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BIMODAL-TRAPS: FROM BASIC RESEARCH TO A COMMERCIAL PRODUCT

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ABSTRACT

Alternative methods for pest control that substitute pesticides are urgent to reduce agricultural damages while safeguarding human health. One innovative method is the behavioral manipulation of insects by means of vibrations. This article resumes the developmental steps taken in designing the first device that uses vibrational signals as a lure for pest monitoring. The brown marmorated stinkbug, *Halyomorpha halys* (BMSB) was used as a model. In BMSB, intraspecific communication relies on pheromones, at long-distance, and on vibrational signals, at short distance, therefore we created a bimodal trap that combined pheromones with vibrations. In the first instance, we designed in the laboratory of biotremology an optimal attractive vibrational signal (namely FS2) that best elicited male attractiveness. Then, we created two traps with different shapes (jellyfish and pyramid) that were tested in semi-field conditions. The jellyfish shape showed higher capture efficacy and thus it was selected to test the duration of the vibration emission (12h vs versus 24h) in field conditions. The 24h emitting trap captured the highest number of BMSB. Overall, these findings allowed us to make an efficient trap for BMSB monitoring and to demonstrate how pest management can be optimized by coupling semiophysicals and semiochemicals.

Keywords: *vibrational signals, applied biotremology, behavioral manipulation, pest control, insects.*

1. INTRODUCTION

Alternative methods for pest control that substitute pesticides are imperative in order to reduce agricultural damage while safeguarding human health [1]. One innovative method is the behavioral manipulation of insects by means of vibrations, which can be applied to species that use vibrational communication [2,3]. For instance, within the Pentatomidae family, vibrational communication serves as a common mechanism for short-range communication during mating behavior. Inside this group, one insect of high interest for pest control is the brown marmorated stinkbug (BMSB), *Halyomorpha halys* (Hemiptera: Pentatomidae), an Asian polyphagous species that has recently invaded North America and Europe, causing severe economic damage to numerous crops [4,5]. In this species, long-range mating communication is mediated by male emitted aggregation pheromones [6,7], whereas vibrational signals mediate behavioral interactions at short distances [8]. In particular, males' interaction with females is associated with the emission of a pulsed signal by the male (Male Song 2 - MS2) followed by a continuous signaling (Male Song 1 - MS1) to which the female replies with two types of signals (Female Song 1 - FS1 and Female Song 2 - FS2), according to the mating stage [8].

Until now, pest control for BSMB heavily relied on pesticide applications that depended on a monitoring system based on the deployment of traps lured with a twocomponent aggregation pheromone [7,9]. However, this trap does not always ensure that the attracted animals are captured, but instead often leads to the "spillover effect", which means the aggregation of individuals in the proximity of the plant where it is positioned, with a consequent significant damage to the crops in the surroundings [10–12]. Therefore, the use of attractive vibrational signals that can guide the insects inside the trap is a promising solution to the spillover effect. This study reports the steps followed in the course of the years for the development of the first bimodal trap (pheromone + vibration) for a more efficient pest monitoring of BMSB. All statistical analyses for this study were performed with R version 4.0.3 [13], and plots were produced using ggplot2 [14].

2. TRAP DEVELOPMENT

2.1 Optimization of the attractive signal

The first step to develop a trap that uses vibrations for behavioral manipulation is to fully understand the species' communication, which was done by [8]. Using this knowledge, we have adopted the strategy of attracting males of BMSB using the female signal FS2 [see 15]. This signal is characterized by trains of short pulses with pronounced downward frequency modulation, frequency at around 80 and 160Hz Hz and separated by approximately 1.4s long pauses [8].

A series of vibrotaxis experiments were set to test the spectral and temporal characteristics of the female calls that best elicited male responsiveness towards the source [16 for details]. Bioassays were conducted in the laboratory of biotremology of the Fondazione Edmund Mach (FEM), San Michele all'Adige, Italy, between March and August 2018-2019, using artificial signals (emitted by electrodynamic mini-shakers) mimicking the natural female calling signal (FS2). Vibrotaxis choice tests (Fig. 1) were performed on 227 BMSB males on a wooden T-stand arena using the FS2 signal in four different modes: a) 76Hz of dominant frequency + fast pulse repetition time (1s); b) 152 Hz of dominant frequency + standard pulse repetition time (1,5s); c) 76Hz of dominant frequency + standard pulse repetition time (1,5s); and d) 152 Hz of dominant frequency + fast pulse repetition time (1s). The experiments registered the rate of attraction of males toward the source of each signal.

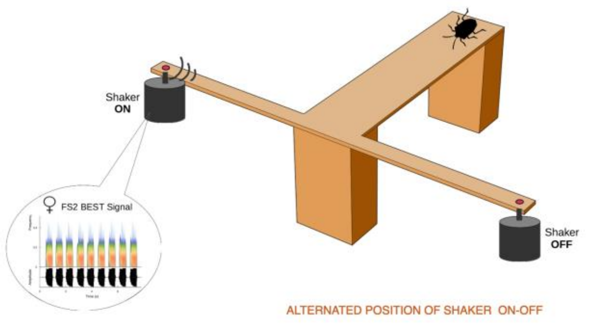

Figure 1. Vibrotaxis choice test on the wooden T-arena [image adapted from 16].

Results showed that males were attracted to FS2 with fast pulse repetition time (1 s), and 76 Hz dominant frequency (equal to the first harmonic of the signal). This resulted in an "optimal" signal (FS2 Best – Fig. 2) that attracted the highest number of males towards a stimulation point on the arena.

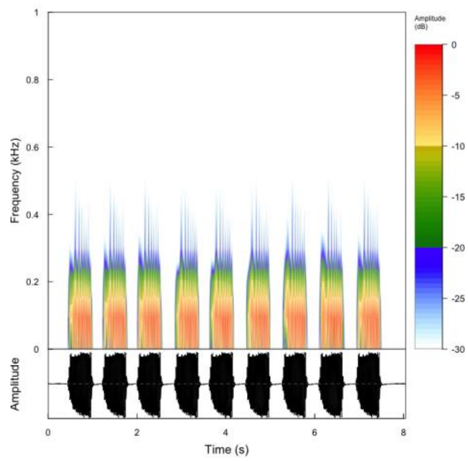

Figure 2. Spectrogram (top) and oscillogram (bottom) of the optimized *Halyomoprha halys* FS2 signal (FS Best).

2.2 Semi-field experiments (bimodal trap prototype)

The next step was to test the "optimal" FS2 signal emitted by a real trap. For this aim, a prototype was designed consisting of a transparent plastic cylinder with a fennel-shape entrance from the bottom base positioned at the top of a black coroplast pyramid 100-cm tall.

A semi-field experiment was conducted in order to compare the efficacy of BMSB captures (adults and nymphs) of a unimodal trap (only pheromone) against the bimodal trap (pheromones + vibrations). Bioassays were performed at FEM under controlled conditions (greenhouse) during June and July 2021 [17 for details]. A total of 15 replicates (with 10 animals each) per category (male/female/nymphs) and per treatment (unimodal/bimodal) were carried out over a seven-week period, totalizing 900 individuals. The results of Wilcoxon test showed that bimodal traps captured a significantly higher number of both males ($p < 0.001$) and females ($p < 0.001$) than unimodal traps, while the number of nymphs did not significantly differ between the two traps (Fig. 3).

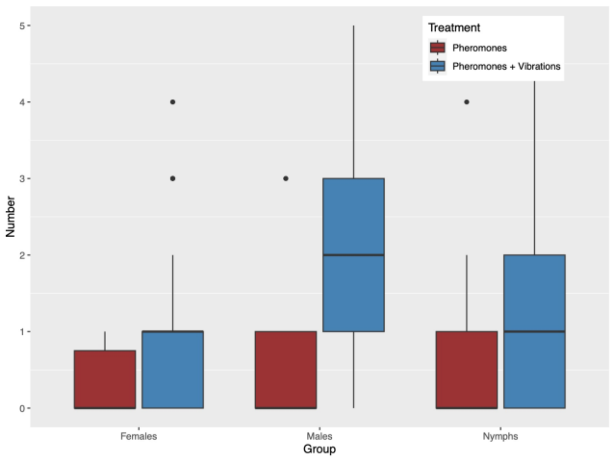

Figure 3. Boxplot of trap capture efficiency (number of individuals) of unimodal traps (pheromones) versus bimodal traps (pheromones+vibrations). The box plot displays the median with a centerline, a variation of 1st and 3rd quartiles represented by the box, a full range of variation (from min to max) represented by "whiskers" and outliers are represented by dots.

2.3 Field experiments (bimodal trap prototype)

The efficiency of the bimodal traps was also tested in the field between May and July 2021 at the external border of two vineyards in FEM [17 for details]. This time four different types of traps were tested, three with only phermones (rescue trap - RR; dual lure - RT; bimodal trap with vibrations off - VOFF) and bimodal with vibration on (pheromone + vibrations - VON). The number of captured BMSB of the bimodal trap was compared to the three unimodal (only pheromone) commercial trap types. The results showed that bimodal traps were highly attractive for BMSB, and again the optimized vibrational FS2 signal (VON) significantly improved the number of captured adults (Fig.4).

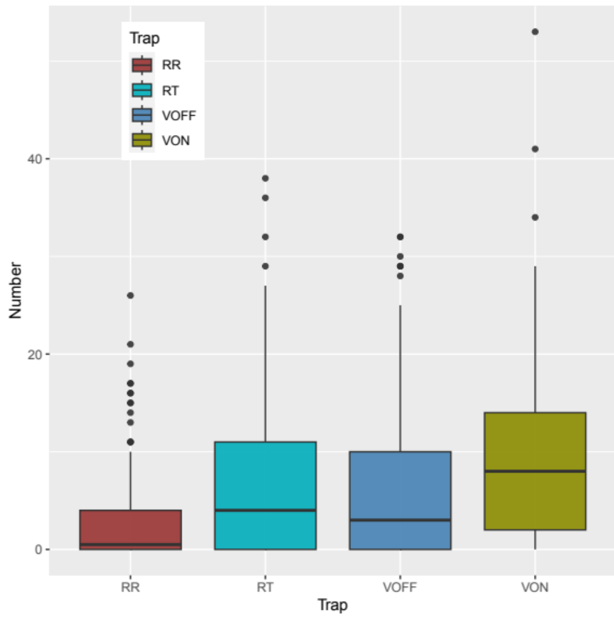

Figure 4. Number of individuals of *Halyomoprha halys* captured with each of the four different type of traps tested in the field: rescue trap (RR), dual lure (RT), bimodal with vibrations off (VOFF) and bimodal vibrations with vibrations on (VON).

2.4 Design efficiency assessment (Jellyfish versus Pyramid)

Since the efficiency of the trap depends also on the ability of the BMSB to climb the vibrational surface and use the directional cue provided by the signal, the shape of the trap is an essential element to consider. Therefore, an experiment was conducted between July and August 2021, inside a greenhouse (FEM) to assess the efficacy of two different models in catching BMSB males: (i) Jellyfish shape and (ii) Pyramid shape (Fig.5).

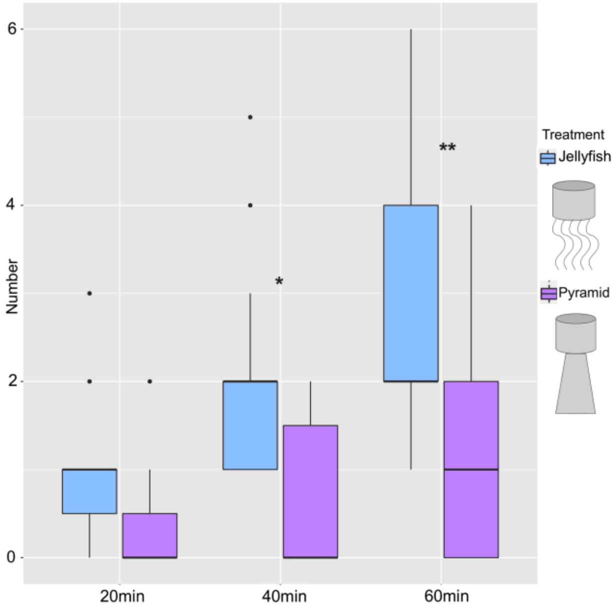

Figure 5. Boxplot of the number of males captured by the Jellyfish and Pyramid traps. The box plot displays the median with a centerline, a variation of 1st and 3rd quartiles represented by the box, a full range of variation (from min to max) represented by "whiskers" above, and below and outliers are represented by the dots. Significant differences among traps were assessed using Wilcoxon test between the two treatments at each time (* $p< 0.05$; ** $p< 0.01$; *** $p< 0.001$).

The jellyfish trap consisted of long plastic stripes that connected the entrance of the funnel to the ground and were used in place of the pyramid panel. The test consisted in placing 10 males inside an insect rearing cage (1m3- Bug-Dorm-6M1010), with either of the two bimodal traps placed at its center (n=15). A total of 300 males were tested. After the introduction of males into the cage, the number of captured individuals was counted every 20 minutes during one hour (three observations per replicate). Insects were not used for more than one trial. The two treatments were always performed simultaneously to minimize any possible effect due to external conditions. Temperature and relative humidity during the experimental time were in the range of 23-29 °C and 50-70 RH. Since data were not normally distributed, to compare the number of individuals captured by the two trap types, a Wilcoxon test for paired data was performed. The results showed that the number of individuals did not differ between the two treatments after 20 min (W=166; p-value=0.15). However, the difference was significant after 40 (W=180; p-value=0.003) and 60 min (W=178.5; p-value=0.005), with a significantly higher number of males captured by the Jellyfish trap (Fig. 5).

2.5 Use of solar panel (12h) versus battery (24h) in field experiments

The next step aimed at describing the BMSB vibrational activity during 24h. To test the effect of the duration of emission of the vibrations by the traps on the capture efficiency, we compared the number of individuals captured by traps emitting the attractive signal for 12 hours and 24 hours. This trial was conducted in the field [see site S2 in 17] and the protocol consisted in deploying traps of (i) control, not connected to energy sources and therefore devoid of any type of vibration, (ii) powered by a solar panel which emitted vibrations for approx. 12 hours and (iii) connected to a battery (Fiamm, model FG21201) capable of supplying electricity (and thus vibrations) for 24 h a day. All the traps were also equipped with a dispenser with specific aggregation pheromone for BMSB (Trécé Inc. Dual Lure). All traps were checked daily at 8:00h and 20:00h. During each check, we removed and counted the number of captured females and males. The experiment was repeated for a total of twenty-two days over a four-week period (September 2022).

To assess the difference among the three types of traps, a non-parametric Kruskal-Wallis test was used. The results showed a significant difference in captures between the different treatments for both sexes (females H=10.6, df=2, p=0.005; males H=17.6, df=2, p=0.0001). Overall, the 24h battery-powered trap was the more effective trap in catching individuals of both sexes (Fig. 6).

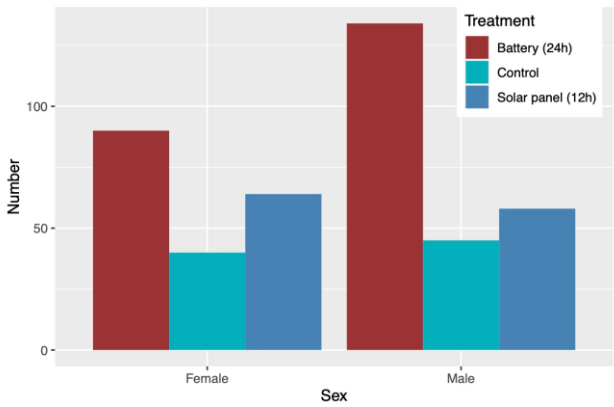

Figure 6. Bar plot of total number of captures of the different traps tested in the field: Control, Solar panel and Battery powered of females and, males forms of *Halyomorpha halys*.

3. CONCLUSIONS

Commercial product: This research allowed us to create an innovative bimodal trap that releases at the same time vibrations and pheromones to capture an insect pest whose mating behavior is mediated by both stimuli that is now commercially available as Shindo Trap (Fig. 7).

Figure 7. Jelly-shaped trap (CBC Europe – Biogard).

We thus demonstrated that pest management can be optimized by integrating semiochemicals and semiophysicals, for a more realistic reproduction of the intraspecific communication of the target species. Furthermore, the use of vibrational for insect attraction towards a trap seems promising also for other species that use these signal for mate attraction, as is the case of other Pentatomids. In fact, species like the Neotropical brow stink bug, *Euschistus heros* or the southern green stink bug, *Nezara viridula*, have already been shown to respond to playback signal [18,19]. These results show the potential of vibratory signals as bait to be incorporated into traps to improve the efficiency of monitoring pests [20]. Moreover, these results represent the conjunction between basic and applied research and are evidence of how the fine knowledge of aspects of behavior and physiology can directly be transferred for the optimization of practical solutions.

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