<u>Title</u>: **Formulation and Optimization of Pheromone Baits for Stink Bugs**

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Abstract:

A new stink bug trap was designed and tested, based on our observations that stink bugs walk towards pheromone sources; that they have a strong tendency to move upwards; that they do not like to enter dark spaces; and that they can escape readily from standard funnel traps. This and other traps were used to field test pheromone components and mixtures for the species *Chlorochroa uhleri*, *C. sayi*, *C. ligata*, *Acrosternum hilare*, and *Nezara viridula*. Further field tests indicated that combinations of plant odors and pheromones were no more attractive than pheromone alone. A possible reason for the relatively low trap catches overall was also found: at shorter ranges, such as on the same plant, male and female bugs locate each other using vibrational signals transmitted through the plant stem. It may be crucial to incorporate these signals into trap designs in order to attract bugs all the way into pheromone traps.

Introduction:

Several species of stink bugs (Heteroptera: Pentatomidae) can cause damage and yield losses to pome fruit crops in the western United States, including the Consperse stink bug *Euschistus conspersus*, species in the genera *Chlorochroa* and *Thyanta*, and *Acrosternum hilare* (UCIPM 1991). Bug feeding damage results in surface dimpling of

the fruit, and white pithy areas in the flesh. Bugs have also been implicated as vectors of pathogens such as *Botryosphaeria* blight in other fruit and nut crops (e.g., Michailides et al. 1996).

Until recently, bug populations were generally controlled by broad-spectrum pesticides applied against primary pests of pome fruit, such as codling moth. However, decrease in the use of broad-spectrum pesticides in favor of "softer" programs, such as pheromone-based mating disruption of codling moth, has resulted in a resurgence in the importance of several secondary pests, including stink bugs, lygus bugs, fruitworms, and leaf rollers (e.g., McGhee and Brunner 1996). For example, apple and pear crops in the Pacific Northwest in the USDA-sponsored areawide project to control coding moth with pheromone-based mating disruption experienced startling increases in the incidence of damage from stink bugs. This rapid buildup of stink bug populations caught growers by surprise because for the past several decades, stink bugs have been sporadic secondary pests. Consequently, there has been little work on their biology and population dynamics in orchard settings. Thus, growers are now faced with rapid buildups of pest species about which little is known. As an example of how rapidly the problem has surfaced, the relative numbers of the different species and their relative importance in causing damage to tree fruits and other crops is not completely clear. Analogous problems have appeared in transgenic crops such as cotton, in which the products of the introduced genes are effective against the targeted pest (e.g., bollworm), but have limited or no efficacy against secondary insect pests in other orders (e.g., Turnipseed and Greene 1996).

Effective control of bugs depends on rapid detection of bugs as they invade an orchard. Monitoring methods for bugs are still relatively primitive, consisting primarily of beating tray samples, or visual inspections to detect damage; unlike the lepidopteran pests, no pheromone-based trapping systems have been developed for most pest bug species. The development of fast, simple, and sensitive detection methods for stink bug pests using traps baited with sex or aggregation pheromones would allow much more effective monitoring of pear orchards for stink bugs.

In an effort to address this problem, over the past several years our research group identified the pheromones of six agriculturally important stink bugs, including three

Chlorochroa and two *Thyanta* spp., and *Acrosternum hilare*. In field trials, all indications are that the pheromone identifications are correct and complete; pheromone-baited traps attract the targeted species. Furthermore, the pheromone lures attracted significant numbers of specialist parasites (tachinid flies) and predators (sphecid wasps), providing further indirect evidence that the pheromone identifications are correct. Our efforts have now shifted to developing effective traps and trapping protocols for stink bugs, and to investigations of the cheapest and most effective ways of using the pheromone chemicals. This report describes our efforts and the efforts of project collaborators and cooperators to move pheromone trapping of stink bugs forward towards commercialization. Furthermore, we describe rapid recent progress in the study of short-range vibrational signals produced by stink bugs, and their implications for trap development.

Methods and Materials:

Insects: Colonies of *Chlorochroa uhleri, C. sayi, C. ligata, Acrosternum hilare*, and *Thyanta pallidovirens* are maintained at ~25°C and 60% RH in an environmental chamber at UC Riverside. Bugs are fed on organic green beans and raw sunflower seeds, supplemented with bouquets of alfalfa and seasonal weeds.

Traps:

In southern California, field trials were conducted from May through September. From June onwards, we used a custom-made trap whose design was based on our observations of bug behavior in response to pheromone in field-cage trials. The trap (henceforth referred to as the screen trap) consisted of a cylinder of hardware cloth mounted on a wooden pole placed upright in patches of host plants, such as alfalfa, with the pole in contact with the plant material. The bugs crawled from the host plant, up the pole, and into the cylinder. The hardware cloth allowed light to pass through the cylinder so the bugs would continue crawling up once they were on the pole; bugs do not like to enter dark enclosures. The bottom of the cylinder was sealed with an upward-pointing screen cone to direct the bugs upward into the trap. The wooden pole was inserted through the center of the cone, with an opening cut in the cone so that the bugs could

enter the trap. Once inside the trap, and continuing to walk upwards, the bugs encountered a second, downward-pointing screen cone which directed them towards the top of the trap. Openings between the top of this cone and the trap lid allowed the bugs to enter the upper chamber of the trap. The use of this second cone reduced probability of escape from the trap. Both cones and the top of the trap were constructed from hardware cloth to ensure light would pass through the trap. Rubber septa loaded with pheromone were pinned inside the top of the trap and protected from direct sunlight by aluminum foil.

Two commercial traps were tested in central California, the Pherocon Japanese beetle trap and a corn rootworm trap (Trécé Inc., Salinas), along with a custom-built Plexiglas tube trap with inward-pointing screen-cone ends.

A collaborator (Russ Myzell) also ran field trials with *Nezara viridula* and *Acrosternum hilare* lures in Florida, using the Tedder's trap. This trap consists of two perpendicular triangles of plywood fastened at right angles to form a pyramid shape, with a screen cage on top to trap the bugs as they climb up the pyramid shape. The trap is painted safety yellow to add a visual component.

Field trials: All field trials used lures consisting of 11 mm rubber septa loaded with 10 mg doses of the appropriate pheromone unless otherwise specified. Septa were heated (100°C for 6 hr) while pumping under high vacuum to remove volatile impurities from the rubber that might interfere with the pheromone's attractiveness. BHT was added as an antioxidant, at 2% of the pheromone dose.

In all trials, the numbers of bugs trapped were counted at intervals of 1-4 days. Unless otherwise specified, trap catch data from all trials was analyzed by ANOVA of the log (x+1) transformed data, followed by application of a means separation test (e.g., Student-Newman-Kuels tests).

Southern California: Trials were run from early May to the end of September, in patches of Russian thistle, buckwheat, and wild mustard on uncultivated land (so that trials would not disturbed or sprayed out), and in plots of pesticide free alfalfa and green beans at Ag. Ops., UC Riverside.

Central California: Trials for *Chlorochroa sayi*, *Chlorochroa uhleri*, and *Thyanta pallidovirens* using different trap designs were conducted in a 160 acre field of volunteer alfalfa approximately 3 miles north of Goshen, Tulare County, California, in July through September 2000. The alfalfa was not irrigated during 2000, consequently it began to dry down severely by mid-July. However, alfalfa continued to flower through September. A pretrial sample on July 5 showed an average of one stinkbug per 10 standard net sweeps; these samples were approximately 50% *Chlorochroa sayi*, 40% *Chlorochroa uhleri*, and 10% *Thyanta pallidovirens*.

Washington State: Trials were conducted in the border areas of apple and pear orchards. Instead of using traps, mullein plants baited with pheromone lures were used as "trap plants". Stink bugs have been found to congregate on mullein plants, possibly because of their height above the surrounding vegetation, or because of other cues, such as color or odor. The numbers of bugs congregating on each plant were then counted. Trials were run with *C. ligata* and *E. conspersus*.

Florida: Trials were conducted around okra fields in August and September, 2000. Traps were checked at ~0800 and 2000 hr daily for a week. Sweep net samples confirmed the presence of significant populations of *N. viridula* and lighter populations of *A. hilare*. Each treatment was replicated in 6 blocks, with one set of treatments constituting a block.

Plant odors plus pheromone.

A blend of the small, common chemicals that constitute the leafy, green odor of alfalfa, a favorite host plant of stink bugs, was formulated, consisting of Z3-hexenyl acetate (91.6%), Z3 hexenol (8.2%), and E2-hexenal (0.2%). This mixture was tested in concentrated form (100%), and in dilutions with mineral oil (25, 5, 1, 0.33, and 0.1%), using open-topped 2 ml vials as dispensers. Thus, one of these dispensers was put out in combination with a standard pheromone lure consisting of a rubber septum lure loaded with 10 mg of pheromone. Two sequential trials were carried out using the screen trap in southern California with *C. uhleri* as the test species, with plant odor concentrations of 100, 25, 5, 1, and 0% being tested in the first trial, and concentrations of 1, 0.33, 0.1 and 0% being tested in the second trial. Another trial in Washington state was carried out with *Euschistus conspersus* as the test species, using plant odor concentrations of 100, 25,

5, 1, and 0%. In these trials, the pheromone and plant odor dispensers were hung on mullein plants, and the numbers of bugs aggregating on each plant was counted.

Recording of substrate-borne vibrational signals:

Recordings were made with virgin, sexually mature bugs between 0900 and 1700 h, in a quiet, fully enclosed room with fluorescent lighting provided with Sylvania Octron 32W lights. Spectral and temporal characteristics of songs were determined from recordings made from bugs singing on the membrane of a 10 cm diam low-midrange loudspeaker (40-6,000 Hz frequency response, impedance 8 Ω, #WS 13 BF, Visaton, Germany) laid flat on a vibration-damping table. A pair of insects was placed on the speaker cone, and prevented from escaping from the speaker by placing a 10 cm diam translucent plastic ` cylinder over the speaker. Signals were amplified with a custom-built amplifier, then digitized and recorded directly onto the hard drive of a computer. For long term storage, digitized data files were rerecorded onto CDs.

Mating behaviors related to song emissions also were analysed from pairs of bugs placed on a potted bean plant (ca. 25 cm high). Signals transmitted through the bean plant were recorded by placing the membrane of a dynamic microphone cartridge (impedance 600 Ω, 22 mm diameter, 40-22000 Hz frequency response ; #D 3800, AKG, Austria) in contact with the stem 3 cm above the ground. The signal from the microphone was amplified, digitized, and recorded. To begin an experiment, a bug was placed on the lower third of the stem. A second bug of opposite sex was placed at the same start position when the first bug stopped walking and stayed in one place for more than 15 s. Recording was started as soon as one of the pair began to sing. Along with the recordings, behavioral observations were recorded in a notebook so that behaviors could be correlated with song characteristics. Recordings from both types of substrate were analysed using Sound Forge version 4.5 software (Sonic Foundry Inc., Madison WI). Data extracted from recordings included the dominant frequencies, durations and repetition of particular pulses or pulse trains.

RESULTS

1. Trap design:

Trap prototypes were built and tested in spring of 2000, based on the following observations and criteria:

- Stink bugs show a strong tendency to walk upwards on a plant, or, if on the ground, they walk towards the nearest vertical object and climb upwards.

- Stink bugs do not like to enter dark spaces.

- At medium range (10 cm to several meters) from a pheromone source, bugs walk towards the pheromone source rather than flying. Thus, traps must be made accessible to walking insects, so they must be in contact with the ground or better, with plant material.

- Bugs are not easily captured in sticky traps. Bugs walk into traps, and when their feet touch the stickum, they stop, and avoid getting caught.

- Bugs move around a lot inside traps, and frequently find their way out. Thus, traps must be easy for the bugs to enter, but difficult for them to find their way out again.

By mid-June, we had a trap design that fulfilled the above criteria, and this trap was used in all field trials in southern California. The details of the design are described in the materials and methods. The key features were that the trap was transparent, that it was mounted on a pole in contact with foliage, providing a "highway" for responding bugs to walk along, that the trap was placed in the upper part or above the plant canopy so that bugs moved upwards towards the trap, and that the double screen cone design hindered the escape of bugs from traps. The latter point must be emphasized: in earlier prototypes with only a single cone, all bugs placed in the trap had escaped by the following day. Furthermore, the trap is constructed from cheap and readily available materials, and it should be possible to incorporate the basic design features into an analogous trap for use in tree crops. For example, an upward-pointing screen cone wrapped around the trunk or a large roughly vertical scaffold limb could be used to direct bugs upwards into a trap container on the top. We will design and build prototypes for tree crops over the winter.

Field trials in central California tested two commercial trap designs, the corn rootworm trap and the Japanese beetle trap. The corn rootworm trap was completely

ineffective, whereas the Japanese beetle trap did work to some degree. However, in our opinion, the prototype stink bug trap described above will be a better trap for stink bugs than the Japanese beetle trap because the prototype design takes the bugs' biology into account. Results from the field trials are given in subsequent sections that describe tests with specific species.

In trials with pheromone-baited Tedder's traps in Florida during August and September, the traps did catch the targeted species (see below). However, the relatively low catches suggested that this trap may not be the most effective trap for catching either *Nezara viridula* or *Acrosternum hilare*. This result was unexpected, because this trap has been used with some success with stink bug species such as *Euschistus heros*. We are fairly sure that the problem is with the trap or the insects' behavior, rather than the pheromone being incorrect, because the traps/pheromone baits attracted significant numbers of specialist stink bug parasitoids that use the pheromones to locate their stink bug hosts (see below).

2. Pheromone blend optimization for individual species.

Chlorochroa uhleri: Males of this species produce a blend of one major component and two minor components. A series of trials were carried out to determine whether all three components were necessary for attraction. In the first trial, we tested the three component blend versus the major component as a single component (Table 1). The results indicated that the single component was at least as good as if not better than the three-component blend.

Table 1. Numbers of *C. uhleri* caught in screen traps baited with lures consisting of the single major component, or a three-component bait consisting of the major component in combination with the two minor components. Traps were out 26 July to 5 August, $n = 10$. Trap counts were transformed by $\log (x +1)$ and analyzed by a 2-way ANOVA, followed by a Student-Newman-Keuls' test. In all cases, $\alpha = 0.05$.

Treatment	Males Mean ± SE	Females Mean ± SE	Total Mean ± SE
Control	3.2 ± 0.6a	2.2 ± 0.6 a	5.4 ± 1.1 a
3-component*	9.1 ± 1.6 b	11.0 ± 2.8 b	20.1 ± 4.0 b
1-component*	15.0 ± 3.0 c	18.8 ± 3.9 c	33.8 ± 6.6 c

* 3-component blend: racemic methyl (*E*)-6-2,3- dihydrofarnesoate, racemic methyl (*E*)-5-2, 6, 10-trimethyl-5,9-undecadienoate, and methyl (2*E*,6-*E*)-farnesoate, 100: 0.09: 0.06. Single component, racemic methyl (*E*)-6-2,3- dihydrofarnesoate only.

However, in a follow-up trial with the 3-component lure, both 2-component lures, and the major component alone, there was no difference between any of the pheromone-containing lures (Table 2).

Two other points stand out. First, in both trials, all of the pheromone-containing lures were significantly more attractive than the controls, demonstrating that the combination of a pheromone lure and a carefully designed trap has promise for monitoring purposes. Second, the traps caught approximately equal numbers of males and females. This was somewhat unexpected because the pheromone is exclusively male-produced, and it biological terms, it is not clear why it should attract other males as well as females.

Table 2. Numbers of *C. uhleri* caught in screen traps baited with lures consisting of the single major component, the major component plus one minor component, or the full three-component. Traps were out 1 August to 11 August, *n* = 6. Trap counts were transformed by log (*x* +1) and analyzed by a 2-way ANOVA, followed by a Student-Newman-Keuls' test. In all cases, α = 0.05.

Treatment	Males Mean ± SE	Females Mean ± SE	Total Mean ± SE
Control	0.8 ± 0.3 a	0.0[****]	0.8 ± 0.3 a
3-component	5.3 ± 2.3 b	9.3 ± 1.4 a	14.7 ± 3.2 b
1-component*	5.0 ± 1.8 b	11.3 ± 3.6a	16.3 ± 5.2 b
2-component[**]	7.7 ± 1.8 b	5.7 ± 1.6 a	13.3 ± 2.9 b
2-component[***]	6.2 ± 1.3 b	10.3 ± 2.5a	16.5 ± 2.8 b

* Racemic methyl (*E*)-6-2,3- dihydrofarnesoate only

** Major component plus methyl (*E*)-5-2, 6, 10-trimethyl-5,9-undecadienoate

*** Major component plus methyl (2*E*,6-*E*)-farnesoate

**** Data for controls excluded from ANOVA for analysis of female counts because all-zeros cannot be included in ANOVA analysis.

Chlorochroa ligata: As far as we have been able to determine, male *C. ligata* produce the same pheromone as *C. uhleri*, so lures containing this pheromone should be attractive to both species. In the Riverside area, *C. ligata* populations were low, with no bugs detected by sweep netting at field trapping sites. Nevertheless, a total of 20 female *C. ligata* were caught in *C. uhleri* traps over the course of the season, versus no *C. ligata* males or females caught in control traps. This fragmentary data suggests that the pheromone should work equally well for both species.

Chlorochroa sayi: *C. sayi* males also produce a mixture of 3 chemicals, with a major component and two minor components. Although *C. sayi* populations were low at our Riverside field sites, several sets of experiments indicated that the pheromone is attractive. In the first experiment (July 11-21), the 3-component lure attracted

significantly more females (23) and males (15) than control traps (0 males and 1 female). In two other experiments that were put out in southern California to test the attractiveness of the major component alone versus blends, and the pure major component versus technical grade material (76% pure), the numbers of bugs attracted to each treatment were too low to analyze statistically, but overall, pheromone-baited traps caught 26 males and 42 females, versus 3 males and 6 females in control traps during the same period. In another trial run in central California using Japanese beetle traps instead of the screen traps, the pheromone baited traps captured 23 females and 18 males, versus a single female in an equal number of control traps. Furthermore, the pheromone-baited traps also attracted 36 *C. uhleri* nymphs, 43 tachinid flies that parasitize stink bugs, and 130 milichiid flies that may feed on stink bug exudates. These fragmentary data again suggest that the combination of a pheromone lure and a reasonable trap show promise for sampling purposes.

Nezara viridula and *Acrosternum hilare*: Field trials were carried out in and around okra fields in Florida by Dr. Russ Myzell, using Tedder's traps. Control traps caught a single *N. viridula* male and 2 tachinid parasites of stink bugs. Traps baited with the *N. viridula* pheromone blend caught 1male and 19 female *N. viridula*, and 42 tachinid parasites. Traps baited with the *Acrosternum hilare* pheromone blend caught a single male *A. hilare*, but also caught 1 male and 4 female *N. viridula*, as well as 59 tachinid parasites. The stink bug catches and the strong attraction of the stink bug parasites to the pheromone-baited traps suggest that pheromone-baited traps have potential as monitoring tools if better traps and trapping protocols can be developed.

3. Combinations of bug pheromones with plant odors.

Combinations of pheromones and alfalfa odors were tested in southern California (test species *Chlorochroa uhleri*) and Washington (test species *Euschistus conspersus*). The data for the *C. uhleri* trials are shown in Tables 3 and 4. In both trials, all treatments with pheromone caught significantly more bugs than untreated controls. Although there were no statistically significant differences between any of the pheromone + plant odor treatments due in part to the high variability between replicates, the treatments with the

higher concentrations of plant odor (1-100%) caught only about half as many bugs as the pheromone alone, suggesting that the higher doses may be repellent. Conversely, the lowest concentrations of plant odor may have increased the attraction slightly. However, to confirm or deny these trends, trials with a much larger number of replicates will be required.

Table 3. Numbers of *C. uhleri* caught in screen traps baited with combinations of the pheromone plus different concentrations of alfalfa odor. Traps were out 20 July to 30 July, $n = 5$. 2-way ANOVA followed by comparison of all treatments to the control using Dunnett's method showed all treatments containing pheromone were better than the blank control. A separate 2-way ANOVA omitting the control from the analysis showed no significant difference among the treatments containing pheromone.

Treatment	Males Mean ±SE	Females Mean ± SE	Total Mean ± SE
Control	2.0 ± 0.9 a	1.8 ± 0.6 a	3.8 ± 1.4 a
Pheromone alone (Ph)	23.2 ± 14.9 b	25.2 ± 16.8 b	48.4 ± 31.6 b
Ph +100% volatiles	10.0 ± 3.2 b	9.8 ± 3.1 b	19.8 ± 6.2 b
Ph + 25% volatiles	12.0 ± 4.8 b	11.4 ± 3.8 b	23.4 ± 8.4 b
Ph + 5% volatiles	15.6 ± 2.8b	13.8 ± 2.5 b	29.4 ± 5.1b
Ph + 1% volatiles	9.2 ± 4.1a	9.0 ± 4.2b	18.2 ± 8.2 b

Table 4. Numbers of *C. uhleri* caught in screen traps baited with combinations of the pheromone plus different concentrations of alfalfa odor. Traps were out 14 August to 19 September, 2000, $n = 5$. 2-way ANOVA followed by comparison of all treatments to the control using Dunnett's method showed all treatments containing pheromone were better than the blank control. A separate 2-way ANOVA omitting the control from the analysis showed no significant difference among the treatments containing pheromone.

Treatment	Males Mean ± SE	Females Mean ± SE	Total Mean ± SE
Control	7.0 ± 3.4 a	2.4 ± 0.8 a	9.4 ± 3.6 a
Pheromone alone (Ph)	17.8 ± 4.9 b	16.8 ± 3.4 b	34.6 ± 7.9 b
Ph + 1% volatiles	24.4 ± 4.4 b	24.4 ± 5.4 b	48.8 ± 9.2 b
Ph + 0.33% volatiles	30.6 ± 7.1 b	29.0 ± 6.5 b	59.6 ± 13.3 b
Ph + 0.1% volatiles	25.8 ± 6.8 b	27.2 ± 8.6 b	53.0 ± 15.1 b

In the Washington State trials, there were no significant differences between any of the treatments, testing plant odors at concentrations from 100 to 1%, for either *C. ligata* or *E. conspersus*. However, because these trials were done without traps, with the odor dispensers simply hung on mullein plants, the possible repellent effects that were seen with higher concentrations of the plant odors in the southern California trials may not have been manifested.

Overall, with the trials in two widely separated geographic areas, with three stink bug species, we were unable to demonstrate a significant effect of alfalfa plant odors in increasing the attraction to pheromone lures. There may be increases in attraction with

the lower concentrations of the plant odors, but the increases are relatively small, i.e., possibly a two-fold increase in captures, but definitely not an order of magnitude increase.

4. Combining pheromones into baits for more than one species.

Washington state collaborators ran a field trial around the borders of pear orchards, comparing the attraction of *Euschistus conspersus* and *Chlorochroa ligata* to baits optimized for each species individually, and to combination baits containing the pheromones of both species. There was no difference in the number of bugs of either species attracted to the individual lures for each species or the combination bait, suggesting that the pheromone of *E. conspersus* is not inhibitory or repellent to *C. ligata*, and vice versa. We were unable to check combination baits for other species because we were not able to locate suitable field sites containing good populations of two or more species simultaneously. Also, because we have only limited amounts of some of the pheromones, we did not want to waste these valuable materials in random trials, in the hope that bugs would be there. As opportunities arise next season, we will pursue this idea, because it would be very useful to have generic stink bug baits attractive to most or all of the most important species in a particular area, rather than having to put out a different bait for each species.

5. Tests of pure versus technical grade pheromones.

Chlorochroa uhleri: Purified *C. uhleri* pheromone was tested versus technical grade material of about 80% purity in a field trial near Riverside. There was no difference in the number of male or female bugs attracted by either the purified or the technical grade material (Table 5), suggesting that this species is relatively tolerant of impurities in its pheromone.

Table 5. Numbers of *C. uhleri* caught in screen traps baited with lures consisting of the single major component, in purified form or in technical grade form (~80% pure)the major component plus one minor component, or the full three-component. Traps were out 1 August to 11 August, $n = 10$. Trap counts were transformed by $\log (x + 1)$ and analyzed by a 2-way ANOVA, followed by a Student-Newman-Keuls' test. In all cases, $\alpha = 0.05$.

Treatment	Males	Females	Total
	Mean ± SE	Mean ± SE	Mean ± SE
Control	2.9 ± 0.9 a	1.6 ± 0.6 a	4.5 ± 1.3 a
92% pure	9.4 ± 1.7 b	8.6 ± 1.2 b	18.0 ± 2.6 b
80% pure	10.6 ± 2.3 b	8.9 ± 1.2 b	19.5 ± 3.3 b

Chlorochroa sayi: The results of field tests near Riverside and in central California with purified and technical grade *C. sayi* pheromone were indeterminate due to the low populations of C. sayi at those test sites at the times the trials were run. Low numbers of bugs were attracted to both treatments in those particular trials.

6. Substrate-borne vibrations as shorter range signals:

As mentioned earlier, our field trapping experiments over the past two years indicate that the pheromones that we have identified are attractive to bugs, but that we may be missing some of the shorter range cues that will pull the bugs all the way into the traps. In particular, we have observed significant numbers of bugs close to pheromone traps and lures, that had not gone all the way into the traps. A couple of reports in the literature suggested that having been attracted to the vicinity of a pheromone source, at shorter ranges the bugs may find each other by using vibrations that they transmit through the plant. With the help of Dr. Andrej Cokl of the Institute of Biology in Ljubljana, Slovenia, an expert on acoustic communication in insects, we were able to jump-start some pilot experiments to look for these types of signals, with spectacular results (Appendices 1 and 2). We were able to record from the two test species, *Acrosternum hilare* and *Nezara viridula*. Several points were noteworthy:

- both sexes of both species produce vibrational songs (Figs 1 and 2);
- the songs are transmitted and received through the substrate, not through the air as airborne sound;
- the songs can be transmitted distances of a meter or more;
- the bugs produce different songs for calling from a distance, and for courtship, once they are actually in contact;
- these songs are clearly involved in the short-range orientation of one bug to the other.

We believe that these songs may provide the key to increasing trap captures by getting the bugs all the way into the traps. In the coming year, we hope to record the songs produced by all of our target species for which we have identified pheromones. Having recorded the songs, , and we will focus our efforts on developing simple devices for playing these songs back, with the aim of developing traps that incorporate both pheromone lures and vibrational signals. We believe that this may be feasible for two reasons. First, the songs are very simple, consisting of a series of low frequency pulses of about 100 Hz, with the main differences between sexes and species being in the temporal pattern of the pulses rather than differences in frequency. Thus, it should be possible to play the songs back using small, cheap devices, similar to the devices in singing birthday cards. Second, the power output from a bug is limited by its small size, so a battery-powered device should be able to produce a signal of similar amplitude for a period of days to weeks.

SUMMARY

In the past year, we focused on a variety of field experiments, the goal of which was the development of more effective pheromone traps and trapping protocols than the single commercially available stink bug trap, the jug trap for *Euschistus conspersus* (Sierra Ag.). Consideration of the parameters that a walking bug uses to find a pheromone source resulted in a prototype trap design which worked quite effectively with the test species *C. uhleri*, with many more bugs being caught in pheromone-baited traps than in untreated controls. Even when bug captures were low, all the results consistently

pointed towards target species being consistently attracted by the pheromone lures. Several experiments also demonstrated that single-component lures are as effective as the more complicated 3-component blends produced by the three *Chlorochroa* species. However, this is not a general rule: *Acrosternum hilare*, *Nezara viridula*, and *Thyanta pallidovirens* all require at least 2 components in their pheromone blends.

One of the most significant findings was that the stink bugs appear to be using vibrational signals to find each other once they are on the same plant. This may explain why we frequently see as many bugs in the vicinity of a trap as inside the trap, i.e., they are attracted to the vicinity of the pheromone lure, but without the vibrational cues to guide them, they do not move into the trap to the pheromone source. These are relatively short range signals, but even if they act only over a meter or less, they may still be required for optimal attraction of bugs into traps or onto trap plants. In the coming year, we evaluate whether these signals can be incorporated into trap designs along with the pheromone baits.

References cited:

McGhee, P. and J.F. Brunner. 1996. Biology and management of stink bugs in tree fruit. Paper 0423, Annual Meeting of the Entomol. Soc. Am., Louisville KY, Dec. 8-12, 1996.

Michailides, T.J., D. P. Morgan, and A. Sullivan. 1996. Spread of Botryosphaeria dothidea in pistachio orchards of the San Joaquin Valley. Cal. Pistachio Industry Annual Report, Crop Year 1995-96.

Turnipseed, S.G. and J.K. Greene. 1996. Strategies for managing stink bugs in transgenic Bt cotton. Proc. Beltwide Cotton Prod. Conf. 1996: 935-936.

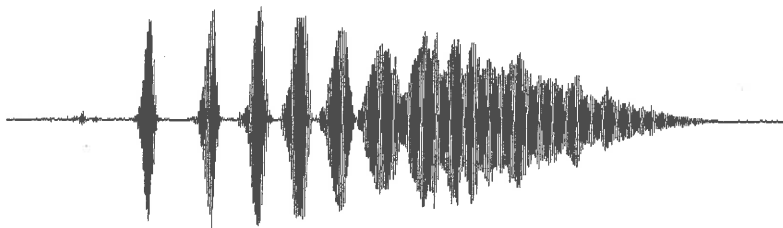UCIPM. 1991. IPM for Apples and Pears. University of California, DANR Pub. #3340.

Appendix 1

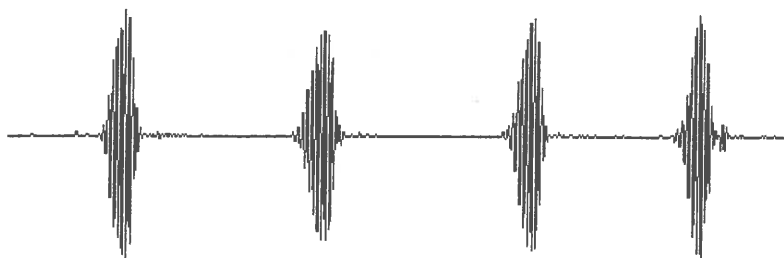## Sonograms of *Acrosternum hilare* substrate-borne vibrational songs

<u>Female calling song</u>
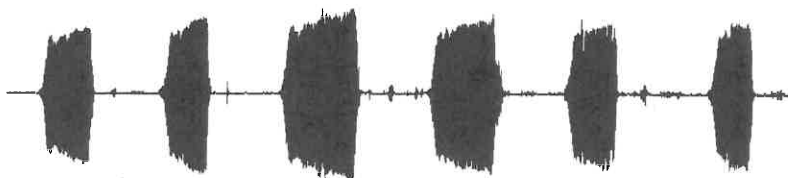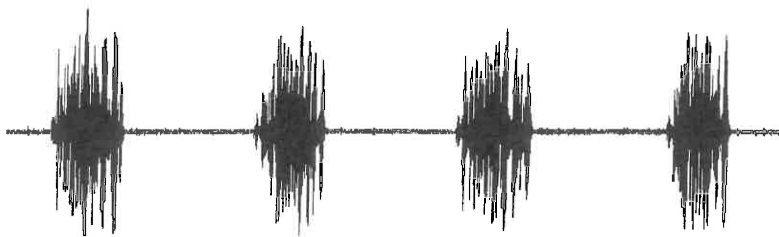
<u>Male calling song</u>

<u>Male courtship song</u>
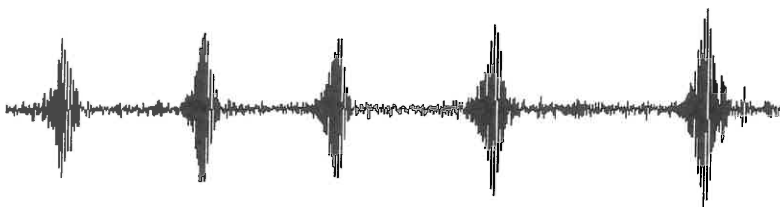
Appendix 2

Sonograms of *Nezara viridula* substrate-borne vibrational songs
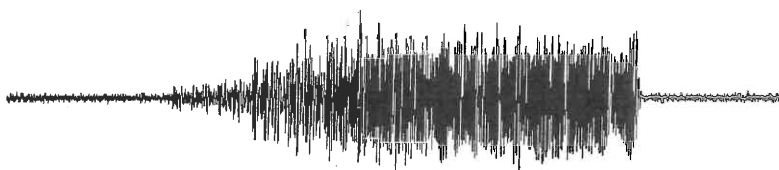
Female calling song

2 s

Male calling song, narrow band

200 ms

Male courtship song

1 s

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