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Effects of aggregation pheromone concentration and distance on the trapping of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) adults

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A B S T R A C T

Rhyzopertha dominica male adults produce an aggregation pheromone that attracts both sexes. While many studies have tested the behavioral response of *R. dominica* adults to its aggregation pheromone, information on the distance of attraction and effective pheromone concentration are lacking. Therefore, the objectives of this study were to determine the recapture rate of *R. dominica* adults released at different distances from a pheromone-baited and pheromone + kairomone-baited trap, as well as the most effective pheromone concentration for *R. dominica*. Experiments were arranged as complete randomized design with four replicates. A commercial pitfall trap containing *R. dominica* pheromone alone or pheromone + kairomone was placed inside an experimental arena. Adult *R. dominica* were released at different distances from the trap and the adults captured were counted. A separate experiment was conducted using pitfall traps containing different concentrations of the aggregation pheromone placed inside the experimental arena. Adult *R. dominica* were released 60 cm away from the pitfall trap and the recaptured adults were recorded. The trapping efficiency was higher when the trap contained both the pheromone and kairomone than the pheromone alone. Trap capture was highest when the beetles were released at distances up to 70 cm and at a concentration of 100 μL/1 m^2. Our findings suggest that food facility managers should take into account the effective pheromone concentration and distance of attraction, while also strongly consider including food kairomones in traps when developing monitoring programs for *R. dominica*.

1. Introduction

Rhyzopertha dominica (F.) (Coleoptera: Bostrychidae) has long been reported as a serious pest of stored food worldwide infesting fresh and processed food resources belonging to 53 plant species in 31 families (Potter, 1935; Crombie, 1941; Cotton, 1956; Aitken, 1975; Edde, 2012; Buonocore et al., 2017). It has been recorded on a variety of raw and processed food products, including cereals and pulses (Sinha and Watters, 1985), other crops such as legumes, tubers, bulbs (Rees, 2004), animal feed (Wijayaratne et al., 2019), products of medical importance, and in some woody and animal-based packaging materials (Riley, 1882; Winterbottom, 1922; Potter, 1935).

General management measures adopted for *R. dominica* and other stored-product insects include inspection of incoming raw materials on a regular basis, following sanitation practices in the maintenance of equipment and structure, chemical control methods (Larson et al., 2008; Martinazzo et al., 2000; Sakka et al., 2020), hermetic storage (Bailey, 1965; Donahaye et al., 1996; Carvalho et al., 2012a,b; Hasaranga et al., 2018). In addition, the management strategies for *R. dominica* may also include the use of aeration (Yang et al., 2017), modified atmosphere (Wijayaratne et al., 2009; Levy-De la Torre et al., 2019), application of ozone (Subramanyam et al., 2017; E et al., 2019), and extreme

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temperatures (Fields, 1992). However, there are some limitations in the currently-available control methods for *R. dominica*. Resistance to the conventional insecticides such as deltamethrin (Attia, 1984; Chen and Chen, 2013) and phosphine (Collins et al., 2017; Yang et al., 2018) as well as novel pest management agents such as spinosad (Chen and Chen, 2013; Yang et al., 2018) has been detected in *R. dominica*. In addition, many of these insecticides may cause deleterious effects on non-target species, human applicators, and the abiotic environment (Fields, 1992; Arthur, 1996; Hagstrum and Subramanyam, 2006; Phillips and Throne, 2010; Wijayaratne et al., 2018). Therefore, the adoption of reduced-risk or biorational methods in the protection of stored food commodities from insect pest infestation remains a high priority at present.

Rhyzopertha dominica emits a male-produced aggregation pheromone that attracts both sexes (Khorramshahi and Burkholder, 1981; Williams et al., 1981; Cheskis et al., 1985; Liu and Lin, 1990; Razkin et al., 1996). In particular, the aggregation pheromone is a mixture of (*S*)-(+)-1-methylbutyl-(*E*)-2-methyl-2-pentenoate (dominicalure-1, or DL-1 hereafter) and (*S*)-(+)-1-methylbutyl-(*E*)-2,4-dimethyl-2-pentenoate (dominicalure-2, or DL-2 hereafter) (Williams et al., 1981) in a natural ratio of 1:2 (DL-1:DL-2),which serves as a lure in baited traps (Cogburn et al., 1984; Leos-Martinez et al., 1987; Fields et al., 1993; Fields and Phillips, 1994; Mills and White, 1994; Trece Inc., 2019). A substantial amount is already known about the basic chemical ecology of *R. dominica*. For example, this includes pheromone emission rates by adults (Edde and Phillips, 2010); effect of trap design (Leos-Martinez et al., 1987; Edde et al., 2005), age, sex, and female mating status (Dowdy et al., 1993), daily rhythm of pheromone release (Bashir et al., 2003a), as well as orientation differences of males and females (Cordeiro et al., 2019). However, the response of *R. dominica* adults when its pheromone components are used in commercial traps is limited.

The monitoring traps commercially available for certain other stored-product insect species such as *Tribolium castaneum* (Herbst) concurrently use kairomone with the pheromone (Campbell, 2012; Dissanayaka et al., 2020b). Similarly, the response of *R. dominica* to its pheromone when used in the traps either alone or in combination with kairomone under warehouse conditions needs to be determined to achieve the maximum benefit of using pheromone technology for monitoring of this species. Furthermore, deployment of correct trap density is required for accurate estimation of an insect population (Buckman and Campbell, 2013). Attraction of *R. dominica* adults located at various distances from the trap and the efficiency of adult attraction to different pheromone concentrations are two basic requisites for such determination of optimum trap density. However, the unavailability of these important information on *R. dominica* limits the potential use of aggregation pheromones in the monitoring programs for this pest within food facilities targeting its management and protection of stored food from its infestation. Therefore, the objectives of this study were to determine the distance of attraction to the traps baited with *R. dominica* pheromone, and to elucidate the effective pheromone concentration that maximizes attraction by *R. dominica* adults.

2. Materials and methods

2.1. Insects

Rhyzopertha dominica were reared in the Entomology laboratory of Rajarata University of Sri Lanka since 2017 using routine culturing procedures (Wijayaratne et al., 2019). The colony was obtained from Anuradhapura, Sri Lanka at a commercial food facility. Cracked rice (var. Red Nadu) was used to rear the insects. One-month-old adults were used in the experiments below.

2.2. Pheromones, kairomones and traps

The two aggregation pheromone components of *R. dominica*, including(*S*)-(+)-1-methylbutyl-(*E*)-2-methyl-2-pentenoate (DL-1) and (*S*)-(+)-1-methylbutyl-(*E*)-2,4-dimethyl-2-pentenoate (DL-2) (Williams et al., 1981) were used in the current experiments. In order to assess the distance of attraction in the first experiment, two rubber septa with DL-1 and DL-2 were fixed inside the top cover of a commercial pitfall trap (Storgard® Dome Trap, Trece Inc., Adair, OK, USA). The pheromone septa were maintained at 5 °C inside a refrigerator before used in the experiment. When testing the effect of kairomones, 20 drops (approximately 100 μL) of the commercial kairomone solution (Storgard® Oil, Trece Inc., Adair, OK, USA) were added to each dome trap in addition to the two pheromone septa. Traps that had neither the pheromone nor kairomone (e.g. an empty trap) were used as controls. In the second experiment exploring the effective concentration, an *R. dominica* aggregation pheromone solution (Trece Inc., Adair, OK, USA) containing a mixture of DL-1 and DL-2. The pheromone-baited trap was placed on the floor to be on the midline along the long axis of the experimental arena and 30 cm inside the left margin, 1 h before beginning the experiment (Dissanayaka et al., 2020a,b).

2.3. Experiment 1: distance of attraction by *Rhyzopertha dominica* adults to pheromone-baited traps

This experiment was conducted on the floor of a warehouse (Fig. 1). Pitfall traps were baited with either pheromone only or pheromone + kairomone. For a given experimental step (replicate), twenty *R. dominica* adults were released at various distances from the trap (30, 40, 50, 60, 70, 80 and 90 cm) on the midline marked along the long axis of the experimental arena. For each distance, a separate experiment was conducted. After 24 h from release, all the *R. dominica* adults inside or underneath each trap were counted. Each treatment (pheromone alone, pheromone + kairomone, or control) and release distance was replicated 4 times. Following each experiment, the experimental arena (floor of the warehouse) was washed with a biodegradable substance (Britol Disinfectant Pine, Antler Industries Pvt. Ltd., Piliyandala, Sri Lanka) before the next experiment. The temperature and humidity profiles during the experiment were recorded every 15 min by using data loggers (TM-305U, Onset Computer Corporation, Bourne, MA).

2.4. Experiment 2: trapping efficiency of *Rhyzopertha dominica* adults at different concentrations of pheromone

Similar to the experiment 1, this experiment was also conducted on the floor inside a warehouse (1 m length x 1 m width) (Fig. 2). The experimental arena was cleaned by using biodegradable substance (Britol Disinfectant Pine, Antler Industries Pvt. Ltd., Piliyandala, Sri Lanka) preceding each experiment. Polytetrafluoroethylene (Teflon, Sigma Aldrich, Saint Louis, USA) was applied along the boundaries of experimental arena to prevent the escape of adults. The pitfall trap (Storgard® Dome Trap, Trece Inc., Adair, OK, USA) was placed 30 cm inside the left edge of the experimental arena and to be on the midline along the long axis of the experimental arena. Using the aggregation pheromone of *R. dominica* (a mixture of DL-1 and DL-2), available as the technical solution dissolved in hexane (Trece Inc., Adair, OK, USA), two rubber septa in the dome trap were impregnated with one of the following concentrations at a time by using a micropipette (Labnet International, Inc., Poland): 25, 50, 100, 150 or 200 μL. The two rubber septa were cleaned twice using hexane before it was impregnated with the aggregation pheromone. The pitfall trap was left inside the experimental arena1 h before the commencement of experiment

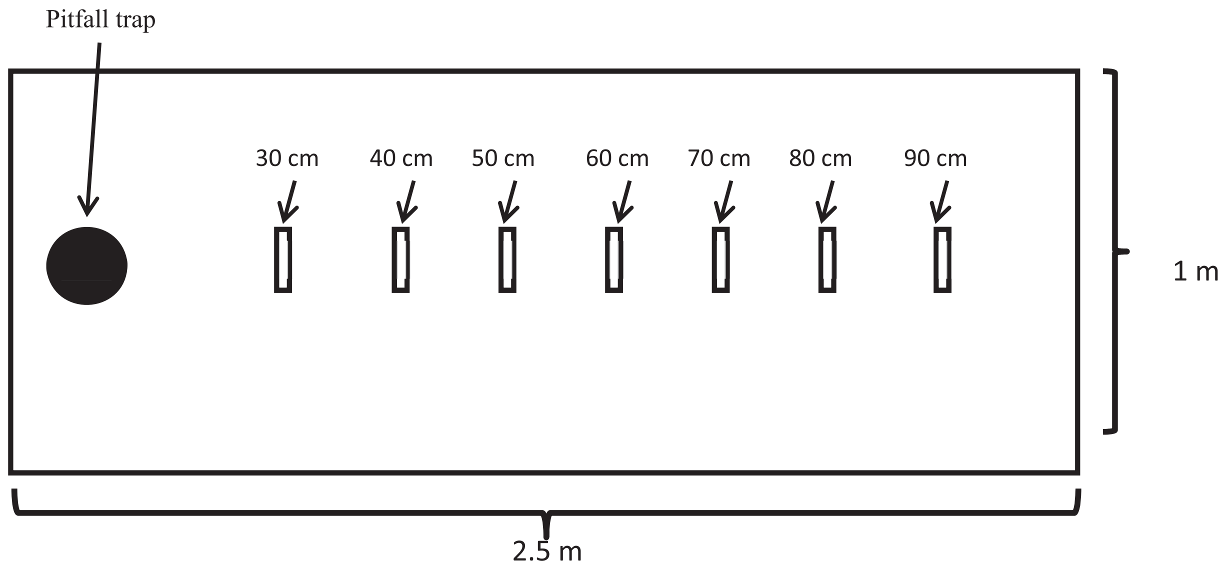

Fig. 1. Experimental arena (not to the scale) showing the placement of trap and locations where *Rhyzopertha dominica* adults were released for the experiment 1.

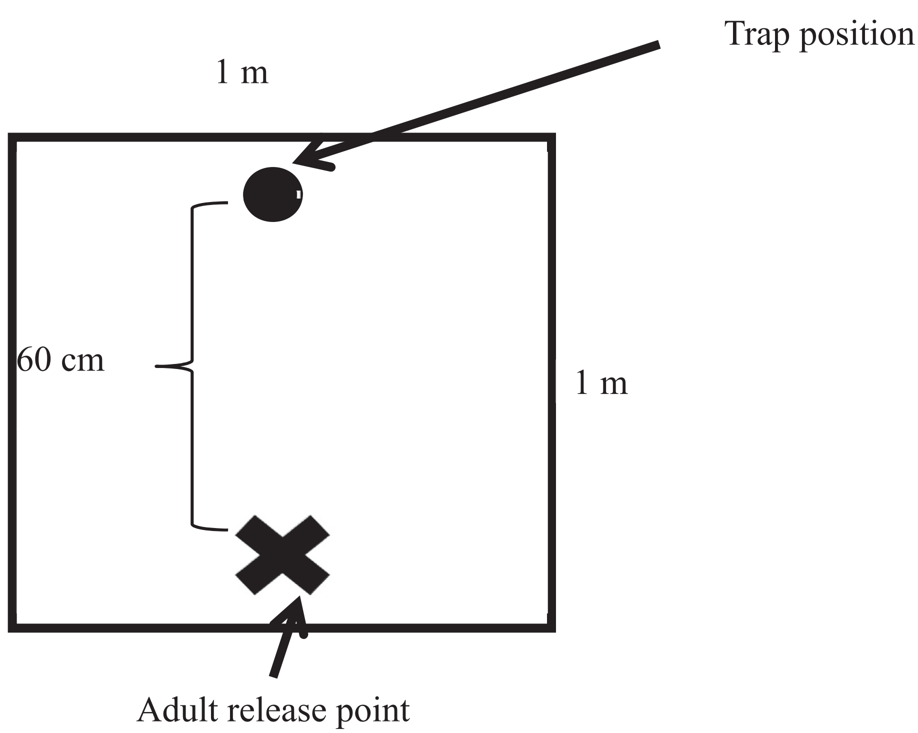

Fig. 2. Experimental arena (not to the scale) showing the placement of trap and locations where *Rhyzopertha dominica* adults were released for the experiment 2.

(Dissanayaka et al., 2020a,b). As the control, the two rubber septa impregnated with hexane (solvent for the pheromone) were used. One-month-old *R. dominica* 100 adults were released 60 cm away from the pitfall trap at a time (the effective distance of attraction by the aggregation pheromone as found during Experiment 1 in Section 2.3). For a given pheromone concentration tested, a separate experiment was conducted with adults released at 60 cm from the trap. For a given pheromone concentration (or control using hexane), four replicate experiments were conducted. The adults captured inside the pitfall trap were counted 24 h following release. Temperature and relative humidity profiles inside the experimental arena during the experiment period were recorded using data loggers (TM-305U, Onset Computer Corporation, Bourne, MA).

2.5. Data analysis

Normality of the data set was proven by the Shapiro-Wilk test. The Bartlett's test confirmed the homogeneity of variances across treatments. The percentage of adults captured when released at a particular distance from the trap (in the experiment 1) or in response to a particular pheromone concentration (in the experiment 2) was transformed using $\sqrt[2]{\arcsin(x)}$ to accommodate the heterogeneous variances (Zar, 1999). The transformed data were analyzed using an analysis of variance (ANOVA) in the Statistical Analysis System (SAS) (SAS Institute, 2002–2008) to determine differences in mean capture among adults released from different distances (in the experiment 1) or different pheromone concentrations (in the experiment 2). Upon a significant result from the overall model, multiple comparisons employed Tukey's test. The significance was tested at $\alpha = 0.05$.

3. Results

3.1. Experiment 1: distance of attraction by Rhyzopertha dominica to pheromone-baited traps

Release distance of *R. dominica* affected recapture in traps baited with pheromone only ($F_{20,63} = 21.21$, $P < 0.001$). While recapture of *R. dominica* linearly declined with increasing distance of release from the trap, the percentage recaptured at 30 cm was not significantly different from recapture up to and including adults released 60 cm away from the trap (Fig. 3). The distance of attraction (e.g. recapture) by traps dropped off for adults released at 70 cm and 80 cm compared with those released 30–60 cm away from the trap. The lowest recapture in traps was observed when adults were released at 80 or 90 cm away, and recapture those traps was similar to using traps without pheromones (control traps).

Likewise, the release distance of adult *R. dominica* significantly affected recapture in traps baited with pheromone + kairomone ($F_{20,63} = 30.59$, $P < 0.001$). Adding a kairomone extended the distance of attraction by 10 cm relative to pheromone-baited traps alone, with recapture of adult *R. dominica* released 30–70 cm away statistically equivalent (Fig. 4). At every release distance except 90 cm, more adults reached the trap having pheromone or pheromone + kairomone than those captured by control traps without stimuli. All the control traps did not recapture *R. dominica* except traps at 30 cm. Furthermore, greater amount of adults were recaptured in traps having pheromone + kairomone (mean ± SE: 6.5 ± 3.3, 6.3 ± 3.1, 4 ± 2, 4 ± 2, 3 ± 1.5, 2 ± 1, 1 ± 0.5, at 30, 40, 50, 60, 70, 80 and 90 cm, respectively) compared with those traps that had only the pheromone (mean ± SE: 5.5 ± 0.6, 4.8 ± 0.5, 3.5 ± 0.3, 3 ± 0.4, 2 ± 0.4, 0.8 ± 0.3, 0.3 ± 0.3, at 30, 40, 50, 60, 70, 80 and 90 cm, respectively).

3.2. Experiment 2: trapping efficiency of Rhyzopertha dominica adults at different concentrations of pheromone

Concentration of pheromone in traps significantly affected recapture of *R. dominica* ($F_{5,18} = 22.4$, $P < 0.001$). Traps with pheromone concentrations between 25 and 200 μL recaptured significantly more adults than control traps (hexane only/0 μL pheromone) (Fig. 5). Control traps without pheromone did not capture any *R. dominica* adults. Increasing the pheromone concentration inside the trap enhanced recapture of *R. dominica* adults until 100 μL, which showed the highest recapture. However, further increases in the pheromone concentrations to 150 and 200 μL actually decreased the percentage of adults recaptured by traps (Fig. 5). Furthermore, the recapture in traps baited with 25, 150 and 200 μL of pheromone were not significantly different among each other and exhibited lower recapture than traps baited with 50 or 100 μL of *R. dominica* aggregation pheromone.

4. Discussion

Capture of insects in pheromone-baited traps may be affected by a variety of factors, including trap design (Morrison et al., 2015), pheromone concentration (Morrison et al., 2016), presence of specific stimuli (Dissanayaka et al., 2018b), plume reach (Kirkpatrick et al., 2019), among other factors. Most notably, here we found that that attraction by *R. dominica* adults to traps quickly drops off with distance, and is significantly lowest once one moves 60 cm or 70 cm away from pheromone-baited or pheromone + kairomone-baited traps, respectively. Similar to *R. dominica*, *T. castaneum* adults released at increasing distances from a baited pitfall trap also showed decreasing attraction (Dissanayaka et al., 2020a), which mirrors the behavioral response of *T. castaneum* adults to pheromone-baited traps in other work (Dissanayaka et al., 2018a). Campbell (2012) found that in the absence of airflow there was no significant difference between pheromone-baited traps and unbaited traps at 10 cm in encounter rates by *T. castaneum*, but that distance of attraction increased to 90 cm in the presence of airflow. Chingoma (2006) reported that *R. dominica* adults were caught up to 1000 m from a pheromone-baited trap in prairie landscapes. Mahroof et al. (2010) found that *R. dominica* were recaptured 261–375 m away from where they

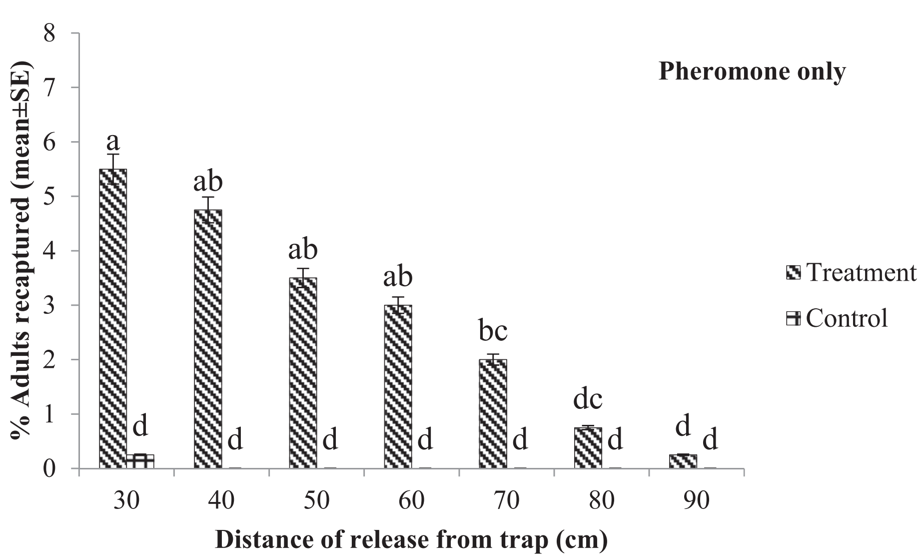

Fig. 3. Mean percentage (±SE) of *Rhyzopertha dominica* adults recaptured by traps baited with pheromone only at increasing distances from the release of insects (n=4) in warehouses. Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$).

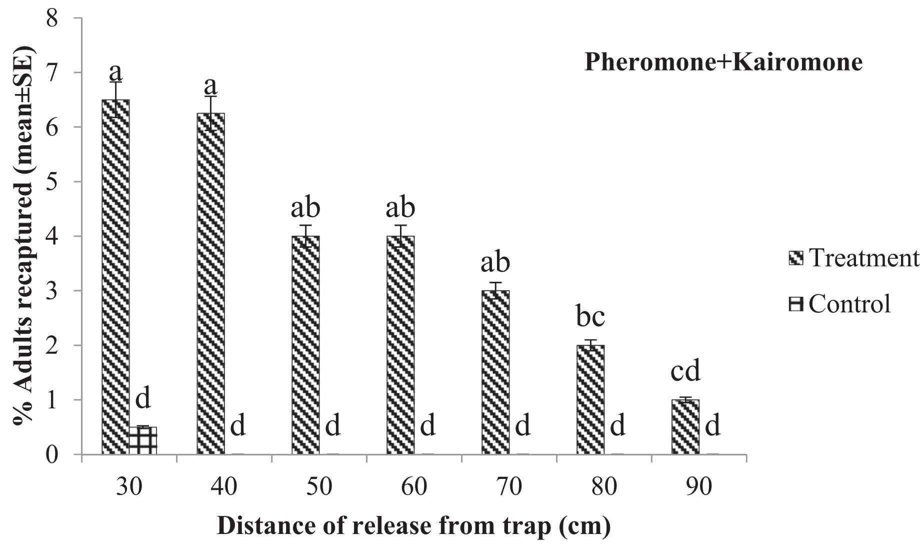

Fig. 4. Mean percentage (±SE) of *Rhyzopertha dominica* adults recaptured by traps baited with pheromone + kairomone at increasing distances from the release of insects (n=4) in warehouses. Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$).

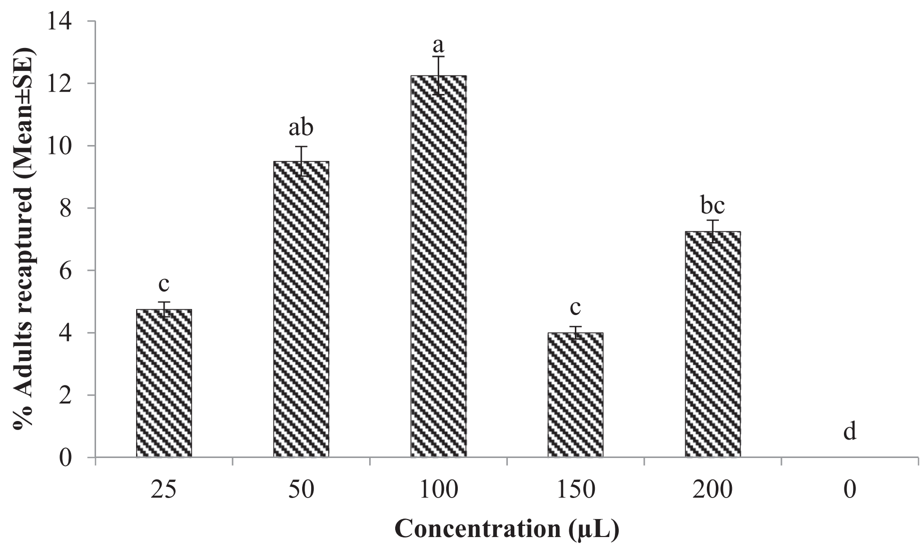

Fig. 5. Mean percentage (±SE) of *Rhyzopertha dominica* adults recaptured by traps baited with different concentrations of the conspecific aggregation pheromone when insects were released 60 cm away. Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). The control is represented by the pheromone concentration 0 µL.

were released, with dispersal greater in forested sites than in open sites. The same study found that the percentage of recaptured beetles significantly decreased between traps deployed 100–1600 m away from release sites. By contrast, we found that distance of attraction quickly drops off with distances of less than 1 m in a warehouse. This behavioral response was based on the walking orientation of *R. dominica* to a ground-deployed pitfall trap, not a flight traps as was used in past landscape-level studies.

One possibility to explain this is that olfactory response and orientation to conspecific pheromones may be more important at the local level with other factors assigned more importance when orienting from a distance by *R. dominica* such as visual cues, habitat-level cues, or internal state (e.g. physiology). If this possibility is true, it suggests that traps deployed in the field may divert *R. dominica* who may already be dispersing close to pheromone-baited traps, as opposed to specifically attracting individuals at a distance. A second possibility is that orientation while walking may be less driven by olfactory orientation than is flight behavior, and thus the range over which *R. dominica* responds to its pheromone is smaller while walking than in flight. While walking, it has been documented in prior research that *R. dominica* appears to encounter hosts by chance, and does not actively use host odors such as those from wheat, rice or maize (Nguyen et al., 2008). In further support of this second possibility, prior work has shown that indoor pheromone-baited flight traps captured more *R. dominica* than ground-deployed traps in warehouses (Toews et al., 2006). The difference between these two possibilities is actually not trivial, because stakeholders often voice a concern about the use of pheromone tools around food facilities attracting more insects than would otherwise be there. Future work testing the distance of attraction over a larger range of distances and using an elevated flight trap baited with pheromones should be able to resolve this question.

The highest trapping efficiency was found for traps baited with

100 μL of *R. dominica* aggregation pheromone, but even this only reached approximately 12% recapture. Toews et al. (2006) showed lower trap captures of *R. dominica* adults inside a warehouse compared to directly outside, but the two captures were correlated, suggesting immigration into the food facility. Prior work has found that the presence of food patches in a warehouse (e.g. poor sanitation) may decrease trapping efficiency and disrupt the correlation of monitoring trap captures with true pest density (Toews et al., 2005). We found that in an indoor environment the trapping efficiency of *R. dominica* adults by the synthetic pheromone is low, but it may be greater in other locations and in different habitats as is the case for other agricultural pests (Kirkpatrick et al., 2019). We also observed that increased concentrations led to increased trap capture, but only up to a certain point. This may have been the result of saturation of the container space with the pheromone such that a pheromone gradient was not able to form that would have allowed the insects to easily find the trap. Another possible explanation is that the response of *R. dominica* adults to its aggregation pheromone may depend more on the relative ratio of DL-1 and DL-2 than on quantitative differences in the overall concentration of the pheromone. The natural ratio of DL1: DL2 in the pheromone emissions of *R. dominica* is 1:2 (Burkholder and Ma, 1985), but the adults can modify this ratio in response to changes in the environmental conditions (Mayhew and Phillips, 1994; Bashir et al., 2001, 2003a,b; Edde et al., 2007; Edde and Phillips, 2010). The relative ratio of components was reported as being more important in other stored-product insect species, such as *T. castaneum* (Boake and Wade, 1984). A third possible explanation deals with the concentrations in this study differing from natural emission rates by *R. dominica* males. For example, *R. dominica* adults can biosynthesize about 0.03 mg of their aggregation pheromone per male per day under a 1:1 sex ratio (Edde et al., 2007), but this may alter with the specific population (Edde, 2012). Responding *R. dominica* may be able to perceive that the synthetic pheromone concentration inside the trap differs from the amount released naturally by the male *R. dominica* adults, which may lead to a low orientation towards the pheromone trap. However, these hypotheses need to be tested in future studies by having pheromone concentrations lower and higher than that released by the males naturally.

Furthermore, we found that the addition of kairomones to the pheromonal stimuli in traps increased attraction by *R. dominica*. The same has been demonstrated for other stored product insects, including *Sitophilus oryzae* (Linnaeus) and *T. castaneum* (Sinclair et al., 1984; Walgenbach et al., 1987; Phillips et al., 1993; Dissanayaka et al., 2018b). Maga (1978) reported that *T. castaneum* trap catch is increased when the pheromone is combined with grain volatiles. Similarly, in a recent study, Dissanayaka et al. (2018b) showed that oils having botanical origin enhances trapping of *T. castaneum* adults to aggregation pheromone 4,8 DMD. In evaluating *R. dominica* response to flight traps, Edde et al. (2011) found that there was enhanced response when ethanol, an odorant signaling plant distress, was included with a conspecific aggregation pheromone lure. This is also in agreement with the current research findings as more *R. dominica* adults moved to the trap and from farther when they were baited with both pheromones and kairomones.

Overall, the distance of attraction and optimal concentration for attraction to traps by *R. dominica* found in this study will contribute to determining the optimum trap density targeting for monitoring programs in grain storage facilities. The low trapping efficiency for *R. dominica* found in this study in simple warehouse settings may be an artifact of trap type and different orientational processes while walking or flying, but merits further investigation. Future research needs to determine the trapping efficiency of *R. dominica* pheromone-baited traps under natural and complex settings in commercial warehouses, and identify limiting factors for the use of pheromones in monitoring *R. dominica*. This information should be used to substantively improve monitoring and management programs for *R dominica*. Trapping of *R. dominica* adults drops off after 60–70 cm from a pheromone only or pheromone + kairomone-baited trap. The maximum trapping occurs when 100 μL/1 m^2 of aggregation pheromone is deployed inside a trap. The presence of kairomones in the trap enhances trapping efficiency and distance of attraction over the presence of the pheromone alone.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

D.M.S.K. Dissanayaka: Conceptualization, Investigation, Data curation, Formal analysis, Writing - original draft. **A.M.P. Sammani:** Investigation. **L.K.W. Wijayaratne:** Conceptualization, Methodology, Validation, Supervision, Writing - review & editing, Resources, Funding acquisition, Project administration. **R.H.S. Rajapakse:** Writing - review & editing. **S. Hettiarachchi:** Writing - review & editing. **W.R. Morrison:** Writing - review & editing.

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