FINAL PROJECT REPORT

Project Title: Chlorochroa ligata pheromone and development of management strategies

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Clive Kaiser, Oregon State University, Milton-Freewater, OR
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Other funding sources

Agency Name: California Pistachio Commission
Amt. requested/awarded: $19,353
Notes: Work to be done in CA

Agency Name: Blue Mt. Horticulture Association
Amt. requested/awarded: $5,000
Notes: Work to be done in eastern OR

Agency Name: SCRI
Amt. requested/awarded: funded at $
Notes: Collaborative national project

Total Project Request: Year 1 & 2 plus BMSB: 67,226

Budget History

Budget 1 – Jay Brunner

Item	2010	2011	2011-BMSB	2011-Total
Salaries (Doerr; 0.0166 FTE)	0	5,247	0	5,247
Benefits (Doerr; 0.0166 FTE)	0	1,590	0	1,590
Wages	7,000	6,400	4,000	10,400
Benefits (14.8%)	1,036	1,036	600	1,636
Equipment	0	0	0	0
Supplies (traps and lures)	5,000	0	5,000	5,000
Travel	500	1,000	500	1,500
Miscellaneous	0	0	0	0
Total	13,536	16,373	10,100	25,373

Budget 2 – Jocelyn Millar

Item	**2010**	2011
Salaries (4 weeks post-doc)	6,240	4,160
Benefits (4 weeks post-doc)	1,750	1,167
Wages	0	0
Benefits	0	0
Equipment	0	0
Supplies	5,000	5,000
Travel	0	0
Miscellaneous	0	0
Total	12,990	10,327

Budget 3 – Peter Shearer

Item	2010	2011	2011BMSB
Salaries	0	0	0
Benefits	0	0	0
Wages	0	0	4,000
Benefits (14.8%)	0	0	600
Equipment	0	0	0
Supplies	0	0	0
Travel	0	0	500
Miscellaneous	0	0	0
Total	0	0	5,100

Budget 4 – Todd Murray

Item	2010	2011	2011BMSB
Salaries	0	0	0
Benefits	0	0	0
Wages	0	0	4,000
Benefits (14.8%)	0	0	600
Equipment	0	0	0
Supplies	0	0	0
Travel	0	0	500
Miscellaneous	0	0	0
Total			5,100

OBJECTIVES

1. Improve and scale up the production of the pheromone of Chlorochroa ligata.
2. Determine the optimized release rate for the Chlorochroa ligata pheromone.
3. Develop life history and ecology information for Chlorochroa ligata.
4. Determine the potential for a dual lure for Chlorochroa ligata and Euschistus conspersus.
5. Evaluate the concept of an attract-and-kill station for stink bugs.
6. BMSB: Evaluate/develop monitoring tools and determine distribution of the brown marmorated stink bug.

SIGNIFICANT FINDINGS:

- Synthesis of C. ligata pheromone was challenging due to two steps in the five-step process that represented bottlenecks in production of high quality pheromone. These synthesis problems will make it a challenge to interest companies in making a C. ligata pheromone lure or the lures will be very expensive. The problem is exacerbated by the fact that it is necessary to use large doses of pheromone (50-100 mg) in each lure.

- There was little difference in release rates of C. ligata pheromone from polyethylene lures of different thickness under laboratory or field conditions.

- Over two years traps baited with C. ligata pheromone lures made of polyethylene of different thickness captured low numbers. The thinnest polyethylene lure, 1.5 mil, captured more C. ligata than a commercially produced fiber lure in 2011. Low capture levels were due to low densities of C. ligata in the areas trapped coupled with a low release (evaporation) rate of the pheromone from lures tested. Placing multiple C. ligata pheromone lures (3 or 10) in the same trap increased captures by five to six times compared to a trap with only one lure.

- E. conspersus pheromone release rates from polyethylene lures increased as the thickness decreased. In 2011 the thinnest lure's release rate was about four times that of a commercial lure. In the spring traps baited with the thinnest polyethylene E. conspersus lures (= highest release rate) captured more adults compared to thicker polyethylene lures and a commercial lure. However, in the summer traps baited with a commercial lure and the thickest polyethylene+foil lure captured more E. conspersus adults than traps baited with thinner polyethylene lures.

- Traps baited with the E. conspersus or C. ligata pheromones captured primarily conspecifics, that is, their own species. When lures of both species (combo lure) were placed into the same trap there was no interference in the capture of either species.

- Trap captures of E. conspersus and C. ligata tracked seasonal activity, indicating that each species has only one generation per season (2010 and 2011).

- Laboratory screening of insecticide residues showed that Thiodan, Lannate and two pyrethroids (Danitol and Warrior) were most effective in killing E. conspersus adults. Treating the panels of pyramid traps with insecticide did not prove to be an effective way of intoxicating stink bugs adults, probably because they tended to crawl up the edges of panels.

- Pyramid trap efficiency is limited because stink bug adults can escape. For example, 70% of the stink bug adults placed inside jugs escaped within four days. Placing a kill strip inside the jug or treating the inside of the jug with an insecticide dramatically reduced the number of escapes.

- Large numbers of E. conspersus adults, 527, were trapped from native habitat near a apple orchard notorious for high fruit injury from stink bugs. The area closest to the high density trapping area had the lowest fruit injury, 0.4% compared to the orchard average of 4.4%.
- When trap captures of summer E. conspersus adults near orchard boarders was compared with fruit injury in the border row there was a good relationship ($R^2 = 0.88$), giving hope that traps could be a useful tool in indicating risk of crop damage.
- BMSB was detected at four sites in southwestern WA, representing a slight increase in its distribution in WA. BMSB was also detected in Hood River, WA. No BMSB were detected in eastern WA or Milton-Freewater, OR.

METHODS

Synthesis of C. ligata pheromone (year 1). Millar's laboratory worked on methods of improving and scaling up the synthesis of the C. ligata pheromone to provide the quantities needed for field trials. In published work, Millar's group has shown that (methyl (R)-3-(E)-6-2,3-dihydrofarnesoate, methyl (2E,6E)-farnesoate, and methyl (E)-5-2,6,10-trimethyl-5,9-undecadienoate) are required for attraction in the field. Furthermore, they have shown an equal mixture of the (R)- and (S)-enantiomers (= the racemic form) is attractive. This is critically important, because the racemic form is much cheaper to produce. Working out an efficient synthesis of the pheromone will greatly increase the possibility of the pheromone being commercialized, so that it will become freely available to growers at an affordable price.

Optimize release rate of stink bug pheromone. Pheromones of C. ligata, E. conspersus and T. pallidovirens were put in sealed polyethylene pouches with a cotton wick (Fig. 1), which were then placed in a fume hood in the laboratory and weighed at regular intervals to determine the evaporation (= release rate). After a range of release rates was identified candidate lures were placed in the field to determine which attract the most of each species. Traps baited with different lures were checked every 2-4 days. Bugs trapped during each sample period were identified, counted and sexed. Lure trials were replicated at four to five locations. Unbaited traps served as controls.

Fig. 1

Traps. In 2010 new pyramid traps were constructed from expanded PVC panels and painted yellow. Traps were 4 feet tall with an 18" base. A one-gallon clear plastic jar was fixed to the top to collect bugs (Fig. 2 - left). In 2011 smaller traps, black in color, were used in the trap out (attract and kill) study and were used to monitor the red-shoulder stink bug, Thyanta pallidovirens, and the BMSB (Fig. 2 - right).

Fig. 2

Life history of C. ligata. Trapping began in May to capture overwintering bugs and continued into September. By monitoring over this period we were able to characterize the seasonal life history of C. ligata and compare it to E. conspersus. Trapping focused on the border areas of orchards. Traps were checked weekly, bugs removed, counted, sexed, and life stages recorded.

Dual attraction of C. ligata and E. conspersus. A new trap was placed at each of four locations where we were already sampling for C. ligata and E. conspersus that was baited with a lure for each species (= combo trap). The number and sex of each stink bug was recorded from August 23 through September 13.

Attract-and-kill station for stink bugs. We established colonies of C. ligata and E. conspersus in our greenhouse from field-collected individuals. We used transplanted mullein plant plus beans as a food source. We caged stink bugs (E. conspersus) in plastic cups treated with insecticides and monitored mortality at 1 h, 1d and 3d, after which mortality in the untreated controls increased beyond limits considered acceptable. Residual activity of candidate insecticides was assessed by treating cups with insecticide and placing them in the field for selected periods after which stink bugs were exposed to aged residues. Mortality was recorded 1 h, 1d and 3d after exposure. Stink bug adults were also exposed to insecticide treated panels to determine if they would become intoxicated. Stink bug adults were also exposed to insecticide residues and an insecticide tape inside the plastic jar on top of traps to determine rates of escape and mortality.

In 2011 we placed 20 traps in an uncultivated area of an orchard, which historically had high pressure from E. conspersus to determine if removal of bugs from this area could reduce fruit injury in the nearby orchard. Traps were checked two times per week and the number and sex of bugs recorded. Fruit injury samples were taken in the nearby orchard, Tillman site, prior to harvest in October by examining 20 fruit per tree in four rows starting with the edge tree and each tree at 25 meter intervals within each row.

BMSB: Evaluate/develop monitoring tools and determine distribution of the brown marmorated stink bug. Additional funding was provided to sample for the BMSB. Most of the trapping was done with small black pyramid traps (Fig. 2) with emphasis on the southern part of WA and northern OR. Traps with pheromone lures (Plautia stali (E,E,Z)- 2,4,6-decatrienoate)) were placed out in August. In addition, Master Gardeners were trained by WSU Extension personnel to trap and search for BMSB in western WA.

RESULTS AND DISCUSSION:

Synthesis of stinkbug (C. ligata and E. conspersus) pheromone. The Millar laboratory began synthesizing C. ligata pheromone in late winter of 2009-10. The pheromone synthesis process must have as few steps as possible to minimize costs. Each step must produce clean product with minimal byproducts. Starting materials and intermediates should be as cheap as possible. The synthesis process was challenging because two of the five steps had low yields. Twenty grams of pheromone were delivered on 1 April, 2010 and we began lure release rates studies and trapping studies with polyethylene lures (Obj. 2). The Millar laboratory synthesized another 16 grams of C. ligata pheromone by 1 July, 2010, which was sufficient to complete planned studies lure testing and phenology studies. The Millar laboratory provided another 50 grams of C. ligata pheromone for 2011 research activities, but it is unlikely that the synthesis process can be tweaked to increase yield. We worked with a commercial company, Scentry Biologicals, interested in producing C. ligata pheromone but they ran into the same barriers to efficient synthesis as experienced by the Millar laboratory. They did, however, provide some pheromone and a fiber lure for testing in 2011. Synthesis obstacles will make production of the C. ligata pheromone expensive and, therefore, it is unlikely available as a commercial product given the relative importance of this species.

In 2010 we obtained commercial *E. conspersus* lures and removed pheromone to make our own polyethylene lures (Fig. 1) used in release rate studies. In 2011 the Millar laboratory converted (pear ester) to the *E. conspersus* pheromone so we could conduct field studies on optimization of lures for *E. conspersus* capture and for trap out (attract and kill) studies.

Millar's laboratory also provided pheromone of another stink bug species, *Thyanta pallidovirens*, the red-shoulder stink bug, which was used in monitoring at some sites in 2010 and 2011.

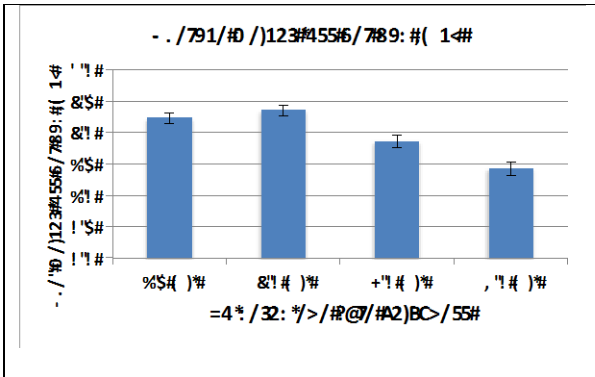


Fig. 3. Average total weight loss (n=4) of *C. ligata* pheromone from polyethylene lures of different thickness.

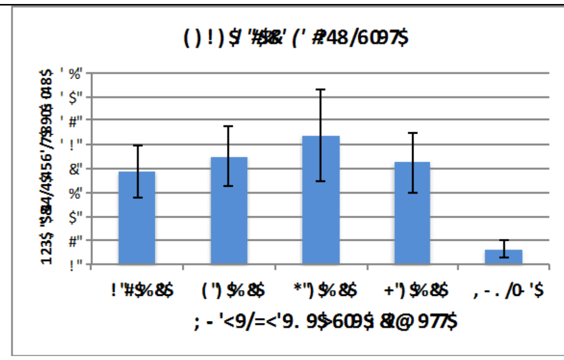


Figure 4. Average cumulative capture of *C. ligata* in traps baited with polyethylene lures of different thickness.

Optimize release rate of stink bug (C. ligata and E. conspersus) pheromone. Under laboratory (fume hood) conditions the release rate of C. ligata pheromone from polyethylene lures of variable thickness showed very little difference (Fig. 3). Evaporation of C. ligata pheromone from a cotton wick was only about 25% higher than the thinnest, 1.5 mil, polyethylene lure. When C. ligata pheromone lures were aged in the field for 49 days and weighed every 7 days pheromone release rate (net weight loss) was low, similar to the laboratory study, with no real differences observed between lures of different polyethylene thickness.

Average (total) capture of C. ligata in traps baited with polyethylene (pheromone) lures was low with no differences in between lures of different thicknesses over a 47-day period (Fig. 4). In 2011 we compared a 1.5 mil polyethylene lure with a C. ligata fiber lure provided by Scentry Biologicals. Captures of C. ligata were low but more adults were captured (LSD p-value = 0.03) in traps baited with the polyethylene lure in the spring, due primarily to a capture of more males while in the summer the same lure captured more adults (LSD p-value = 0.02) due to capturing more females.

Fig. 5. Average weight loss of *E. conspersus* pheromone from polyethylene lures of differing thickness, 2010.

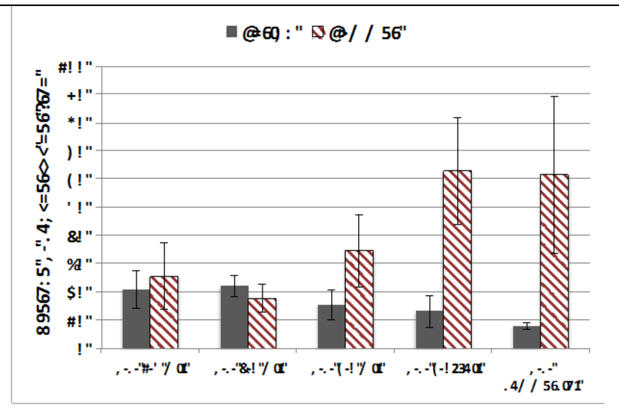


Fig. 6. Average capture of *E. conspersus* adults in pyramid traps baited with polyethylene lures of differing thickness loaded with aggregation pheromone during the spring and summer of 2011.

In an attempt to enhance the attraction of C. ligata pheromone we baited traps with a single lure, three lures, and ten lures. The trap baited with three and ten lures captured between five and six times more C. ligata adults than the trap baited with the single lure. There is some hope that the attraction of C. ligata could be enhanced by using multiple lures in the same trap, but the expense of producing the pheromone could limit this approach.

E. conspersus pheromone is a smaller molecule relative to the C. ligata pheromone and therefore evaporates faster from a substrate or lure. Polyethylene lures of varying thickness provided a very good release profile (Fig. 5). The thinnest E. conspersus lure, 1.5 mil, released 95% of the total pheromone in 10 days while the thickest lure, 6.0 mil, released 42% and a commercial lure released 27% of the total pheromone.

In 2011 we evaluated E. conspersus polyethylene lures of different thickness along with a commercial lure from AlphaScents during the spring and summer. In the spring, more E. conspersus adults were captured in the thinnest lures, 1.5 and 4.0 mil (Fig. 6). Most of the E. conspersus adults captured were females, 70%. In the summer the commercial lure and the thickest polyethylene lure, 6.0 mil+foil, which captured the most E. conspersus adults (Fig. 6), but the female bias was diminished to only 55% during this period. It is unclear as to why there would be a difference in the capture of E. conspersus adults in traps baited with lures of different release rates in the spring versus summer. The most important time for monitoring E. conspersus adults is in the summer so lures releasing pheromone at rates similar to the thickest polyethylene lure with foil and the commercial lure would be the best choice.

We conducted some preliminary release rate studies with the T. pallidovirens pheromone using polyethylene lures. The release rate of this pheromone was even faster than that of E. conspersus with the thinnest lure releasing 81% of the pheromone in only five days in the laboratory. We monitored T. pallidovirens using its pheromone in pyramid traps in several southern WA sites in 2011 but very few bugs were captured.

Stink bug pheromones, at least for the species we have worked with, are highly specific, that is, they primarily attract members of their own species with very little cross over attraction.

Life history of C. ligata and E. conspersus. By combining data from all trapping studies in 2010 and 2011 we were able to characterize the seasonal life history of E. conspersus and C. ligata. Both species have only one generation per year, confirming what has been reported in the literature and earlier studies in WA. New C. ligata adults began appearing in early July in 2010 with captures peaking in late July and early August (Fig. 7). New E. conspersus adults did not appear until late July with a definite peak in mid-August. In 2011 the cool year delayed the appearance of new C. ligata adults and peak activity was in late August and early September. Similar delays in the summer appearance of new E. conspersus adults and peak activity were observed in 2011. (Fig. 7) These data could have been influenced by orchard border spraying, which began in mid- to late-July in most orchards.

Dual attraction of C. ligata and E. conspersus. There appeared to be no impact on placing lures of C. ligata and E. conspersus in the same trap (combo trap). Over a 21 day period in August-September the total number of E. conspersus captured in four combo traps was 96, while the number caught in a trap baited with the commercial lure was 88. The number of C. ligata captured during this period was low but slightly higher in the combo trap, 12, compared to the trap baited with the 1.5 mil polyethylene C. ligata lure, 6 bugs. Certainly there was no negative impact of putting the pheromones of both species in the same trap.

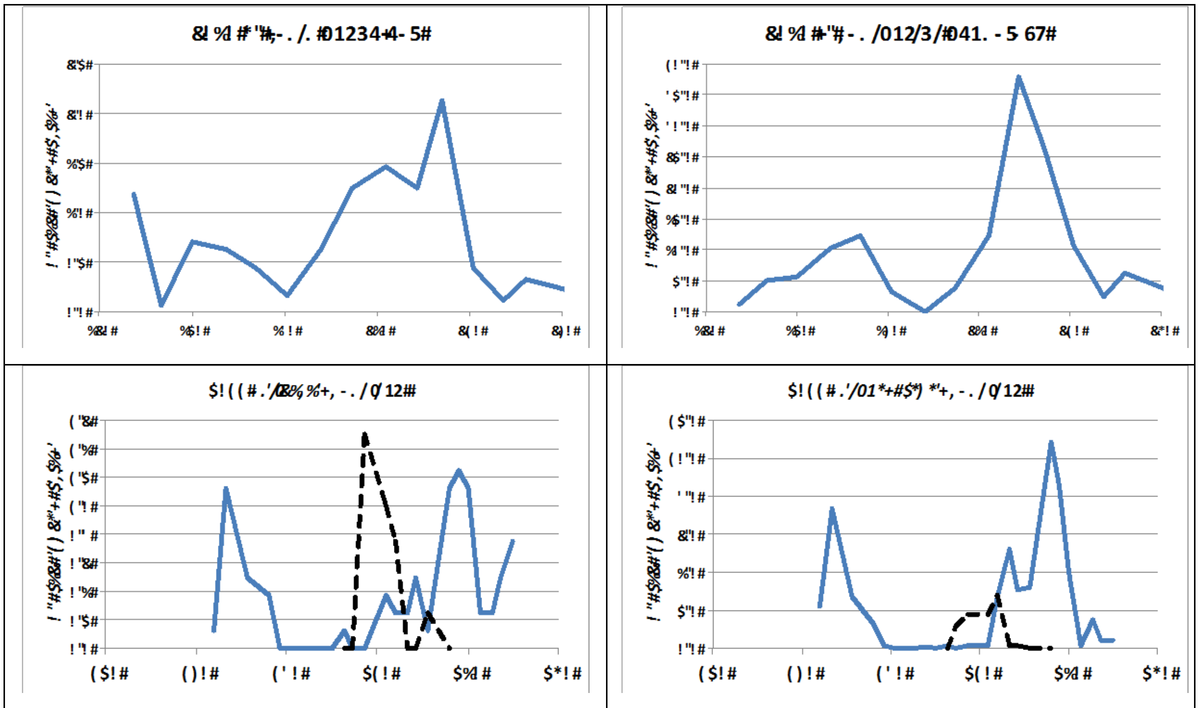


Fig. 7. Seasonal capture of *C. ligata* and *E. conspersus* in traps baited with aggregation pheromone. Dashed line data from Milton-Freewater, OR.

Attract-and-kill station for stink bugs. We evaluated several different insecticides against stink bugs to gain an understanding of which might be the best candidates for attract-and-kill studies. We established colonies of both C. ligata and E. conspersus, but because more E. conspersus were captured early in the season and they began to reproduce sooner we used this species in all our insecticide studies. The initial screen was to expose E. conspersus adults to residues on plastic cups and record mortality over three days. We examine residues of the full field rate in a dilute concentration (1X – typical of a handgun application), a concentration 20% of the full rate (0.25X) and a 4X concentration (equivalent to a typical airblast sprayer application).

Insecticide residues that were most toxic in the initial laboratory screen were the carbamates Lannate (methomyl) and Carzol (formetanate hydrochloride), the chlorinated hydrocarbon, Thiodan (endosulfan) and the synthetic pyrethroids Danitol (fenpropathrin) and Warrior (lambda-cyhalothrin). Most of the newer insecticides were not effective.

While pyrethroids provided a fast 'knock down', that is the bugs appeared dead, but within a short time they had recovered and more were "alive" on day 1 compared to 1h after exposure. We took some of the best insecticides from the initial screen and exposed residues in cups in the field then exposed adult E. conspersus to these. Thiodan provided residues that lasted over 28 days. Warrior residues were not as effective and had a short longevity under field conditions. There was some promise of the Thiodan+Warrior combination giving a fast knock down and good mortality.

Fig. 8

We treated trap panels with insecticides, Thiodan, Warrior or a Thiodan-

Warrior mix, and put the traps in a collecting tray and released 15 E. conspersus adults on the tray (Fig. 8). The release was repeated 5 times for each treatment (75 total bugs). Twenty-four hours after release the location of bugs and mortality was recorded. Many bugs left the trays and were not found. In the control 41% were accounted for after 24h. Few bugs were found dead in the trap, indicating that they were not intoxicated while climbing panels on the way to the trap. This may in part be due to their behavior of climbing primarily on the edges of the panels when moving towards the trap. A few bugs were found dead in the trays suggesting that they became intoxicated when climbing on the panels.

We next treated the inside of the jugs atop the traps with a Thiodan-Warrior mixture or a kill strip containing DVDP. Twenty or twenty-five adult E. conspersus were placed into the jug. This activity was repeated three times. The number of bugs alive and dead in each jug was recorded each day for six days. In the untreated control only 68% of bugs remained in the jug after one day, only 3% were dead. After four days only 30% remained in the untreated control jug and half of these were dead. We therefore know that given the present trap design bugs that enter the jug can escape. The good news was that after day one those bugs remaining in the jug treated with the kill strip (78%) and Thiodan-Warrior (95%) treatments were dead. If traps are to be used as an attract-and-kill device the insecticide should be placed inside the jug to kill bugs after they enter.

Fig. 9

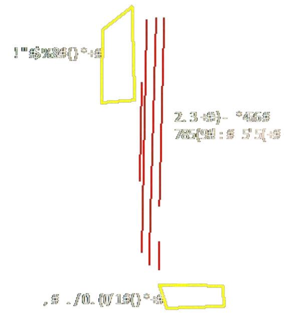
We monitored stink bugs at ten locations in WA during 2010, five over the entire season and five only in the second generation. Since previous research had shown that the highest level of fruit injury from stink bug feeding occurs on orchard borders we sampled ten trees in on the border closest to where traps were placed. We then compared the total capture only of E. conspersus with the fruit injury. There was a good relationship between total captures for the entire season, and for capture only in the summer, and fruit injury (Fig. 9). While these data should be considered preliminary they show promise of using trap captures of E. conspersus as an indicator of the risk of an orchard for fruit injury.

Twenty small black pyramid traps (Fig. 2) were placed in an area of approximately 2 acres of native habitat near an apple orchard with a notorious problem with stink bug damage, Pittman orchard near Manson (Fig. 10 A, C). The 20 traps in the native area captured 527 E. conspersus adults, an average of 26.3 per trap. Traps associated with a lure test located at the southern end of the orchard captured another 410 E. conspersus adults. Monitoring traps placed near the orchard border captured an average of 33.0 E. conspersus adults per trap. These data suggest that the high density of traps had little impact on movement of E. conspersus adults into the orchard.

Just prior to harvest three of the eight rows were sampled, plus a partial row which was closest to the native area, for stink bug injury to fruit. The average percent fruit injury across the entire block was 4.4%. The average percent fruit injury on the northern border, edge and trees in 25 meters in from the edge, was 14.2% (Fig 10D). There was an area of relatively low fruit injury that was closest to the high density trapping area, 0.4%, which suggests an impact of removing a large number of E. conspersus adults. While it is unwise to place too much weight on results from a single location these results may point to a hope of using border trapping at high density as a way to mitigate the impact of E. conspersus adults moving into orchards in late summer.

A

B



C

D

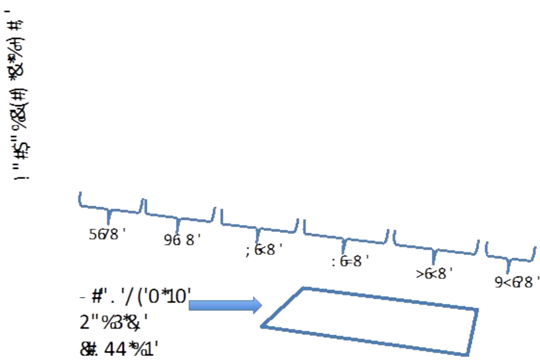


Fig. 10. A - Native area where 20 *E. conspersus* pheromone baited traps were placed (June - Sept.). B - Aerial view of the Pittman orchard site. Red lines show rows of apple sampled for fruit injury. C - View of the Pittman orchard looking southeast to northwest. The site is surrounded by native habitat and is long an narrow. D - Percent fruit injury in the Pittman orchard by tree and by section of orchard.

BMSB: Evaluate/develop monitoring tools and determine distribution of the brown marmorated stink bug (BMSB), Halyomorpha halys (Stal). This is an exotic stink bug species first discovered in the US in the mid-1990s in Pennsylvania (Allentown). In 2004 it was reported from OR, and is now well established in the Portland, OR area. It has since spread north to Vancouver, south to Corvallis, east to Sandy, and has recently been found in Arlington, OR, just across the river from Roosevelt, WA. In 2010 the eastern US reported high levels of damage to many soft fruit and apple orchards and researchers in the mid-Atlantic region report high captures in traps.

In 2010, BMSB was confirmed in Vancouver WA. In 2011, Master Gardener volunteers were recruited to host the modified panel traps through the I-5 corridor in three counties (Clark, Cowlitz and Lewis) and into the Columbia River Gorge (Skamania County). Twenty-seven volunteers (13 in Clark, 6 in Cowlitz, 5 in Lewis, and 3 in Skamania) were recruited and given trap instructions and maintenance requirements. Since no pesticides were used in the survey, volunteers were instructed to check traps each morning. Identification factsheets were also provided to help volunteers distinguish possible BMSB specimens from other true bugs. Along with maintaining the traps, volunteers were directed to make observations of susceptible hosts for the presence BMSB in the immediate area.

Trapping occurred from mid-September to late October. By November 10, 66% of the traps have been returned with potential specimens of BMSB and collections from the immediate area. No BMSB were caught in traps, however, BMSB adults were hand-collected at 4 trapping sites documenting its presence. At the low densities that BMSB is currently present in southwest WA, traps baited with the Plautia stali pheromone do not effectively attract adults. While densities of BMSB in WA are low, the volunteer survey confirmed an expanded distribution to northern Clark County (La Center WA) and as far east as Prindle WA in Skamania County.

No BMSB were collected in traps or from visual observations in eastern WA (Brunner and Walsh) or northeastern OR (Milton-Freewater). A single BMSB was detected in Hood River, OR and numerous BMSB were trapped and collected in the Portland, OR area and south in the Willamette Valley of OR.

Executive summary

This project met the objectives outlined in the original proposal. While we demonstrated that the aggregation for *Chlorochroa ligata* was attractive selective it was very difficult to synthesize which will likely limit its commercial availability due to high cost. The *C. ligata* pheromone is a large molecule and, therefore, has a slow release rate from different lures tested. Efforts to optimize capture of *C. ligata* by using lures with different pheromone release rates was not successful, as all lures captured roughly the same number of bugs. Using multiple lures in a trap did increase capture of *C. ligata* but the cost of using multiple lures seems impractical because of the expected cost of lures. We were able to show that the optimized pheromone release rate for *Euschistus conspersus* was different in the spring, overwintered adults, compared to the summer adults. The good news is that the best commercially available *E. conspersus* lure has an optimized attraction in late summer to detect adults moving into orchard from native habitats. We also showed that putting lures of two stink bug species, *E. conspersus* and *C. ligata*, in the same trap did not inhibit capture of either species. Monitoring *E. conspersus* adults in late summer and fall appeared to be a good indicator of risk of the orchard to fruit injury, that is the more *E. conspersus* adults captured the more injury was detected to fruit on the orchard border. Laboratory screening showed that the carbamates Lannate (methomyl) and Carzol (formetanate hydrochloride), the chlorinated hydrocarbon, Thiodan (endosulfan) and the synthetic pyrethroids Danitol (fenpropathrin) and Warrior (lambda-cyhalothrin) caused highest mortality of *E. conspersus* adults. Treating the panels of pyramid traps with toxicants did not prove to be a good method for attract and kill as bugs tended to crawl up the edges of the panels and were exposed to very little toxicant. Bugs entering traps were able to escape, but putting a Vaportape II kill strip in the trap or coating the inside of the trap with a toxicant reduced or eliminated bugs escaping traps. A mass trapping study provided some encouragement that this approach could provide some protection from orchards at high risk from immigrating stink bug adults in late summer and fall.