Research Article

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Evaluating the efficacy of two insect detection methods with *Riptortus pedestris* (Hemiptera: Alydidae): portable harmonic radar system and fluorescent marking system

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Abstract

BACKGROUND: Although portable harmonic radar system and fluorescent marking system are two widely used detection methods, their comparative effectiveness has not been studied. Therefore, we first tested the applicability of fluorescent marking system on *Riptortus pedestris* (Hemiptera: Alydidae). Then, we evaluated the efficacy of the two methods used either alone or combined in a grass field and bean field, varying with complexity, during day and night.

RESULTS: Fluorescent marking did not affect the behavior or fitness of *Riptortus pedestris* except for vertical walking, while allowing the detection from >25 m when paired with a handheld laser. Generally, the portable harmonic radar system and both methods combined were more successful in sample detection, although the fluorescent marking system in the bean field at night was as competitive as the two. Combining both methods made sample retrieval easier at night than the portable harmonic radar system. Nevertheless, the total detection time showed a large variance across the methods.

CONCLUSION: The portable harmonic radar system can be an effective detection method in either landscape during both day and night. Furthermore, the fluorescent marking system can be a reliable tool at night as well. Lastly, combining both methods can improve the night detection.

Keywords: movement; dispersal; detectability; tracking; bean bug

1 INTRODUCTION

Field tracking of an organism can provide valuable information on the life history and the behavior of the target in natural settings.[1–3] Two of the key components that determine the efficacy of detection methods are conspicuousness of the marker on target and the effect of detection technique application on the organism.[4] Tracking of large vertebrate organisms has been studied using various tracking methods including radar tracking devices, bands, brands and tags.[5,6] However, smaller organisms including insects and other invertebrates require a more delicate tracking method than vertebrates because their behavior and physiology can easily be altered by the marker and tags.[7–10] For example, Kim *et al.*[10] observed a significant change in the flight behavior of *Ricania* sp. (Hemiptera: Ricaniidae) and *Apis mellifera* (Hymenoptera: Apidae) due to a radar tag attachment although the same tag did not affect the flight behavior of *Riptortus pedestris* (Hemiptera: Alydidae), *Lycorma delicatula* (Hemiptera: Fulgoridae), and *Bombus terrestris* (Hymenoptera: Apidae). Furthermore, the small size of the organisms makes visual detection more challenging.[11] Radar system[9,12] and fluorescent marking[13,14] are generally used to detect insects or other invertebrates[4] among the numerous insect detection methods such as mutilation marking,[15] paint and ink marking,[16,17] genetic marking,[18,19] and elemental marking.[20,21]

Harmonic radar system has been demonstrated as a useful tool to track terrestrial insects.[1,2,22] Conventional radio transponders require batteries to operate, increasing its weight and the loading on the target insects thereby limiting the application only to larger insects.[9] For example, tags used by Hedin and Ranius[23] weighed at least 0.48 g, taking up to 20–25% of the body weight of *Osmoderma eremita* (Coleoptera, Scarabaeidae), a large beetle that weighs 2.2 g for females and 2.4 g for males. Boiteau and Colpitts[8] demonstrated that the flight of *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) which weighs 117 mg might be altered if the weight of the tag exceeded 2.4% of its body weight.[8] However, the tag of the harmonic radar system does not require a battery to operate, leading to minimal loading on the target; Roland *et al.*[7] were able to reduce the weight of the tag down to 0.4 mg.[2,22,24] A transponder attached to a target organism utilizes the signal from the radar as its energy source, amplifying the frequency to a harmonic frequency and re-emitting it.[25,26] Indeed,

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a portable version of the harmonic radar, harmonic direction finder, has been recently used to study various insects such as terrestrial beetles and hemipteran insects.[9,24,27–29] Although it has a shorter range than conventional harmonic radar and only gives the direction without the implication of the location of the tagged individual, it is more affordable and accessible, allowing the portable device to be applicable in various landscapes.[9,30]

Fluorescent marking has been used to observe and study the behavior and dispersal of a large number of insects such as mosquitoes and bees because it is easy to access and apply to large groups of insects.[4,14,31] Traditional pairing of fluorescent marking with ultraviolet (UV) lamps is labor-intensive and inefficient when target animals have high mobility due to its short range of illumination.[13,32] Recently, Rice *et al.*[33] suggested the use of a handheld laser which enabled detection of fluorescent-marked *Halyomorpha halys* (Heteroptera: Pentatomidae) located 40 m away while creating a 1-m diameter field of detection. With its increased range of detection, this new fluorescent marking system allows the researchers to quickly scan larger areas and hence, increasing the potential to be an effective insect detection method.

Although both the portable harmonic radar system and fluorescent marking are widely used detection methods, effectiveness of the techniques may vary in different settings.[4,12,14,27,34] Harmonic direction finder allows remote sensing of tagged individuals behind obstacles such as soil or vegetation.[27] However, as mentioned earlier, the operator is not able to locate the tagged individuals just with the signal from the device because it can only give the direction of the target.[30,35] Furthermore, the effectiveness of the devices reduces in the presence of water or metal.[36] Though, a handheld laser makes fluorescent-marked individual conspicuous, making the detection intrinsic and efficient.[13,33] However, only the marked object in an unobstructed line of sight can be detected by UV-light source. Also, the efficacy of UV lamps or handheld lasers decreases immensely under bright light.[13]

In our study, we assessed the detection efficacy of the portable harmonic radar system and fluorescent marking paired with a handheld laser on a small agricultural insect pest, *Riptortus pedestris*. This insect is a major crop pest in East Asia, causing severe economic damage on multiple leguminous crops, gramineous crops, and fruit trees.[37–39] According to anecdotal evidence, *Riptortus pedestris* may actively seek host plants and avoid chemical pesticides, implying the need for the understanding of its dispersal capacity and pattern to enhance management tactics.[40] The insect is 16 mm long[41] and weighs 41 mg.[10] Previous studies have demonstrated that attachment of 9 mm long harmonic radar tags that weigh 3 mg does not have a significant effect on the longevity, vertical mobility and flight capacity of *Riptortus pedestris*, suggesting its potential in studying the population dynamics of the insect.[10,40] However, how *Riptortus pedestris* may be affected by fluorescent marking is still yet to be understood. Therefore, we first investigated the effect of fluorescent dye on behavior (feeding, walking and flight) and fitness (longevity and fecundity) of adult *Riptortus pedestris* to ensure that it does not adversely affect the organism.[4,42] Furthermore, we measured the detection distance of fluorescent-marked *Riptortus pedestris* to validate if harmless levels of fluorescent marking is viable in insect detection. Finally, we assessed the detection efficacy of each method for both day and night to test the effectiveness of the methods under different light level. We also assessed if using both methods can improve the detection compared to using either method alone. Because a handheld laser can make fluorescent-marked individuals more conspicuous, we assumed that it can assist harmonic direction finder in locating the tagged object especially at night. The experiment was conducted in two agricultural landscapes varying with complexity and obstructiveness, grass field and bean field, both of which are potential habitats of *Riptortus pedestris*.

2 MATERIALS AND METHODS

2.1 Insect

Adult *Riptortus pedestris* individuals used in the experiment were obtained from a laboratory colony collected from wooded areas in Gachon University, Seongnam-si, Gyeonggi-do, South Korea (37° 27′ 2.44″ N 127° 7′ 50.74″ E) using aggregation pheromone traps (Green-Agrotech Co. Ltd, Gyeongsangbuk-do, South Korea). Insects were kept inside of mesh cages (BugDorm-4, MegaView Science Education Services Co., Ltd, Taiwan) and provided with dried soybeans and distilled water containing 0.05% ascorbic acid at 25 ± 2 °C, 30% ± 10% relative humidity (RH) with a photoperiod of 16 h:8 h light:dark. Second instar nymphs of *Riptortus pedesris* was supplied with *Burkholderia* strains (RPE 75),[43] a gut symbiont of *Riptortus pedestris* which is known to enhance the fitness of the insect.[41,44]

2.2 Portable harmonic radar system

For the portable harmonic radar system, a harmonic direction finder manufactured by B. Colpitts and his laboratory at the University of New Brunswick, Fredericton, New Brunswick, Canada was used for the experiment. The harmonic direction finder (*ca*. 10 kg) transmits 9.41 GHz signal which is absorbed and re-emitted in a harmonic frequency by a dipole radar tag (*ca*. 3 mg) that consists of a 9 mm long and 0.3 mm diameter copper wire and a transponder (Schottky diode – Agilent HSCH-5340, Agilent Technologies Inc., Santa Clara, CA, USA). The tag has a 1 mm diameter loop and is bent to 90° at the bottom for the convenience of attachment to the insect. Detailed information on the harmonic direction finder and the tags can be obtained from Boiteau *et al.*[45] and Lee *et al.*[29] When we tagged the insect sample, the pronotum of adult *Riptortus pedestris* was carefully sanded first, and a droplet of cyanoacrylate glue (Loctite 401 Prism Instant Adhesive, Henkel Corporation, Rocky Hill, CT, USA) was applied to ensure a solid attachment.[10,40]

2.3 Fluorescent marking system

For fluorescent marking on *Riptortus pedestris,* red fluorescent paint (Luminous Paint Red 4 Oz, 1166R, BioQuip Products, Rancho Dominguez, CA, USA) was diluted with equal volume of distilled water. Then, the paint solution was sprayed thoroughly on the insect for approximately 2 s (10 mg) using airbrush kit (BBM-001 Beetle Bug Mini Package, YAMATO COMP, Seoul, South Korea). Furthermore, when the insect needed to be both fluorescent-marked and radar-tagged, the insect was first fluorescent-marked and given an hour for the paint to dry before radar tag attachment. A handheld laser (PX 600 mW, class IIIB purple laser, 405 nm, Big Lasers, New York, NY, USA), introduced by Rice *et al.*,[33] was used for the detection of fluorescent-marked insects (Fig. 1).

2.4 Assessment of survivorship, feeding activity, and fecundity

The potential impact of fluorescent marking on survivorship was assessed for 30 unmarked and 30 marked insect groups each consisting 15 males and 15 females in a laboratory experiment. Each individual was placed in a transparent plastic cup [10.5 cm × 9.0 cm

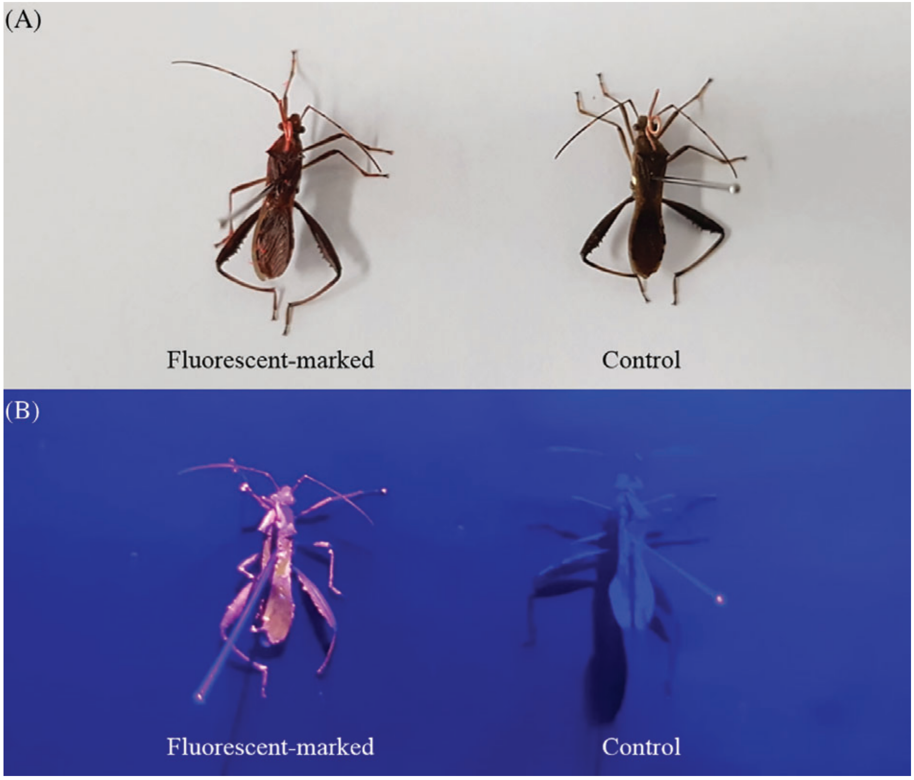

Figure 1. Fluorescent marked and untreated control *Riptortus pedestris* with radar tag attachment under (A) white light, and (B) UV-light in dark.

(diameter × height)] and provided with two dried soybeans and a cotton ball soaked with distilled water containing 0.05% ascorbic acid in a 40 mm petri dish at 25 ± 1 °C and 45% ± 5% RH. Survival of each insect was checked every 12 h over seven days. In the same experiment, we examined the total number of stylet sheaths left on dried soybeans, which is often employed as a measure of feeding activity for hemipteran insects.[46,47] To dye the soybeans, we first soaked each soybean in lactophenol blue solution containing fuchsine acid and distilled water (1:1:1). Lastly, the fecundity of female *Riptortus pedestris* was evaluated by counting the total number of eggs laid inside of each plastic cup after the seven days of observation period; when nymphs already hatched from the eggs, we included the number of empty shells when measuring the fecundity.

2.5 Assessment of walking and flight ability

The potential effect of fluorescent marking on two mobility parameters, vertical walking and flight abilities were evaluated. First, the vertical walking of untreated control and fluorescent-marked *Riptortus pedestris* consisting 15 males and 15 females each was evaluated under laboratory conditions at 25 ± 2 °C and 40% ± 10% RH. Each group was prepared a day before and provided with dried soybeans and distilled water containing 0.05% ascorbic acid in a 40 mm petri dish. At the onset of each trial, an adult *Riptortus pedestris* was randomly selected and placed inside the bottom of a transparent polycarbonate cylinder [6 cm × 36 cm (diameter × height)] with wire mesh attached to the inner surface and subsequently evaluated for its vertical walking distance over 5 min.[46] When the insect reached the 30 cm mark at the top, the recorder reversed the cylinder, so that the adults could continue moving upwards.

Second, the potential influence of fluorescent marking on *Riptortus pedestris* flight behavior was evaluated under field conditions. The experiment was conducted at a soccer field of Gachon University, Seongnam-sii, South Korea from 15:00 to 17:15 h (sunrise at 05:44 h and sunset at 19:30 h) at 33 ± 2 °C and 50% ± 10% RH. The experiment was conducted with 30 untreated control and 30 fluorescent-marked individuals, after three individuals from each group, which failed to take off within the first 5 min were excluded. Tested insects were prepared the day before with same method as the individuals from vertical walking evaluation. When the experiment started, the recorder placed adult *Riptortus pedestris* at the top of a Dead-Inn Pyramid Trap (AgBio Development Inc., Westminster, CO, USA) [49.0 cm × 108.8 cm (base × height)] which was set at the center of the soccer field. When adult *Riptortus pedestris* initiated flight within 5 min, time until takeoff, flight direction and whether adults made over 30 m of sustained flight were recorded.

2.6 Detection distance of fluorescent marking system

To assess the potential of the fluorescent marking system on *Riptortus pedestris*, maximum detection distance of fluorescent-marked individuals was measured in an indoor gym at Gachon University under dark. Two Dead-Inn Pyramid Traps were placed 3 m apart. Each trap was vertically divided into three even sections. A recorder pinned a marked insect on random one of six sections. An operator with a handheld laser stood 30 m away from the center of the two traps. Once the experiment started, the operator aimed the laser to the traps and scanned them carefully. If the operator could not spot the marked insect, the operator moved 1 m towards the traps until the location of the marked insect was correctly identified. The distance from the marked insect to the operator was recorded. Three operators participated in the experiment and it was replicated 30 times with batteries changed after every five replications.

2.7 Detection efficacy

The efficacy of three detection methods, the portable harmonic radar system, the fluorescent marking system and both methods combined was assessed in two different landscapes varying with visual obstructiveness. In each landscape, the effect of light availability (day or night) on the detection efficacy was evaluated. The first landscape chosen was a grass field [25 m × 10 m (width × height)] with grass approximately 10 cm tall in Gachon University (37° 27′ 2.44′′ N 127° 7′ 50.74′′ E). The second landscape chosen was a right-triangle-shaped bean field [28 m × 18 m (base × height)] in Mugap-ri, Chowul-up, Gwangju-si, South Korea (37° 25′ 27.11′′ N 127° 19′ 40.48′′ E). The bean plants were 0.5–1 m tall and at R6–R7 growth stage.

The experiment was conducted as follows. Starting from a fixed position for each landscape, an operator attempted to detect a hidden adult *Riptortus pedestris* sample in the arena using either one of three methods. For the portable harmonic radar system, the operator searched for a radar-tagged *Riptortus pedestris* using a harmonic direction finder. During the night experiment, the operator was assisted with a handheld flashlight. For the fluorescent marking system, the operator used a handheld laser to locate fluorescent-marked samples. Finally, when using both methods, the operator first sought a radar-tagged and fluorescent-marked sample using a harmonic direction finder. Then, after the device recognized a signal from the sample, the operator used a laser to detect the insect.

In each trial, a recorder randomly placed an insect sample inside of the experimental arena. In the grass field, the insect was pinned on the ground whereas it was pinned on the adaxial surface of bean plant in the field. Whether the operator successfully retrieved the insect sample within 10 min and the time until the detection were recorded. Finally, when using the portable harmonic radar system or both combined, the operator notified the recorder when the operator recognized a positive signal (signal detection) for the first time. The experiment was replicated 30 times each in different landscape for both during day and night for all three detection methods. The day experiment was conducted after sunrise (06:45–07:00 h) between 10:00 and 16:00 h. For the night experiment, the trial was conducted past sunset (17:30–17:55 h), between 19:00 and 23:00 h to minimize the level of light. From the experiment, the detection rate, total detection time and time delay (the time between signal detection by the harmonic direction finder to sample retrieval) were obtained to analyze the efficacy of the detection method.

2.8 Data analysis

Effect of fluorescent marking on *Riptortus pedestris* was first analyzed by comparing the performances of untreated control and fluorescent-marked group. The survivorship, proportion of successful flights and flight directions of the two groups were analyzed using Pearson's chi-square test. However, feeding activity, fecundity of the females, vertical walking, and the time until takeoff were compared between the two groups using a two-tailed t-test with α level set at 0.05. For the results with non-significant differences, we retrospectively calculated the minimum differences which could have been detected using t-test with the following equation[48]:

$$D = t \times \sqrt{\left(\mathrm{SE}_1^2 + \mathrm{SE}_2^2\right)}.$$

where D is the minimum difference between the performance means of the untreated control and fluorescent-marked groups, t is a t-value for each corresponding degrees of freedom at $\alpha = 0.05$, and SE_1 and SE_2 are the standard errors for the untreated control and fluorescent-marked groups respectively.

The detection rate, total detection time and time delay were analyzed to assess the efficacy of each detection method at different settings. For the detection rate, a nominal logistic regression was used to investigate the individual and interactive effects of three fixed factors: (i) the landscape (grass field *versus* bean field), (ii) time of experiment (day *versus* night), and (iii) detection method (portable harmonic radar system, fluorescent marking system *versus* both methods combined). For the total detection time, a general linear model was used to assess the significance of the three factors as well as the interaction among the three. Additionally, we assessed if the fluorescent marking system can improve the portable harmonic radar system when used together; we evaluated if the time delay can be reduced when the two methods were combined compared to using only the harmonic radar system. The effect of the three factors and the interactive effects among them were analyzed using a general linear model. Then, the detection rate and total detection time were evaluated across detection methods using analysis of variance (ANOVA) whereas the time delay was compared using t-test for each setting (landscape and time of experiment). All the statistical analysis were executed using JMP software (JMP®, Version 12. SAS Institute Inc., Cary, NC, 1989–2007).

3 RESULTS

3.1 Assessment of survivorship, feeding activity, and fecundity

The survivorship of *Riptortus pedestris* adults did not significantly differ between the untreated control and fluorescent-marked groups (Table 1). For the feeding activity, there was no significant difference in the number of stylet sheathes remaining on the soybeans between the two groups (Table 1). In addition, the fecundity of *Riptortus pedestris* female was not significantly affected by fluorescent marking (Table 1). Retrospective analyses showed that the differences in the means that could have been detected by t-test were 4.0 and 15.8 for feeding activity and fecundity respectively.

3.2 Assessment of walking and flight ability

The vertical walking of *Riptortus pedestris* was significantly increased in the fluorescent-marked group compared to untreated control ($t = 3.55$, df = 1, $P < 0.05$) (Table 1). However, the flight ability was not affected by fluorescent marking; neither the time until takeoff nor the number of individuals which successfully made a 30-m sustained flight was significantly different between the two groups (Table 1). The minimum difference to be detected for the time until takeoff was 14.2 s. When the flight directions of all the insects that took off were compared between the two groups, the flight direction of fluorescent-marked *Riptortus pedestris* leaned towards west, whereas prevailing flight direction of the untreated control group was along the northwest and southeast axis (Fig. 2). However, no statistical difference was observed between the two groups ($\chi^2 = 11.33$, df = 7, $P = 0.12$).

3.3 Detection distance of fluorescent marking system

The mean detection distance of fluorescent-marked *Riptortus pedestris* individuals (distance between operator and marked insect) was 27.1 ± 1.2 m [mean ± standard error (SE)] (Fig. 3).

Table 1. Summary of the effects of fluorescent marking on the fitness and behavior of *Riptortus pedestris*

Performance	Measure	Control group (Mean ± SE)	Fluorescent marked group (Mean ± SE)	Effect of marking(P)
Survivorship	Proportion of surviving insects (%)	93.3 ± 4.6	86.7 ± 6.2	0.39
Feeding activity	Number of sheaths remaining on beans	8.8 ± 1.3	9.8 ± 1.5	0.52
Fecundity	Number of eggs laid	38.9 ± 5.8	29.5 ± 5.1	0.23
Vertical mobility	Vertical distance traveled (cm)	93.2 ± 12.1	234.6 ± 42.8	< 0.05
Flight behavior	Proportion of sustained flights over 30 m (%)	90.0 ± 5.5	93.3 ± 4.6	0.64
	Time until takeoff (s)	10.9 ± 6.9	4.3 ± 1.7	0.36

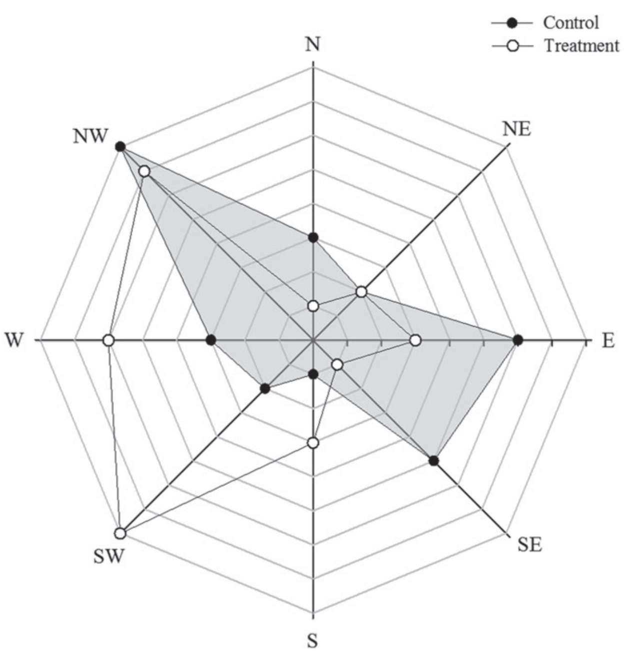

Figure 2. Flight direction of *Riptortus pedestris* in untreated control and fluorescent marked groups.

Overall, the detection distance became longer as the trials had progressed suggesting that operators might have become more effective in finding the marked target over the duration of experiment.

3.4 Detection efficacy

Regression analyses were used to evaluate whether the three factors (landscape, time of experiment, and detection method) affected the detection efficacy to find marked insects (detection rate, total detection time, and time delay). First, the detection rate was significantly affected by all three fixed factors ($P < 0.05$) (Table 2), whereas none of their interaction terms were significant. Especially for the detection method, the detection rate of the fluorescent marking system was significantly lower compared to either the portable harmonic radar system (odds ratio = 400.50, $P < 0.05$) or both methods combined (odds ratio = 390.65, $P < 0.05$). However, the detection rates of the other two detection methods did not differ significantly (odds ratio = 0.97, $P = 1.00$). Likewise, the three fixed factors significantly affected the total detection time ($P < 0.05$) (Table 3), but their interaction terms did not. Especially, only the total detection time of combined methods did not differ with either of the methods (Tukey's HSD, $P < 0.05$). For the time delay (the time between signal detection by the harmonic direction finder to visual detection of the insect sample), the detection methods significantly affected the period between the signal detection and sample retrieval ($P < 0.05$) (Table 3), but neither landscape nor time of experiment did. Among the interaction terms of the three factors, the interaction between time of experiment and detection method was significant ($P < 0.05$) (Table 3).

We further compared the detection efficacy across the detection methods tested for each setting, which consists of the landscape and time of experiment. In the grass field, the fluorescent marking system yielded the lowest detection rate compared to the portable harmonic radar system or both methods combined, regardless of time of experiment (day: $\chi^2 = 37.43$, df = 2, $P < 0.05$; night: $\chi^2 = 15.18$, df = 2, $P < 0.05$) (Fig. 4(A)). In the bean field, the results were similar to the grass field during the day but they were not at night: the detection rate was lowest with the fluorescent marking system during the day ($\chi^2 = 29.20$, df = 2, $P < 0.05$) whereas there was no significant difference among the three methods at night ($\chi^2 = 1.58$, df = 2, $P = 0.46$) (Fig. 4(B)). For the total detection time, there was no significant difference among the three detection methods in all settings (Fig. 5). Lastly, the time delay significantly differed between the two detection methods tested only at night, regardless of the landscape (grass field: $t = 2.25$, df = 1, $P < 0.05$; bean field: $t = 3.16$, df = 1, $P < 0.05$) (Table 4).

4 DISCUSSION AND CONCLUSION

Although fluorescent marking system has been employed when monitoring multiple insect species, Rice *et al.*[33] were the first to evaluate and demonstrate the potential of a handheld laser in detection of fluorescent-marked insects from a long distance.[13,14,31] Nevertheless, although the results by Rice *et al.*[33] suggest the effectiveness of a handheld laser among the other detection devices on fluorescent-marked insects, whether the method was viable in detecting living insects, especially *Riptortus pedestris*, was not provided because the experiment was conducted with dead *H. halys* samples. The response of insects to chemicals may vary respective of the compounds and the application method,[49] and likewise, the effect of the fluorescent marking on a living organism varies with the species of the insect and the method used to mark the insect. However, the effect of the spraying method on *Riptortus pedestris* was not tested previously. Therefore, our study first tested the impact of fluorescent-marking on adult *Riptortus pedestris* and subsequently assessed its applicability.

In our experiment, although the vertical walking of fluorescent marked insects increased by an average of 2.5 times compared with the untreated control, fluorescent marking did not affect the fitness (longevity and fecundity), feeding behavior, or flight ability of *Riptortus pedestris*. Tagging and marking may affect the

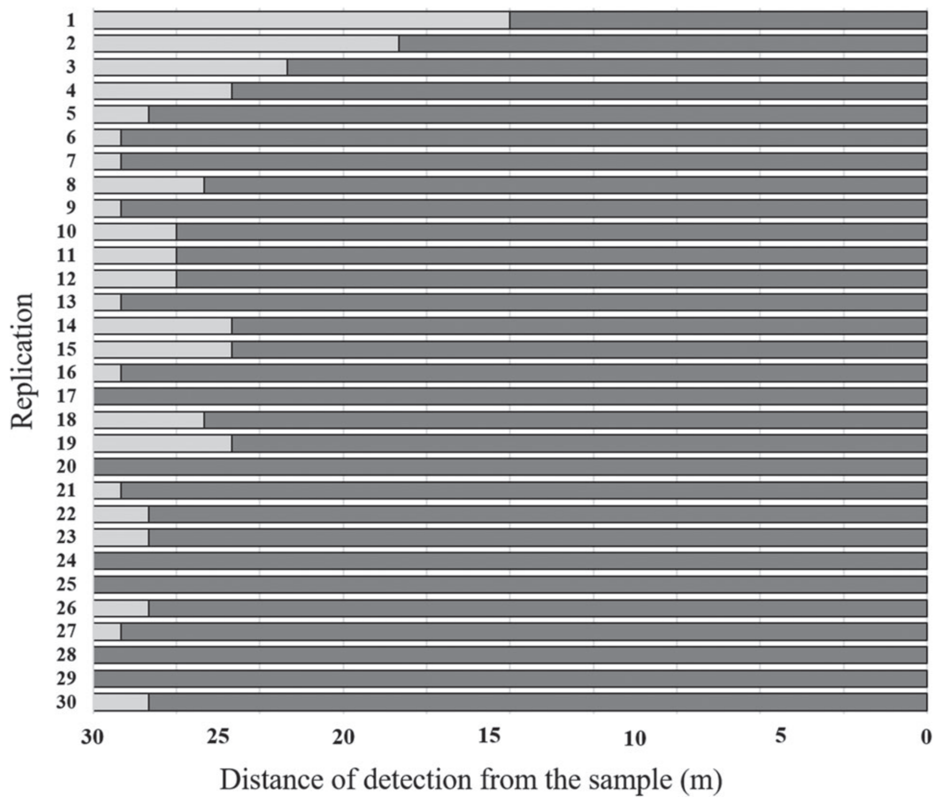

Figure 3. Detection distance of fluorescent marked *Riptortus pedestris* (distance between operator and marked insect) when operator attempted to find the marked individual using a handheld UV laser under dark.

Table 2. Analysis of effect of three fixed factors (landscape, time of experiment and detection method) on detection efficacy (detection rate, total detection time, and time delay) using regression models

Detection efficacy	Factor	df	χ^2	P
Detection rate	Landscape	1	10.97	< 0.05
	Time of experiment	1	36.28	< 0.05
	Detection method	2	55.41	< 0.05
	Landscape × Time of experiment	1	1.37	0.24
	Landscape × Method	2	5.81	0.06
	Time of experiment × Method	2	0.19	0.91
	Landscape × Time of experiment × Method	2	4.34	0.11

Table 3. Analysis of effect of three fixed factors, landscape, time of experiment and method, on total detection time using general linear model for detection time and time delay

Detection efficacy	Factor	df	F	P
Total detection time	Landscape	1, 261	8.41	< 0.05
	Time of experiment	1, 261	9.28	< 0.05
	Detection method	2, 261	4.49	< 0.05
	Landscape × Time of experiment	1, 261	0.03	0.86
	Landscape × Method	2, 261	2.35	0.10
	Time of experiment × Method	2, 261	1.39	0.25
	Landscape × Time of experiment × Method	2, 261	0.16	0.86
Time delay[a]	Landscape	1, 205	1.58	0.21
	Time of experiment	1, 205	1.08	0.30
	Detection method	1, 205	5.59	< 0.05
	Landscape × Time of experiment	1, 205	0.16	0.69
	Landscape × Method	1, 205	0.01	0.98
	Time of experiment × Method	1, 205	11.93	< 0.05
	Landscape × Time of experiment × Method	1, 205	0.03	0.85

[a] For time delay, only two methods (portable harmonic radar system and both methods combined) were compared.

physiology or behavior of the insect depending on the suitability of their materials and methods.[10,50–52] When they influence the target organisms they tend to reduce or hamper the mobility or survivorship; Kim *et al.*[10] observed a decrease in flight capacity of *Ricania* sp. and *A. mellifera* due to a radar tag attachment. Also, when different levels of fluorescent dye was applied to *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae), only the dye application of the highest amount reduced the number of insects that successfully flew.[50] However, to our knowledge, this was the first case of increased mobility upon marking. Often times, the researchers only evaluate the flight response of the target insects when testing the feasibility of the marker because it is

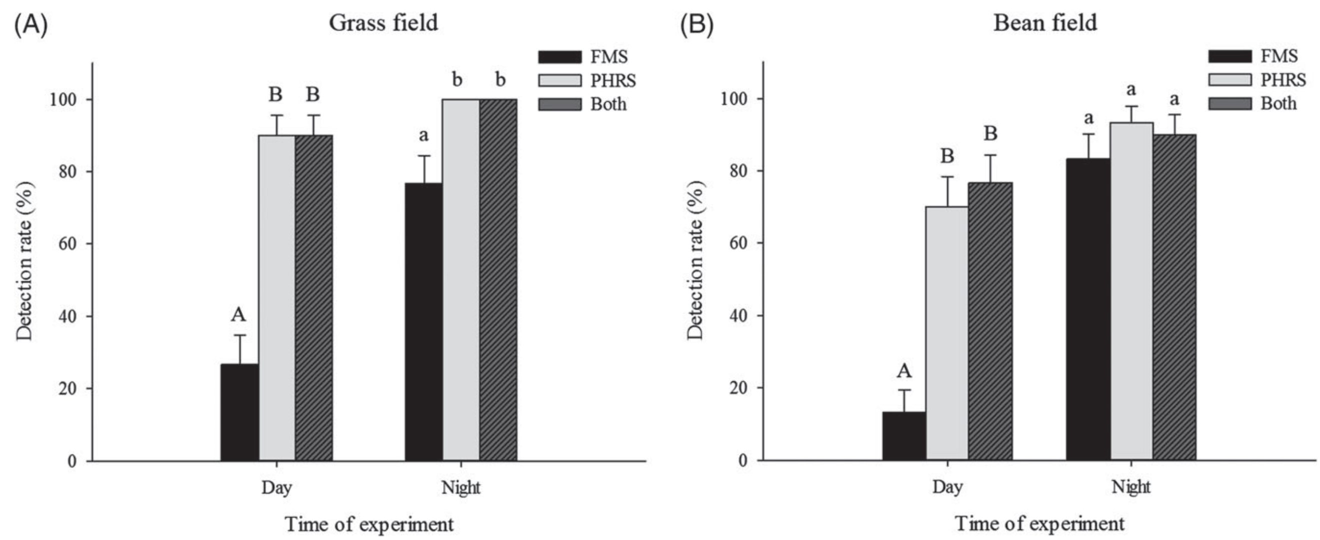

Figure 4. Detection rate between day and night using three different detection methods in (A) grass field and (B) bean field. Different letters indicate significant difference among the tested groups (ANOVA followed by Tukey's test, $P < 0.05$). FMS: Fluorescent marking system, PHRS: Portable harmonic radar system, Both: Both methods combined.

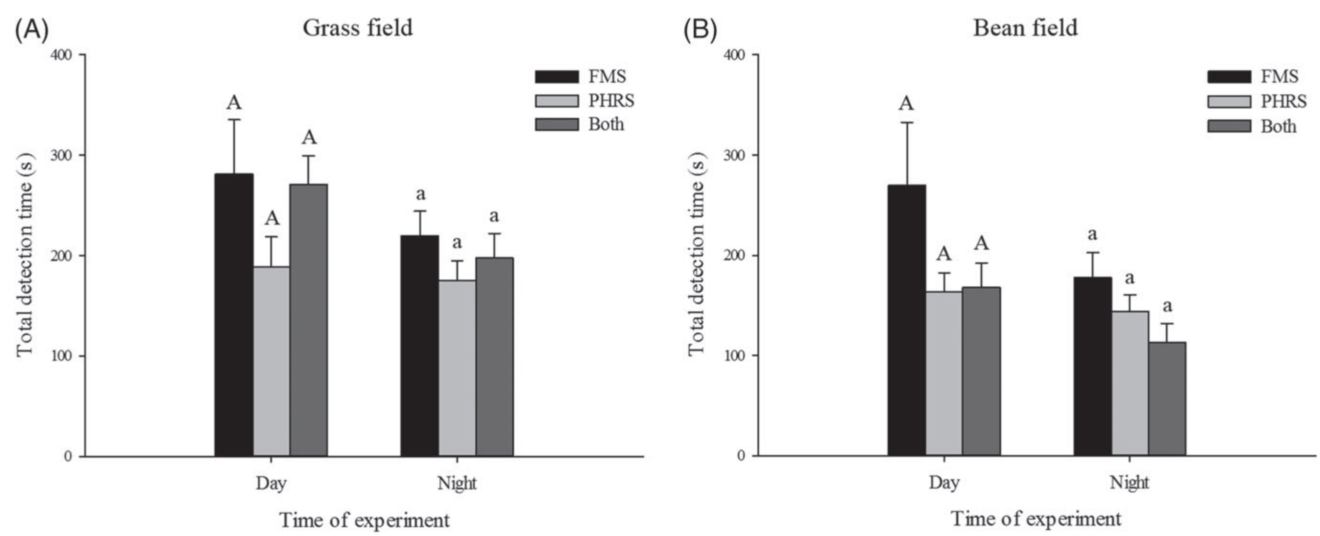

Figure 5. Total detection time in day and night using three different detection methods in (A) grass field and (B) bean field. Different letters indicate significant difference among the tested groups (ANOVA followed by Tukey's test, $P < 0.05$). FMS: Fluorescent marking system, PHRS: Portable harmonic radar system, Both: Both methods combined.

the main method of dispersal for flying insects.[8,50,51,53] Nevertheless, as our study shows, diverse aspects of insect mobility need to be considered with regards to how they may affect the dispersal. Furthermore, when such influence is discovered, a proper adjustment and calibration compared to untreated control are necessary to properly interpret the results.

In addition, our retrospective analyses demonstrated that even the non-significant data may need to be interpreted with caution. First, the *t*-test might have not been able to detect the difference in the fecundity of the two treatments due to the low sample size. Because only the females that reproduced were used in the statistical analysis, fecundity assessment had relatively smaller sample size than other assessments did. Nevertheless, even with a larger sample size, variation within treatments might have yielded the non-significant results; especially, the mean time until takeoff of the fluorescent-marked group was two times smaller than that of the control group. Indeed, it is not uncommon to observe such large variations in behavioral assessments of insects.[29,45,54] However, the number of *Riptortus pedestris* that successfully made a 30-m sustained flight was similar in the two groups, which suggests that fluorescent marking does not significantly affect the flight of the insect.

In our experiment, the detection distance of a marked insect with a handheld laser was approximately 27 m, but it kept increasing from initial 15 m until fourth trial from which it remained >25 m. This increase in detection distance can be attributed to the effect of learning by the operators, suggesting that it can allow a more reliable and effective detection of fluorescent-marked insects when used by trained personnel.

As the fluorescent marking system showed its viability in tracking of *Riptortus pedestris*, we then compared its efficacy with the portable harmonic radar system in two different landscapes during day and night. It was previously demonstrated that the radar tag attachment does not negatively affect adult *Riptortus pedestris*.[10,40]

Table 4. Time delay in detection (the time between signal detection by the harmonic direction finder to visual detection of the insect sample) across landscapes and time of experiment

Landscape	Time of experiment	Time delay (s) respective to the detection method[a] Portable harmonic radar system	Both methods combined
Grass field	Day	22.01 ± 4.85 A	26.38 ± 3.63 A
	Night	43.96 ± 10.17 a	17.84 ± 5.60 b
Bean field	Day	17.29 ± 3.90 A	23.09 ± 6.97 A
	Night	37.25 ± 8.72 a	9.10 ± 1.83 b

[a] Different letters indicate significant difference between the two methods within time of experiments ($P < 0.05$).

In addition, we evaluated if combining two methods can enhance the detection efficacy.

In general, the detection rate was significantly affected by detection method as well as landscape and time of experiment. For the detection methods tested, the overall detection rate of using fluorescent marking system was significantly lower than the portable harmonic radar system or both methods combined. This difference attributes to the fact that the detection rate of fluorescent marking was significantly lower than the other two methods in all settings, except for the bean field experiment at night. For the landscapes tested, the overall detection rate was significantly higher in the grass field compared to the bean field. Visual detection of the marked insect is required prior to the sample retrieval for all the detection methods used. In this study, the sample retrieval was more difficult in the bean field because bean plants created obstacles for line of visual detection. In addition, although the portable harmonic radar system can detect tagged objects behind plant materials,[11] the detection efficacy can decrease in the presence of obstacles,[55] which resulted in lower detection rate in the bean field.[24,56,57] Lastly, the overall detection rate was significantly lower during day than at night. This can be attributed in part to the low detection rates of fluorescent marking over daytime.[13,58] Undoubtedly, sunlight makes it difficult to locate laser illumination reflected from fluorescent-marked insect, resulting in substantially lower detection rates by the fluorescent marking system during daytime. However, it is also noteworthy that the detection rate substantially was enhanced at night regardless of landscapes to the level that there was no significant difference among the three methods at night in the bean field, a more complex landscape. Finally, there was no evidence supporting that combining the two methods significantly increased the detection rate compared to when the portable harmonic radar system is used alone. However, the use of both methods combined contributed to easier sample retrieval of marked insects at night compared to the use of radar system alone. In our experiment, the combined use reduced the time between signal detection and retrieval of the sample by more than 50% at night. This enhancement was not observed during the daytime experiment.

In general, the total detection time was affected by the three factors tested with a similar pattern to the results of the detection rate. Overall, the fluorescent marking system yielded the longest amount of time for final detection of marked insects; the difference was significant compared to using the radar system alone whereas it was not significant with the both methods combined. However, there was no significant difference in the total detection time across the three detection methods in any cases when it was compared in each landscape and time of experiment. This was in part due to large variations in the data within method. These variations are thought to be the results of handling of the devices by the operators. Because a thorough and systemic search is required when operating the harmonic direction finder or laser, the level of training can affect the efficiency of the detection.

To our knowledge, our experiment was the first to (i) demonstrate the feasibility of using the fluorescent marking system in detecting *Riptortus pedestris*, and (ii) compare the efficacy of the portable harmonic radar system, fluorescent marking system, and the combined use in detecting marked insects. Our results provide fundamental information which allows a more efficient and reliable detection of marked insects in the field conditions. First, the fluorescent marking did not negatively affect the fitness and behavior of adult *Riptortus pedestris*; however, caution is still warranted because the application of fluorescent paint elevated the insect's walking distance in this experiment. In addition, the fluorescent marking on *Riptortus pedestris* was distinguishable and easily detected *ca*. 20 m away at night, suggesting its great potential as a night time detection tool. It is noteworthy the cost required for the fluorescent marking system (*ca*. \$260) is substantially lower compared to the harmonic radar system (*ca*. \$40 000). Moreover, the training required to operate the fluorescent marking system is much easier, and it is more accessible in the market. To our knowledge, no study has reported the viability of using the radar system at night. Interestingly, our results demonstrate that the detection rate of the portable harmonic radar system was not compromised even when light was lacking, compared to using the system under daylight. Furthermore, combining the fluorescent marking system to the radar operation at night conferred more efficient detection of marked insects. Therefore, combining both methods would allow the visual detection of target insect at night in a more efficient manner.

To conclude, our study demonstrates that the portable harmonic radar system can serve as a reliable detection tool during both day and night, and its efficacy can be enhanced with the aid of fluorescent marking system at night. As expected, the fluorescent marking was not viable during daytime but it can be operated reliably and efficiently at night, especially in a complex landscape such as a bean field. Even with a substantial increase in crop damage by *Riptortus pedestris*, dispersal capacity and patterns of *Riptortus pedestrs* are largely unknown. Therefore, the detection methods tested in this study can be a useful addition to conventional monitoring tools such as pheromone traps.

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REFERENCES

1 Riley JR, Greggers U, Smith AD, Reynolds DR and Menzel R, The flight paths of honeybees recruited by the waggle dance. *Nature* **435**:205–207 (2005).

2 Ovaskainen O, Smith AD, Osborne JL, Reynolds DR, Carreck NL, Martin AP *et al.*, Tracking butterfly movements with harmonic radar reveals an effect of population age on movement distance. *Proc Natl Acad Sci USA* **105**:19090–19095 (2008).

3 Ramírez PA, Bell BD, Germano JM, Bishop PJ and Nelson NJ, Tracking a small cryptic amphibian with fluorescent powders. *N Z J Ecol* **41**:134–138 (2017).
4 Hagler JR and Jackson CG, Methods for marking insects: current techniques and future prospects. *Annu Rev Entomol* **46**:511–543 (2001).
5 Basavaraju Y, Devi BR, Mukhatyakka G, Reddy LP, Mair GC, Roderick EE *et al.*, Evaluation of marking and tagging methods for genetic studies in carp. *J Biosci* **23**:585–593 (1998).
6 Walker KA, Trites AW, Haulena M and Weary DM, A review of the effects of different marking and tagging techniques on marine mammals. *Wildl Res* **39**:15–30 (2011).
7 Roland J, McKinnon G, Backhouse C and Taylor PD, Even smaller radar tags on insects. *Nature* **381**:120–120 (1996).
8 Boiteau G and Colpitts B, Electronic tags for the tracking of insects in flight: effect of weight on flight performance of adult Colorado potato beetles. *Entomol Exp Appl* **100**:187–193 (2001).
9 O'Neal ME, Landis DA, Rothwell E, Kempel L and Reinhard D, Tracking insects with harmonic radar: a case study. *Am Entomol* **50**:212–218 (2004).
10 Kim J, Jung M, Kim HG and Lee D-H, Potential of harmonic radar system for use on five economically important insects: radar tag attachment on insects and its impact on flight capacity. *J Asia Pac Entomol* **19**:371–375 (2016).
11 Nathan R, The challenges of studying dispersal. *Trends Ecol Evol* **16**:481–483 (2001).
12 Morrison WR, Lee DH, Short BD, Khrimian A and Leskey TC, Establishing the behavioral basis for an attract-and-kill strategy to manage the invasive *Halyomorpha halys* in apple orchards. *J Pest Sci* **89**:81–96 (2016).
13 Narisu LJA and Schell SP, A novel mark-recapture technique and its application to monitoring the direction and distance of local movements of rangeland grasshoppers (Orthoptera: Acrididae) in the context of pest management. *J Appl Ecol* **1**:604–617 (1999).
14 Verhulst NO, Loonen JA and Takken W, Advances in methods for colour marking of mosquitoes. *Parasit Vectors* **6**:200–206 (2013).
15 Unruh TR and Chauvin RL, Elytral punctures: a rapid, reliable method for marking Colorado potato beetle. *Can Entomol* **125**:55–63 (1993).
16 Wojcik DP, Burges RJ, Blanton CM and Focks DA, An improved and quantified technique for marking individual fire ants (Hymenoptera: Formicidae). *Fla Entomol* **83**:74–78 (2000).
17 Anderson CN, Cordoba-Aguilar A, Drury JP and Grether GF, An assessment of marking techniques for odonates in the family Calopterygidae. *Entomol Exp Appl* **141**:258–261 (2011).
18 Berghammer AJ, Klingler M and Wimmer EA, Genetic techniques: a universal marker for transgenic insects. *Nature* **402**:370–371 (1999).
19 Ramdya P, Schaffter T, Floreano D and Benton R, Fluorescence behavioral imaging (FBI) tracks identity in heterogeneous groups of Drosophila. *PLoS One* **7**:e48381 (2012).
20 Van Steenwyk RA, Ballmer GR, Page AL and Reynolds HT, Marking pink bollworm with rubidium. *Ann Entomol Soc Am* **71**:81–84 (1978).
21 Van Steenwyk RA, Kaneshiro KY, Hue NV and Whittier TS, Rubidium as an internal physiological marker for Mediterranean fruit fly (Diptera: Tephritidae). *J Econ Entomol* **85**:2357–2364 (1992).
22 Osborne JL, Clark SJ, Morris RJ, Williams IH, Riley JR, Smith AD *et al.*, A landscape-scale study of bumble bee foraging range and constancy, using harmonic radar. *J Appl Ecol* **36**:519–533 (1999).
23 Hedin J and Ranius T, Using radio telemetry to study dispersal of the beetle Osmoderma eremita, an inhabitant of tree hollows. *Comput Electron Agric* **35**:171–180 (2002).
24 Lee DH, Park CG, Seo BY, Boiteau G, Vincent C and Leskey TC, Detectability of *Halyomorpha halys* (Hemiptera: Pentatomidae) by portable harmonic radar in agricultural landscapes. *Fla Entomol* **97**:1131–1138 (2014).
25 Riley JR and Smith AD, Design considerations for an harmonic radar to investigate the flight of insects at low altitude. *Comput Electron Agric* **35**:151–169 (2002).
26 Tahir N, Brooker G, Recent developments and recommendations for improving harmonic radar tracking systems, in *Proceedings of the 5th European Conference on Antennas and Propagation (EUCAP)* 11–15 April 2011, Rome, Italy. New York: IEEE, pp. 1531–1535 (2011). Available: IEEE Xplore [5 March 2018].
27 Mascanzoni D and Wallin H, The harmonic radar: a new method of tracing insects in the field. *Ecol Entomol* **11**:387–390 (1986).
28 Boiteau G, Vincent C, Meloche F and Leskey TC, Harmonic radar: assessing the impact of tag weight on walking activity of Colorado potato beetle, plum curculio, and western corn rootworm. *J Econ Entomol* **103**:63–69 (2010).
29 Lee DH, Wright SE, Boiteau G, Vincent C and Leskey TC, Effectiveness of glues for harmonic radar tag attachment on *Halyomorpha halys* (Hemiptera: Pentatomidae) and their impact on adult survivorship and mobility. *Environ Entomol* **42**:515–523 (2013).
30 Drake VA and Reynolds DR, *Radar Entomology: Observing Insect Flight and Migration*. CABI, Wallingford (2012).
31 Hagler J, Mueller S, Teuber LR, Van Deynze A and Martin J, A method for distinctly marking honey bees, Apis mellifera, originating from multiple apiary locations. *J Insect Sci* **11**:143–156 (2011).
32 Longland WS and Clements C, Use of fluorescent pigments in studies of seed caching by rodents. *J Mammal* **76**:1260–1266 (1995).
33 Rice KB, Fleischer SJ, De Moraes CM, Mescher MC, Tooker JF and Gish M, Handheld lasers allow efficient detection of fluorescent marked organisms in the field. *PLoS One* **10**:e0129175 (2015).
34 Pellet J, Rechsteiner L, Skrivervik AK, Zürcher JF and Perrin N, Use of the harmonic direction finder to study the terrestrial habitats of the European tree frog (*Hyla arborea*). *Amphibia-Reptilia* **27**:138–142 (2006).
35 Chapman J, Reynolds D and Smith A, Migratory and foraging movements in beneficial insects: a review of radar monitoring and tracking methods. *Int J Pest Manage* **50**:225–232 (2004).
36 Lövei GL, Stringer IA, Devine CD and Cartellieri M, Harmonic radar-a method using inexpensive tags to study invertebrate movement on land. *N Z J Ecol* **1**:187–193 (1997).
37 Brier HB and Rogers DJ, Susceptibility of soybeans to damage by *Nezara viridula* (L.)(Hemiptera: Pentatomidae) and *Riptortus serripes* (F.)(Hemiptera: Alydidae) during three stages of pod development. *J Aust Entomol* **30**:123–128 (1991).
38 Wada T, Endo N and Takahashi M, Reducing seed damage by soybean bugs by growing small-seeded soybeans and delaying sowing time. *Crop Prot* **25**:726–731 (2006).
39 Lim U, Occurrence and control method of *Riptortus pedestris* (Hemiptera: Alydidae): Korean perspectives. *Korean J Appl Entomol* **52**:437–448 (2013).
40 Lee DH, Evaluating effects of harmonic radar tag attachment on the survivorship and dispersal capacity of *Riptortus pedestris* (Hemiptera: Alydidae). *Fla Entomol* **99**:110–112 (2016).
41 Kikuchi Y, Hosokawa T and Fukatsu T, Insect-microbe mutualism without vertical transmission: a stinkbug acquires a beneficial gut symbiont from the environment every generation. *Appl Environ Microbiol* **73**:4308–4316 (2007).
42 Nakata T, Effectiveness of micronized fluorescent powder for marking citrus psyllid, *Diaphorina citri*. *Appl Entomol Zool (Jpn)* **43**:33–36 (2008).
43 Kikuchi Y, Hosokawa T and Fukatsu T, Specific developmental window for establishment of an insect-microbe gut symbiosis. *Appl Environ Microbiol* **77**:4075–4081 (2011).
44 Kikuchi Y, Meng XY and Fukatsu T, Gut symbiotic bacteria of the genus *Burkholderia* in the broad-headed bugs *Riptortus clavatus* and *Leptocorisa chinensis* (Heteroptera: Alydidae). *Appl Environ Microbiol* **71**:4035–4043 (2005).
45 Boiteau G, Meloche F, Vincent C and Leskey TC, Effectiveness of glues used for harmonic radar tag attachment and impact on survival and behavior of three insect pests. *Environ Entomol* **38**:168–175 (2009).
46 Bowling CC, The stylet sheath as an indicator of feeding activity by the southern green stink bug on soybeans. *J Econ Entomol* **73**:1–3 (1980).
47 Jung M, Kim S, Kim HG and Lee DH, Lethal and sublethal effects of synthetic insecticides on the locomotory and feeding behavior of *Riptortus pedestris* (Hemiptera: Alydidae) under laboratory conditions. *J Asia Pac Entomol* **21**:179–185 (2018).
48 Glover T and Mitchell K, *An Introduction to Biostatistics*. McGraw-Hill, New York, NY, pp. 164–165 (2008).
49 Kim S, Lee JK, Song YJ, Kang SC, Kim B, Choi IJ *et al.*, Evaluating natural compounds as potential insecticides against three economically important pests, *Bemisia tabaci* (Hemiptera: Aleyrodidae), *Frankliniella occidentalis* (Thysanoptera: Thripidae), and *Myzus persicae* (Hemiptera: Aphididae), on greenhouse sweet peppers. *Appl Biol Chem* **61**:313–323 (2018). https://doi.org/10.1007/s13765-018-0362-8.
50 Garcia-Salaza C and Landis DA, Marking *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) with fluorescent marker dust and its effect on survival and flight behavior. *J Econ Entomol* **90**:1546–1550 (1997).

51 Toepfer S, Levay N and Kiss J, Suitability of different fluorescent powders for mass-marking the Chrysomelid, *Diabrotica virgifera virgifera* LeConte. *J Appl Entomol* **129**:456–464 (2005).
52 Reid TG and Reid ML, Fluorescent powder marking reduces condition but not survivorship in adult mountain pine beetles. *Can Entomol* **140**:582–588 (2008).
53 Stephens AEA, Barrington AM, Bush VA, Fletcher NM, Mitchell VJ and Suckling DM, Evaluation of dyes for marking painted apple moths (*Teia anartoides* Walker, Lep. Lymantriidae) used in a sterile insect release program. *Aust J Entomol* **47**:131–136 (2008).
54 Morrison WR, Poling B and Leskey TC, The consequences of sub-lethal exposure to insecticide on the survivorship and mobility of Halyomorpha halys (Hemiptera: Pentatomidae). *Pest Manag Sci* **73**:389–396 (2017).
55 Boyarski VL, Rodda GH and Savidge JA, Evaluation of harmonic direction-finding systems for detecting locomotor activity. *J Wildl Manage* **71**:1704–1707 (2007).
56 Boiteau G, Vincent C, Meloche F, Leskey TC and Colpitts BG, Harmonic radar: efficacy at detecting and recovering insects on agricultural host plants. *Pest Manag Sci* **67**:213–219 (2011).
57 Gui LY, Boiteau G, Colpitts BG, MacKinley P and McCarthy PC, Random movement pattern of fed and unfed adult Colorado potato beetles in bare-ground habitat. *Agric For Entomol* **14**:59–68 (2012).
58 Turchin P, Odendaal FJ and Rausher MD, Quantifying insect movement in the field. *Environ Entomol* **20**:955–963 (1991).

