



Distance and height of attraction by walking and flying beetles to traps with simultaneous use of the aggregation pheromones from *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae)

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ABSTRACT

Adults of *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (F.) biosynthesize aggregation pheromones 4,8-dimethyldecanal and dominicalure-1/dominicalure-2, respectively. These pheromones are commonly used independently, and their simultaneous use has not been adequately studied. Furthermore, information on trapping flying *R. dominica* in pheromone traps is minimum. Therefore, the objectives were to evaluate distance of attraction of *T. castaneum* and *R. dominica* adults to traps having both pheromones, and height of *R. dominica* adult attraction to traps with its pheromone lure alone. In first experiment, both pheromones were deployed simultaneously inside a commercial pitfall trap. One-month-old 20 *T. castaneum* and *R. dominica* adults were released every 30 cm from the pheromone trap. The adults trapped were recorded at 4 and 24 h following their release. Adults of both species released were captured in higher percentages at 24 h than 4 h. At 30 cm distance, these values were 45.5% for *T. castaneum* and 10–12% for *R. dominica* for 24 h whereas they were 40.5% for *T. castaneum* and 5–7.5% for *R. dominica* following 4 h exposure. The maximum trap capture was at 30 cm for *T. castaneum* and 30–60 cm for *R. dominica*. In second experiment, a trap with two rubber septa containing dominicalure-1 and dominicalure-2 was placed at different heights inside cage, and *R. dominica* adults were released at the bottom. In each experiment, four replicates were tested. After 24 h, flying *R. dominica* adults were captured in progressively lower percentages as trap height increased up to 40 cm above the bottom of cage. Trap heights above 10 cm exhibited decreased trap capture of *R. dominica* compared to those at 3 cm. We conclude that simultaneous use of both aggregation pheromones better facilitates trapping of walking *T. castaneum* and *R. dominica*. Traps above 10 cm show decreased captures of flying *R. dominica*.

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1. Introduction

Tribolium castaneum (Herbst), red flour beetle and *Rhyzopertha dominica* (F.), lesser grain borer are devastating insect pests of different types of raw and processed agricultural commodities stored after harvest, and coexist in different habitats of the post-harvest supply chain (Hagstrum and Subramanyam, 2006; Hagstrum et al., 2012; Dissanayaka et al., 2018c; Sajeevani et al.,

2018; Kumari et al., 2020). The mobility of *T. castaneum* (Semeao et al., 2013) and *R. dominica* (Toews et al., 2006) increases the likelihood that they will infest stored products, and presents challenges to their management (Arthur et al., 2020; Sakka et al., 2020). Due to the increased concerns caused by the use of insecticides on the biotic and abiotic environment, alternative integrated pest management (IPM) methods have actively been sought (Phillips and Throne, 2010; Wijayaratne and Rajapakse, 2018; Wijayaratne et al., 2018; Agrafioti et al., 2019). Synthetic analogs of pheromones produced and released by insects have widely been used in monitoring programs to determine presence and changes in

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the abundance of the stored product insect population (Phillips et al., 2000; Campbell, 2011).

In *T. castaneum*, a male-produced aggregation pheromone consisting of 4,8-dimethyldecanal (4,8 DMD) attracts both males and female conspecifics (Suzuki, 1980), and is used in traps for monitoring population levels (Campbell, 2012). However, commonly only a portion of the actual insect population is detected by the commercially-available traps (Campbell, 2012; Dissanayaka et al., 2018a). Previous studies have explored the production and perception of the *T. castaneum* aggregation pheromone by conspecifics. Following the isolation of 4,8 DMD several decades ago (Suzuki and Sugawara, 1979), the initial research investigated the effect of food quality (Ming and Lewis, 2010) and food ingestion (Hussain et al., 1994b) on pheromone production; beetle age on the perception of pheromone (Faustini et al., 1981); the release rate of the pheromone in lures (Hussain et al., 1994a); the sensitivity of different *T. castaneum* populations to aggregation pheromone (Boake and Wade, 1984); and, the biosynthesis pathway for the aggregation pheromone (Kim and Matsuyama Suzuki, 2005). More recent studies have concentrated on issues related to practical application of *T. castaneum* aggregation pheromones. For example, some of the topics covered include effect of pest and trap density on behavioral response (Buckman and Campbell, 2013), nutrition, sex and mating status of *T. castaneum* in response to pheromone-baited traps (Fedina and Lewis, 2007); effects of landscape structure and flour residues (Campbell, 2013); variability in the behavioral response by different populations in response to pheromones and kairomones (Gerken et al., 2018); effects of pheromone concentration (Dissanayaka et al., 2018a, 2020a), air flow (Dissanayaka et al., 2018a), distance (Dissanayaka et al., 2020a), population size (Dissanayaka et al., 2020b) and influence of kairomones (Dissanayaka et al., 2018b) on trapping efficiency of *T. castaneum*.

The male adults of *R. dominica* produce (S)-(+)-1-methylbutyl (*E*)-2-methyl-2-pentenoate (dominicalure-1, or DL-1) and (S)-(+)1-methylbutyl (*E*)-2,4-dimethyl-2-pentenoate (dominicalure-2, or DL-2) that attract both sexes (Williams et al., 1981). Different facets of *R. dominica* pheromone system have been studied in prior work. These include orientation of unmated and mated females to pheromone (Dowdy et al., 1993), diurnal and sex-specific variation of pheromone titer (Bashir et al., 2003), and influence of pheromone release rate on boring ability through grain (Bashir et al., 2003). It has also included examining the response of male and female adults to synthetic blends of DL-1 and DL-2 under field and laboratory conditions (Edde et al., 2005), how feeding status and sex density affect pheromone emission rates (Edde and Phillips, 2010), and how green leaf volatiles and plant essential oils synergize pheromone response (Edde et al., 2011). Most recently, work has evaluated the effects of pheromone concentration and distance (Dissanayaka et al., 2020c) on trapping walking *R. dominica* adults over short distances, but not on flying conspecifics to traps placed at different heights.

While there have been a multitude of studies on the pheromone systems of these two species, all have used either *T. castaneum* or *R. dominica* pheromone alone, but not typically in combination. However, generally under realistic contexts, these two species may often co-occur in large post-harvest facilities that have both processing components and bulk storage areas. Furthermore, the use of pheromones from more than one species in a trap will economize on traps and speed adoption of monitoring programs. In addition, it may be particularly important to assess both pheromones together in a trap, because there may be cross-attraction and/or interference in perception (Cox, 2004). For example, there appears to be strong cross-attraction among the pheromones produced by *Trogoderma* spp. (Coleoptera: Dermestidae) (Vick et al., 1970), and traps containing 92% (*Z*)-14-methyl-8-hexadecenal and 8% (*E*)-14-methyl-8-

hexadecenal captured 3000 individuals, including nine species of *Trogoderma*, *Megatoma*, *Orphilus*, *Orphinus*, and *Thaumaglossa* (Olson et al., 2013). By contrast in other contexts, when two stereoisomers are deployed together that have antagonistic effects, one compound may inhibit response to the other as reported in *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) response to (4S, 5S, 7S)-serricornin and (4S, 5S, 7R)-serricornin, where the latter inhibits the behavior of this species (Okada et al., 1992). Thus, it is important to assess if pheromones are deployed together in a trap that do not possess any antagonistic interactions.

An open question in stored product entomology has been the distance of attraction to pheromonal and other stimuli by target pest species. Concern has often been voiced by stakeholders about deploying pheromones too close to food facilities for fear of attracting more insects than would otherwise be there. Additionally, information about distance of attraction can help determine plume reach of pheromone traps, optimal trapping density, and other important variables (Kirkpatrick et al., 2019) that may optimize trapping programs for stored product insects.

Additionally, it is important to take into account the life history traits of the species that are being targeted. For example, *R. dominica* adults are strong fliers, but weak walkers (Stejskal et al., 2003; Khan and Marwat, 2004), while *T. castaneum* are weak fliers, but strong walkers (Morrison et al., 2018; Wilkins et al., 2020a). Thus, it is not just important to assess distance of attraction to traps while walking, but also while flying, especially for *R. dominica*. Despite the studies conducted on the behavior of *R. dominica* in response to its aggregation pheromone (Edde et al., 2005, 2011), little information is available on trapping at different heights. Knowing the optimum height of deployment for traps to elicit maximal attraction will improve the efficiency of monitoring and detection for *R. dominica*. Moreover, success of simultaneous deployment of pheromone lures of both *R. dominica* and *T. castaneum* has not been sought previously. Such attempt would provide insights on how the two species would react under the influence of pheromone from the other species as well as practical-base for economical use of pheromone lures. Therefore, the objectives of this research were to determine distance of attraction by walking *T. castaneum* and *R. dominica* adults when the aggregation pheromones of both species were combined in a trap and the height of attraction of flying *R. dominica* to its aggregation pheromone used alone.

2. Materials and methods

Experiment 1. Distance of attraction for walking *Tribolium castaneum* and *Rhyzopertha dominica* to traps with 4,8 DMD and DL-1/DL-2.

The experiment was conducted on cement floor measuring 2 m² in a warehouse (Anuradhapura, Sri Lanka). The commercial pitfall dome traps (Storgard™ Dome® trap, Trécé, Inc., Adair, OK, USA) were used in the study to place the pheromones. One rubber septum containing 4,8-DMD (Trécé, Inc., Adair, OK, USA) was fixed on to one knob on the inner surface of the top cover of dome trap whereas the second rubber septum containing mixture of DL-1 and DL-2 (Trécé, Inc., Adair, OK, USA) was attached to the second knob on the trap. In addition, 15 drops of commercial kairomone oil (Storgard™ Oil, Trécé, Inc., Adair, OK, USA) were added to an absorbent piece of filter paper (4 cm diameter) and placed in the trap's internal reservoir. Then the trap was placed inside a 2 m² experimental arena (30 cm from the left perimeter) under still air condition 2 h prior to the start of the experiment. A total of 20 one-month-old *T. castaneum* and 20 *R. dominica* adults were released at 30, 60, 90, 120 and 150 cm distances from the dome trap in 4 replicate parallel experimental arenas. Adults of both species were

released at a particular distance at the same time. The adults trapped were counted at 4 and 24 h following their release. The experiment was replicated four times per trapping distance and species; a new trap was used for each replicate experiment. The control experiments for *T. castaneum* were conducted without 4,8 DMD or kairomones inside the traps. Similarly, for *R. dominica*, the control experiments used only the dome trap without DL-1/DL-2 or the kairomones. The temperature and r. h. profiles of the warehouse during the experiments were measured every 15 min by using data loggers (TM-305U, Onset Computer Corporation, Bourne, MA), and were 30.2 ± 1 °C and $60.1 \pm 1\%$, respectively.

Experiment 2. Height of attraction for flying *Rhyzopertha dominica* adults to traps baited with a mixture of Dominicalure-1 (DL-1) and Dominicalure-2 (DL-2).

The experimental arena used for this experiment was a cage (70 cm \times 50 cm \times 50 cm L:W:H) that was covered with insect proof netting (approximately 400 holes/cm²) made of nylon. The bottom of the cage was covered with Teflon (Polytetrafluoroethylene; Sigma Aldrich, Saint Louis, MO, USA) to avoid the surface horizontal movement by *R. dominica* adults. One MicroDot trap (Trécé Inc., Adair, OK, USA) (rectangular trap having internal hollow and accommodates two pheromone lures on one side) commercially available for *R. dominica* was used in this experiment. Two septa each containing a mixture of dominicalure-1 or DL-1 [(S)-(+)-1-methylbutyl (E)-2-methyl-2-pentenoate] and dominicalure-2 or DL-2 [(S)-(+)-1-methylbutyl (E)-2,4-dimethyl-2-pentenoate] (Trécé Inc., Adair, OK, USA) were fixed underneath the top cover of the trap. A trap was placed on the left wall of the cage at different heights, including 3, 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 cm from the bottom of the cage. The trap was placed inside the cage 1 h before the beginning of the experiments. A total of 100 one-month-old *R. dominica* adults were gently released at the center on the bottom of the cage. After 24 h, the number of adults inside the trap was counted. Replicates at each height were repeated after rotating the cage by 180° to control for positional effects. A total of 4 replicates were conducted for each height. In the control experiments, only the dome trap was used without DL-1/DL-2.

2.1. Statistical analysis

In experiment 1, the data on percentage of adults trapped at a particular distance was transformed using square root of the arcsine to accommodate the unequal variances associated, then analyzed using ANOVA in SAS (Zar, 1999). Separate models were run for each species, but in each case the horizontal distance of the trap away from the release point was treated as a fixed, explanatory variable. Upon a significant result from the model, multiple comparisons were done by a Tukey HSD test. The significance was tested at $\alpha = 0.05$ for both experiments. In experiment 2, the percentage of adults that reached the trap was converted into the square root of the arcsine value to resolve the unequal variances associated with the percentage data. These transformed data were analyzed with ANOVA in SAS (SAS Institute, 2002-2008), using trap height as a fixed, explanatory variable. Upon a significant result from the model, multiple comparisons employed a Tukey HSD test.

3. Results

3.1. Distance of attraction for walking *Tribolium castaneum* and *Rhyzopertha dominica* to traps with 4,8 DMD and DL-1/DL-2

There was a significant difference in the percentage of walking *T. castaneum* captured after 4 and 24 h at each of the distances, indicating that more beetles were captured 24 h following their release ($F_{19,60} = 26.21$, $P < 0.001$). The percentage of adult

T. castaneum captured in controls at each distance was significantly lower than the when baited traps were present after 4 h ($F_{9,30} = 27.37$, $P < 0.001$) and 24 h ($F_{9,30} = 12.74$, $P < 0.001$) durations (Fig. 1). The greatest recapture of adults occurred when baited traps were only 30 cm (4 h–40.5% and 24 h–45.5%) away from the release point. Moderate recapture of *T. castaneum* persisted at 60–120 cm (4 h–19.5–18.5% and 24 h–23–18.5%) with half as many adults captured as at 30 cm, but significantly dropped off further by 150 cm (4 h–5.5% and 24 h–8%). This same pattern of recapture was found at both 4 and 24 h after release.

Overall, there was a significant difference between *R. dominica* adults trapped at 4 and 24 h ($F_{19,60} = 29.21$, $P < 0.001$; Fig. 2), with almost twice as many adults recaptured at 24 h compared to 4 h after release. This is evident from the percentage adults trapped at 30 and 60 cm distances varied as 10 vs. 5, 12 vs. 7.5, respectively. Furthermore, the percentage of *R. dominica* adults captured by control traps was significantly lower than those captured in baited traps after 4 h ($F_{4,15} = 50.07$, $P < 0.001$) and 24 h ($F_{4,15} = 72.70$, $P < 0.001$). Walking adult *R. dominica* captures were reliably high when baited traps were 30–60 cm (4 h–5–7.5% and 24 h–10–12%) away, but precipitously dropped off when adults were released 90 cm (4 h–0.5% and 24 h–0.5%) away, and none were recaptured in traps that were 120–150 cm (4 h–0% and 24 h–0%) away. This pattern was similar for recapture of adult *T. castaneum* after both 4 and 24 h.

3.2. Height of attraction for flying *Rhyzopertha dominica* adults to traps baited with a mixture of Dominicalure-1 (DL-1) and Dominicalure-2 (DL-2)

Overall, there was a significant difference in the percentage of *R. dominica* recaptured between the baited and control traps ($F_{21,66} = 22.73$, $P < 0.001$; Fig. 3). In fact, there were no *R. dominica* adults trapped in the unbaited control traps. The percentage of *R. dominica* adults captured in traps placed at different heights from the bottom of the cage progressively decreased with the increased distance to the trap from the bottom of the cage. Flying *R. dominica* adults first exhibited a significantly not decreased recapture percentage from 3 cm until 10 cm (3–2.25%). Captures continued to decrease until a 40 cm (1.75–0.25%) trap deployment height, after which no *R. dominica* adults were recaptured.

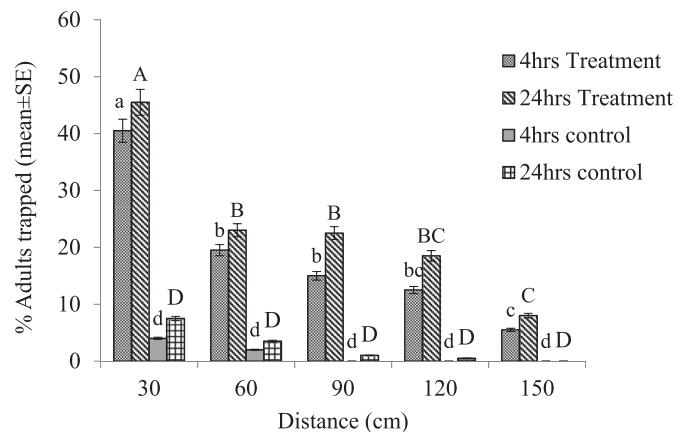


Fig. 1. Percentage (mean \pm SE) of 20 walking *Tribolium castaneum* adults trapped 4 and 24 h following release at increasing distances from traps containing both 4,8 DMD and DL-1/DL-2 (treatment) or neither (control). Within each exposure period, means with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$).

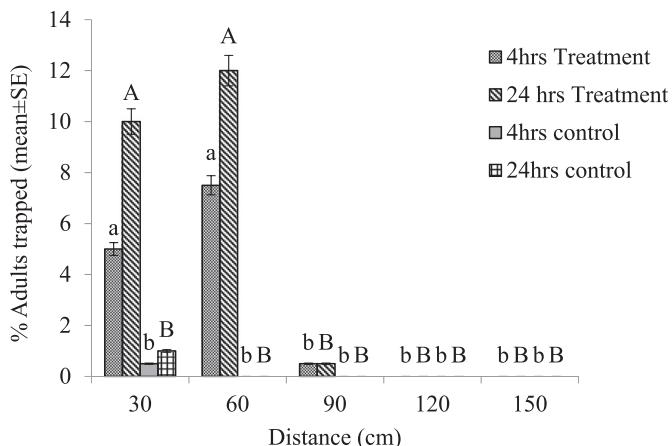


Fig. 2. Percentage (mean \pm SE) of 20 walking *Rhyzopertha dominica* adults trapped 4 and 24 h following release at increasing distances from traps containing both 4,8 DMD and DL-1/DL-2 (treatment) or neither (control). Within each exposure period, means with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$).

4. Discussion

In this study, we have documented that the distance of placement of a trap away from insects in both the horizontal and vertical plane determines the strength of attraction and percentage of insects recaptured. Indeed, prior work has also determined that the distance from the pheromone or kairomone affect the movement of *T. castaneum* adults (Elkinton and Carder, 1984; Dissanayaka et al., 2018a), as well as other stored product insects (Wilkins et al., 2020b). For example, the percentage of *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) showed 2% recapture at 60 cm distance from the pheromone (Hawkin et al., 2011). Our current work has demonstrated a 45.5% recapture rate 30 cm away, 18.5–23% at 60–120 cm and 8% at 150 cm away. The current findings are partially consistent with a previous study that reported *T. castaneum* is maximally captured up to 60 cm away from the pheromone (Dissanayaka et al., 2018a). In the current study, there may be a lower efficiency of recapture at greater distances by

T. castaneum because of the slow diffusion of 4,8 DMD as a result of the size of the compound and the fact that the experiment was performed in a still-air setting.

Likewise, walking *R. dominica* attraction to traps with both stimuli were significantly affected by distance, and perhaps more dramatically than *T. castaneum*. Prior work has found that trap design is a major factor affecting the response of *R. dominica* to their aggregation pheromone. For example, lindgren four-unit funnel traps and Japanese beetle traps recaptured 80% of flying *R. dominica* released (Edde et al., 2005). In the current study, we used a commercial pitfall trap designed specifically for walking stored product beetles and is used regularly by stakeholders. In this study, *R. dominica* were recaptured at a rate of 5–12% when released 30–60 cm away from baited traps. The difference in recapture rates between the different kinds of traps is likely the result of differences in perception modality during flight versus walking for each species, as well as differences in overall movement ecology, with *R. dominica* adults being much weaker walkers. Lindgren and Japanese beetle traps capture insects during flight, while pitfall traps capture insects while on the ground. In addition, trapping systems are often designed by companies as holistic systems, with each part (e.g. trapping mechanism, kill mechanism, attractants) reinforcing the effects of others. Furthermore, little information is available about the response of *R. dominica* to the host plant volatiles (Fadamiro et al., 1998); therefore, it is possible that *R. dominica* adults did not respond well to the kairomones inside the trap. Future research should also be designed to distinguish if the release of both *T. castaneum* and *R. dominica* adults at the same time or separately from a particular distance affects the recapture rate.

Recapture of flying *R. dominica* in pheromone-baited traps was significantly affected by trap height. Similar to our findings, Edde et al. (2005) reported that more stored product beetles were caught at lower heights under storage conditions, while forest beetle capture was doubled with traps deployed at twice the height (Borden et al., 1986). Edde et al. (2005) reported that pheromone-baited traps placed near the vegetation canopy trapped more *R. dominica* adults than the traps at lower heights and that optimal flight occurs for the pheromone-baited traps placed <4 m above the ground. However, our study suggests the optimal trap height may only be up to 10 cm from the surface. The differences between these

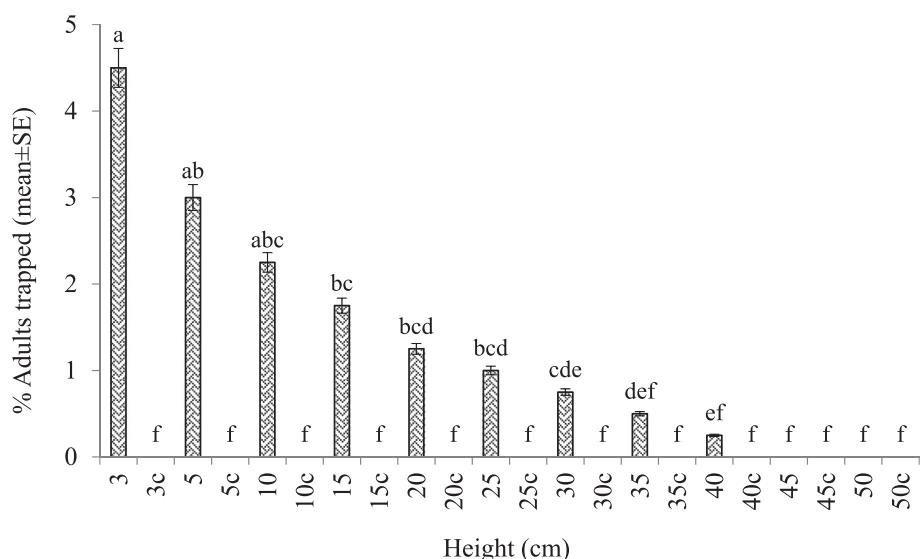


Fig. 3. Percentage (mean \pm SE) of 100 flying *Rhyzopertha dominica* adults captured in traps containing DL-1/DL-2 when placed at increasing heights. Means with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$).

two findings may arise from differences in the tested population size, confounding physical and volatile stimuli from vegetation, and landscape patterns of dispersal (Mahroo et al., 2010), which need to be investigated in the future research. It is also possible that *R. dominica* prefers a landing site near the pheromone trap as observed in relation to feeding in some other insect species (eg. scolytid beetles) (Raffa et al., 1993; Nguyen et al., 2008). The absence of such a structure made the traps used in the present study less behaviorally compatible, leading to a lower percentage of adults that were recaptured which should be considered during future experimental design. Furthermore, trapping of both species was higher at 24 h than 4 h. Therefore, future research can also be designed to investigate how the duration of exposure beyond 24 h affect the trap catch.

Both the distance of the trap in the horizontal and vertical plane affected attraction and recapture by *T. castaneum* and *R. dominica*. Increasing the trap deployment height from the bottom surface reduced trapping. The maximum capture of flying *R. dominica* occurred at 3–10 cm height from the bottom of the cage. Because both *T. castaneum* and *R. dominica* may co-occur in food facilities, it is advantageous to develop traps that target multiple species. As a result, added information on the distance of attraction to traps with both species' pheromones greatly aids in the development of monitoring programs for stored product pests within food facilities, and supports the use of semiochemicals in stored product protection.

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. **T.C. Bamunuarachchige:** Writing - review & editing. **W.R. Morrison:** Writing - review & editing, All authors read and approved the manuscript.

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.

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