



## A trap baited with multiple pheromones attracts sympatric hemipteran pests of sweet persimmon



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### ABSTRACT

Sympatric hemipterans such as *Riptortus pedestris* Thunberg (Hemiptera: Alydidae), *Plautia stali* Scott (Hemiptera: Pentatomidae), and *Halyomorpha halys* Stål (Hemiptera: Pentatomidae) are reported as economic pests of persimmon fruits in Korea and Japan. Multi-attractiveness of the aggregation pheromones (AP) of *R. pedestris* and *P. stali* mounted on a single sticky trap was evaluated for the sympatric hemipteran pests of persimmon fruit. Four trap types were prepared; traps mounted with *R. pedestris* AP alone (lure R), *P. stali* AP alone (lure P), *R. pedestris* and *P. stali* AP mixed in a septum (lure R + P), and traps with two parallel lures (lure R/P). *R. pedestris* was equally captured in traps carrying lures R + P and R/P. *P. stali* and *Gymnosoma rotundatum* (Diptera: Tachinidae), a parasitoid of *P. stali*, were attracted to the traps included *P. stali* AP lures; P, R + P, and R/P. All traps carrying lure P attracted *H. halys* and *Glaucias subpunctatus* (Hemiptera: Pentatomidae), a pest of Japanese cedar and cypress, with no significant difference from control. This study shows that a single trap baited with pheromones of *R. pedestris* and *P. stali* together, mixed, or set in parallel, is effective to attract sympatric hemipteran pests of sweet persimmon, *R. pedestris*, *P. stali*, *G. subpunctatus*, and *H. halys*, in addition to *G. rotundatum*. Attracting multispecies simultaneously could be a viable option in managing these sympatric pest species.

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### Introduction

A system of attracting multiple species simultaneously by housing pheromone components of different species in one trap could be useful to manage sympatric pest species. Such a system has been studied to monitor populations of multiple species simultaneously. In coleopteran species, a single trap baited with a single lure containing pheromone components of several species has successfully caught sympatric pests (Dowdy and Mullen, 1998; James et al., 2000; Wong et al., 2012). Attempts to attract sympatric lepidopteran and hymenopteran pests were performed with a single trap baited with a single lure carrying mixed pheromones of different species or with two or more lures set in parallel at a single trap. A single lure containing pheromones of *Malacosoma disstria* (Lepidoptera: Lasocimipidae) and *Choristoneura conflictana* (Lepidoptera: Tortricidae) was as attractive to *M. disstria* and *C. conflictana* males as were traps baited with each species' pheromone alone (Jones et al., 2009). However, other combinations of species

showed that combining lures may not always be possible (Schwalbe and Mastro, 1988; Johansson et al., 2002). Therefore, pheromone-based multispecies trapping system could be applied to some species, but not to all.

Sympatric hemipterans such as *Riptortus pedestris* Thunberg (Hemiptera: Alydidae), *Halyomorpha halys* Stål, and *Plautia stali* Scott (Hemiptera: Pentatomidae) are economic pests of persimmon fruits in Korea and Japan (Chung et al., 1995; Kim et al., 1997; Lee et al., 2002; Kikuhara and Tsutsumi, 2013). These bugs damage by piercing and sucking fruits; the area fed on becomes concave and the color changes from orange to dark blue (Chung et al., 1995). Male-produced aggregation pheromones have been identified for *R. pedestris* and *P. stali*. A mixture of (*E*)-2-hexenyl (*E*)-2-hexenoate (E2HE2H), (*E*)-2-hexenyl (*Z*)-3-hexenoate (E2HZ3H), tetradecyl isobutyrate (C14iBu), and octadecyl isobutyrate (C18iBu) has been identified as aggregation pheromone for *R. pedestris* (Leal et al., 1995; Huh et al., 2005; Yasuda et al., 2007). Methyl (*E,E,Z*)-2,4,6-decatrionoate (EEZ-10Me) has been identified as an aggregation pheromone for *P. stali* (Sugie et al., 1996), and is also attractive to *H. halys* (Khirmian et al., 2007). *Gymnosoma rotundatum* (Linnaeus) (Diptera: Tachinidae), a parasitoid of pentatomid bugs, *P. stali*, *Nezara viridula*, and *N. antennata*, is attracted to EEZ-10Me and is considered to be an important biological control agent of *P. stali* (Higaki, 2003; Shima, 2006; Jang and Park, 2010; Jang et al., 2011).

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Although these hemipteran pests are sympatric, synthetic pheromone lures for *R. pedestris* and *P. stali* have been separately developed by determining optimal ratios and amounts of the pheromone components and dispenser types for attraction (Huh et al., 2005; Jang et al., 2010; Yamanaka et al., 2011). At present, pheromone traps for mass attraction of *R. pedestris* and for monitoring *P. stali* are set separately, which is cost ineffective and impractical too. Using pheromone traps as a management strategy showed the possibility to suppress *R. pedestris* population in persimmon orchard (Kim, 2012). Thus, it would be imperative if a single trap baited with pheromones of more than two species could attract sympatric hemipteran pests without deteriorating their respective efficacy.

In this study, pheromone lures of *R. pedestris* and *P. stali*, mixed or set in pairs, were evaluated for their attractiveness to corresponding species and to the sympatric hemipteran pests such as *H. halys* and *Glaucias subpunctatus* (Hemiptera: Pentatomidae), and also to *G. rotundatum*.

## Materials and methods

### Chemicals

The aggregation pheromone (AP) components of *R. pedestris*, E2HE2H (purity: 90.0%), E2HZ3H (purity: 97.0%), C14iBu (purity: 98%), and C18iBu (purity: 98%) were synthesized in our laboratory according to previous papers (Huh et al., 2005; Kim and Park, 2013). The pheromone of *P. stali*, EEZ-10Me, was purchased from Green Agrotech (Gyeongsan, Korea).

### Lure

A rubber septum (sleeve stopper, 20 mm id; Korea Ace Scientific Co., Seoul, Korea) was used as the pheromone dispenser. Before applying hexane solution of the synthetic chemicals to the rubber septum, the septum was washed with acetone. Once the applied synthetic chemical had been soaked into the septum, it was air-dried in fume hood for 2 h at room temperature, packed into aluminum foil-laminated package and stored in refrigerator until use.

Four types of pheromone lures were prepared; 75 mg of *R. pedestris* AP in a septum (lure R), 40 mg of *P. stali* AP in a septum (lure P), a lure set A and B in parallel on a same sticky board (lure R/P), and a lure mix of 75 mg of *R. pedestris* pheromone and 40 mg of *P. stali* pheromone in a septum (lure R + P) (Fig. S1). In each septum, butyrate hydroxytoluene (BHT) of the same amount to pheromone was used as an antioxidant. The lures for *R. pedestris* contained E2HE2H, E2HZ3H, C14iBu, and C18iBu at the ratio of 1:5:1:0.5.

### Traps

Double-sided yellow sticky traps (350 mm × 250 mm; Green Agrotech, Gyeongsan, Korea) were used. Traps were set at least 12 m apart from each other, suspended approximately 1.5 m above the ground by using wire hung to tree branches. Sticky boards were replaced after checking the catches every week. Pheromone septa

were replaced every 4 weeks. The trap position was re-randomized within each block every week.

### Preliminary field experiment

Attractiveness of two lures, lures R/P and R + P, was compared with the lure of R in the spring of 2011 and with the lures of R and P in the summer of 2011 in the field. A rubber septum impregnated with only BHT was used as a control. In the experiment, one trap for each treatment was placed at a farm of the Gyeongnam National University of Science and Technology, Jinju (35° 9'18.90"N 128° 5'3.63"E) during 22 April to 8 June 2011 (spring season), and at a persimmon orchard (variety: Fuyu), Jinju (35°9'49.82"N, 128°12'4.39"E) during 14 July to 16 September (summer season) in 2011.

### Field experiment

This experiment was performed to confirm the attractiveness of R/P and R + P lures in the field. Lure R was excluded because we confirmed that the single lure R did not attract other hemipteran species except *R. pedestris* from the preliminary field experiment in 2011. Three traps for each lure were placed over the periods, from 18 August to 14 October 2012 at a persimmon orchard, Jinju (35°9'57.86"N 128°11'47.30"E).

### Chemical and quantitative change in pheromone components

To monitor chemical and quantitative changes of the pheromone components in the field, lures were prepared using 2 mL micro-centrifuge tube (Simport, Beloeil, Canada). Because the pheromone of *R. pedestris* in a micro-centrifuge tube is sold in Korea as a lure, we used the micro-centrifuge tube for monitoring chemical and quantitative changes of the pheromone components. Amount and ratio of the pheromone components in lures R, P, and R/P were same with those of the above field tests. After pheromone solutions were poured into centrifuge tubes, hexane was removed by nitrogen gas. The lures in the capped tubes were installed in the field. Quantitative changes of the pheromone components were checked by weighing the lures with an electronic balance (Adventuraer, Ohaus) every 10 days interval for 30 days after trap installation. For monitoring chemical change, 1 mg of the pheromone was taken from the lure at 0 and 30 days after installation. The pheromone taken was dissolved in 1 mL of hexane and was subjected to gas chromatography-mass spectrometry (GC-MS) in electron impact (EI) ionization mode with a GC-2010 (Shimadzu, Kyoto, Japan) interfaced to a GCMS-QP2010 fitted with a HP-Innowax column (30 m × 0.25 mm i.d. × 0.25 μm, J & W Scientific Co.). The oven temperature was programmed as: isothermal at 40 °C for 1 min, then rising at 6 °C/min to 250 °C, and held this temperature for 4 min. Injector and transfer line temperatures were 250 °C.

**Table 1**

Total number<sup>a</sup> of insects attracted to each pheromone treatment during 2011.

Treatment (lure)	Total number of insects caught					
	Spring, 2011 (for 6 weeks)		Summer, 2011 (for 8 weeks)			
	<i>R. pedestris</i>	<i>G. rotundatum</i>	<i>R. pedestris</i>	<i>P. stali</i>	<i>G. rotundatum</i>	<i>H. halys</i>
<i>R. pedestris</i> AP (R)	34	0	253	0	0	0
<i>P. stali</i> AP (P)	– <sup>b</sup>	–	0	0	19	2
Mixed (R + P)	30	15	355	1	12	4
Parallel (R/P)	42	17	227	0	9	9
Control	0	0	0	0	0	0

<sup>a</sup> Total number of captures is presented, because there were no sex-biased captures for the two bugs and a tachinid species.

<sup>b</sup> Not tested.

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