations were low or moderate.

**OBJECTIVE 2**

In 2013, USDA #20 lures were obtained from the West Virginia ARS laboratory for use in field monitoring associated with the light trapping study (Obj. 1). In addition, ChemTica International provided *Plautia stali* (MDT) lures for use in the light trap study. Ten lures of each type were placed in a fume hood and weight loss was measured over time. The average weight loss in the #20 lures on day 4 was about 0.8 mg/day after which weight loss declined sharply and from day 7 through 25, 0.1 mg/day or less was released. Weight loss from the MDT lures averaged almost 3.5 mg/day between day 0 and day 3. Weight loss declined to about 1.5 mg/day between day 3 and 10 and declined only slightly to 1.3 mg/day between day 10 and day 21. These weight loss data agree with results of weight loss from a different MDT lure assessed in 2011. The #20 BMSB pheromone

seemed to be released very quickly or it is not being released at all or at very low levels after only a few days bringing into question the longevity of these lures in the field.

In 2014 a commercial lure provided by the makers of the Rescue trap were evaluated for release rate based on lure weight loss. The lures came as two-component packets, a green and yellow. The company would not tell us what chemicals were in each packet, but one was thought to contain MDT and the other the USDA pheromone. Weight loss was assessed over 35 days. The average release rate of the yellow packet was 8.9 mg/day while the average release rate of the green packet was 3.0 mg/day. These lures were use in Rescue traps to monitor BMSB in Yakima and Wenatchee areas but no bugs were trapped in 2014.

While we have been able to evaluate the release rates of some commercial or USDA provided lures we have not been able to address the specific objective as stated. The main issue complicating achieving full completion of this objective has been access to technical USDA#20 BMSB pheromone in amounts that can be used to make polyethylene lures for release rate testing and then field evaluations. We have now received a small amount of technical USDA#20 BMSB pheromone and are evaluating release rates from polyethylene lures. We have been promised additional USDA#20 pheromone that can be made up into lures for trapping BMSB in 2015 at different locations throughout WA. There is residual funding in the commission funded project and we are requesting use of those funds to complete this objective and submit an amended final project report in 2016.

**OBJECTIVE 3**

*Olfactometer bioassays.* In 2013, initial studies were conducted evaluating different host plant volatiles using a Y-tube bioassay. Two compounds, hexyl formate (hex) and cis-3-hexen-1-ol (cis3), showed greatest activity in Y-tube bioassays (Fig. 3). In 2014, additional studies were conducted using a four-tube olfactometer setup. Choices included heptanol, MDT, USDA#20 and a blank. Out of positive choices BMSB adult moved to the heptanol 57% of the time, compared to 21% to USDA#20 and 14% to MDT. The four-tube olfactometer appears to provide a good tool for continued assessment of chemicals or plants in the search for kairomones attractive to BMSB.

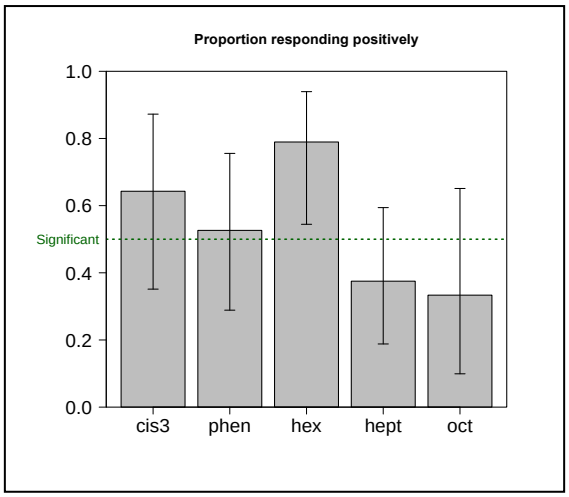


Fig. 3. Proportion response of BMSB in Y-tube bioassay to compounds derived from host plants.

*Field evaluation of kairomones.* At the end of the summer in 2013 some kairomone lures were deployed to the field to determine if they had any biological activity. Kairomone lures were combined with MDT and USDA#20 lures to examine potential synergy. Lures were placed into Rescue ® stink bug traps and hung in hazelnut trees in an abandoned orchard near Tualatin, OR. This orchard was located as a site of high BMSB activity in 2012. Traps were maintained at 50 ft spacing and were rotated weekly. Lures were changed every two weeks. Although the USDA#20+MDT (pheromone + synergist) captured the most BMSB, the kairomone lures cis3

and hex enhanced trap capture when used in combination with MDT compared to MDT alone or un-baited controls (UBC). The compounds cis3 and hex were not attractive on their own (Fig. 4).

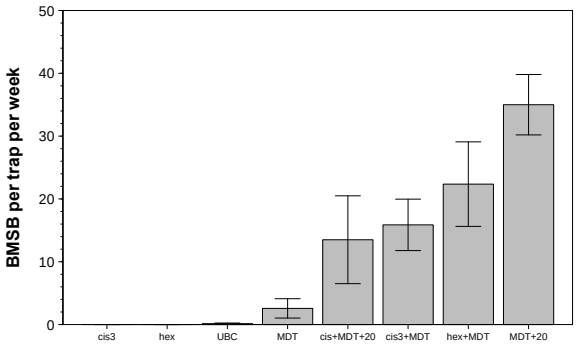


Fig. 4. Average capture of BMSB in traps baited with lures containing candidate plant volatile compounds, pheromones or combinations of volatiles and pheromones.

In 2014 a group of kairomones were placed in sealed polyethylene pouches and evaluated for their relative release rate based weight loss. Table 3 shows the average longevity of the different chemicals. Some like hexyl formate and 6-hexyl formate had fast release while most others had relative slow release. A sub-group of these kairomones were made up in lures and then combined with the BSMB pheromone (USDA#20) or the BMSB synergist (MDT) and evaluated in the field throughout the summer for capture of BMSB. Fig. 5 showed the average BMSB capture in traps baited with different attractants. The USDA#20 plus MDT captured the most BMSB followed by traps baited with only MDT. The addition of any of the kairomone chemicals appeared to suppress captures of BMSB. The combination of cis3+MDT and hex+MDT that showed synergy of BMSB capture were not evaluated in 2014. In addition, heptanol (hept) that looked promising in olfactometry studies did not provide an synergy of BMSB capture when combined with USDA#20 and MDT.

Table 3. Longevity of kairomones placed in polyethylene lures.

| Chemical | Duration in days |
|---|---|
| hexyl formate | 84 |
| cis 3 hexen 1 ol | 69 |
| heptanol | 82 |
| octanol | 95 |
| phenylethanol | 117 |
| 6 cis 3 hexen 1 ol | 89 |
| 6 hexyl formate | 13 |
| hexyl formate | 9 |

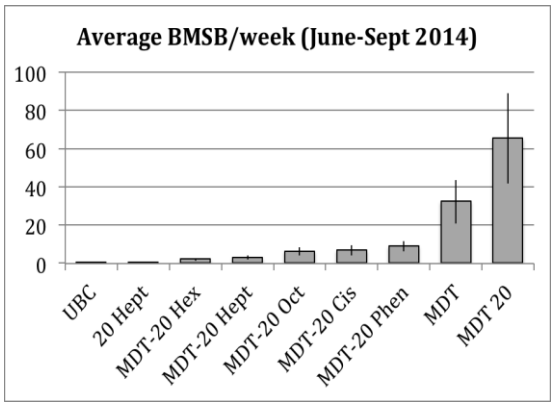


Fig. 5. Average capture of BMSB in traps baited with kairomone lures and USDA#20 and MDT.

The portion of objective 3 associated with the USDA laboratory (Dr. Ashot) was not addressed in this proposal. There was an issue with funding arriving in his laboratory until 2014 when he was fully involved in other aspects of the SCRI national project so he was not able to address his portion of the objective, analyze organic compounds produced in three of the most important early-season host plants for BMSB from the northwest: English holly (*Ilex aquifolium* L.), tree of heaven (*Ailanthus altissima* (Mill.)), and various maple species (*Acer* spp.). Dr. Ashot has assured us that his responsibility for objective 3 will be addressed in 2015 and his will findings will be submitted as part of an amended report to the Technology committee in 2016.

**EXECUTIVE SUMMARY**

Detecting BMSB in an area where populations were low could help improve detection or estimates of this new pest in or near tree fruit crops. Studies in the eastern US pointed to the potential to increase capture of BMSB when including a light source in a pyramid trapping system. This premise was tested in 2013 in areas of OR and WA where BMSB were considered to be at low relative population densities. When pyramid traps with a light source were paired with traps baited with pheromones (USDA#20 plus MDT) the pheromone-baited traps capture more bugs and in a relative sense reflected the expected BMSB populations in a location. In 2014, pheromones were included with a light in one trap while the other trap remained with a light as the only attractant. In addition, other traps in the same area had pheromones as an attractant or were blank. This study showed that indeed when pheromone attractants were added to a trap with a light source more BMSB were captured that in the trap with only a light source. However, the traps with only the pheromone attractants captured more BSMB than the trap with both light and pheromone. The conclusion from this study is that adding a light source to a pyramid tapping system for BMSB did not enhance detection/captures of this pest over a two-year period and is, therefore, not considered a good approach for detecting low-level BMSB populations in an area.

Lures containing the USDA#20 BMSB pheromone and MDT, the pheromone of a closely related stink bug species, were evaluated for release rates by following weight loss. The lures containing USDA#20 pheromone had a low release rate and after about one week appeared to stop releasing pheromone. The lure containing the MDT lasted for at least 21 days. The release rate of a commercially produced two-component lure (Sterling Rescue) for BMSB showed good release of both chemicals over 35 days. Preliminary evaluation of BMSB pheromone USDA#10 using polyethylene packets suggested a high release rate but the amount of pheromone received was so small we were unable to repeat the study. Release rates from polyethylene packets of USDA#20 are being evaluated in the laboratory. These data will inform which lures will be used in 2015 to trap BMSB.

Chemicals identified from BMSB host plants were evaluated in an olfactometer. Initial results indicated two compounds might be attractive to BMSB, hexyl formate (hex) and cis-3-hexen-1-ol (cis3). When these chemicals were put into polyethylene lures and tested in the field in 2013 there was no attraction. However, when each chemical was included with MDT, capture of BMSB was enhanced over MDT alone. In 2014, heptanol was tested in a four-tube olfactometer and showed to be attractive to BMSB relative to MDT and USDA#20 pheromone. When heptanol and four other chemicals, including hex and cis3 evaluated in 2013, were evaluated in the field by combining them with MDT+USDA#20 all reduced capture of BMSB. It is possible that some of these chemicals could enhance capture of MDT but not USDA#20.