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Evaluation of Potential Attractants for Six Species of Stored-Product Psocids (Psocoptera: Liposcelididae, Trogiidae)

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ABSTRACT Psocids have emerged as worldwide pests of stored commodities during the past two decades, and are difficult to control with conventional management tactics such as chemical insecticides. Therefore, it is necessary to investigate alternative management strategies, such as the use of attractants for monitoring and controlling psocids, which can be incorporated into integrated pest management programs for psocids. Using a two-choice pitfall test, we studied the response of adults of different ages and sexes of *Liposcelis entomophila* (Enderlein) (Psocoptera: Liposcelididae), *Liposcelis paeta* Pearman, *Liposcelis decolor* (Pearman), *Liposcelis brunnea* Motschulsky, *Liposcelis corrodens* (Heymons), and *Lepinotus reticulatus* Enderlein (Psocoptera: Trogiidae) to volatiles from different potential attractants including grains, grain-based oils, brewer's yeast, wheat germ, and commercially available kairomone lures. For all species tested, sex and age did not have a major influence on response to the different potential attractants. Brewer's yeast most consistently elicited the strongest response for psocids, but this response frequently was not different from that to wheat germ and wheat germ oil. The percentage response to brewer's yeast varied among the psocid species tested: *L. decolor* (73–78%), *L. entomophila* (62–73%), *L. brunnea* (64–68%), *L. paeta* (42–57%), *Lep. reticulatus* (40%), and *L. corrodens* (15–19%). Two psocids species (*L. corrodens* and *Lep. reticulatus*) had low responses to all the potential attractants evaluated compared with the other four species. These results show there is high potential for using these attractants in a psocid-monitoring program.

RESUMEN En las últimas dos décadas los psócididos han emergido como plagas cosmopolitas. Los psócididos son difíciles de controlar con métodos convencionales de control como los insecticidas químicos. Por lo tanto, es necesario investigar estrategias de manejo alternativas, como el uso de atrayentes para monitorear y/o controlar psócididos, que puedan ser incorporadas a un programa de manejo integrado de plagas (MIP) para psócididos. Por medio de experimentos de libre selección, se estudió la respuesta de adultos (diferentes edades y sexos) de *Liposcelis entomophila* (Enderlein) (Psocoptera: Liposcelididae), *Liposcelis paeta* Pearman, *Liposcelis decolor* (Pearman), *Liposcelis brunnea* Motschulsky, *Liposcelis corrodens* (Heymons), y *Lepinotus reticulatus* Enderlein (Psocoptera: Trogiidae) a volátiles de diferentes atrayentes potenciales que incluyeron granos, aceites de diferentes granos, levadura de cerveza, germen de trigo y cebos con kairomonas disponibles comercialmente. En todas las especies evaluadas, el sexo y la edad no tuvieron una mayor influencia en la respuesta a los diferentes atrayentes potenciales. La levadura de cerveza fue el material que consistentemente provocó la respuesta más fuerte por parte de las especies de psócididos evaluadas, pero en general esta respuesta no fue diferente a la de el germen de trigo y el aceite de germen de trigo. El porcentaje de respuesta a la levadura de cerveza varió entre las especies de psocidos evaluadas: *L. decolor* (73–78%), *L. entomophila* (62–73%), *L. brunnea* (64–68%), *L. paeta* (42–57%), *Lep. reticulatus* (40%), y *L. corrodens* (15–19%). Dos especies de psocidos (*L. corrodens* y *Lep. reticulatus*) tuvieron baja respuesta a los diferentes atrayentes potenciales evaluados comparados con las otras cuatro especies. Estos resultados indican un alto potencial para incorporar estos atrayentes a un programa de monitoreo de psócididos.

KEY WORDS booklice, attractant, stored product

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Several psocid species have emerged as important pests of stored grain commodities worldwide since the 1990s (Mills et al. 1992, Leong and Ho 1994, Rees 1994, Turner 1994) and in the United States in the 2000s (Phillips and Throne 2010, Throne 2010) causing contamination of food commodities (Turner 1994, Athanassiou et al. 2010b). Psocids also can cause significant damage and reduction in weight and quality of rice, *Oryza sativa* L. (McFarlane 1982, Rees and Walker 1990), and wheat, *Triticum aestivum* L. (Gautam et al. 2013, Kučerová 2002). Moreover, chemical insecticides used to control other insect pests of stored products are often not effective for control of psocids (Athanassiou et al. 2009). Several psocid species have shown resistance to phosphine (Ho and Winks 1995, Nayak et al. 1998, 2003, Nayak and Collins 2008, Nayak et al. 2014), contact insecticides such as the carbamate carbaryl (Nayak et al. 1998), the pyrethroids deltamethrin (Nayak et al. 1998), permethrin (Nayak et al. 2002), and esfenvalerate (Opit et al. 2012), the organophosphate dichlorvos (Ding et al. 2002, Dou et al. 2006), pyrethrin (Athanassiou et al. 2009), spinosad (Nayak et al. 2005, Athanassiou et al. 2009), and the insect growth regulator methoprene (Athanassiou et al. 2010a).

Psocids are stored-product pests of increasingly significant economic importance, so it is critical to develop effective integrated pest management (IPM) strategies for their management. A critical part of IPM programs is to have a monitoring program, but monitoring tools for psocids are limited. Opit et al. (2009) observed that corrugated cardboard refuges placed on wheat bin walls or on the surface of grain were an efficient method for sampling psocid species, *Liposcelis entomophila* (Enderlein) (Psocoptera: Liposcelididae) and *Liposcelis decolor* (Pearman), although these refuges are not reported to attract psocids and do not capture psocids that enter the cardboard. Research is limited on evaluating specific materials that are attractive to psocids that might be used in monitoring devices. In a choice study, Green and Turner (2005) observed a significant preference by *Liposcelis bostrychophila* Badonnel adults for ground buckwheat or ground yellow millet with yeast over the same materials without yeast, and they also found that adults of this insect preferred yellow millet to other grains. Green (2008) found that *L. bostrychophila* preferred materials with fungal extracts to controls. However, in these studies, psocids came in contact with the materials, so the results may not have been due to a response to volatile cues from the food.

Diaz-Montano et al. (2014) performed a systematic evaluation of different potential attractants and found that *L. bostrychophila* strongly responded to volatile odors coming from brewer's yeast, wheat germ, and wheat germ oil in the pitfall trap. Because a range of psocid species are stored-product pests and they may not all share the same response to volatile cues, we wanted to determine if the same potential attractant(s) could be used for multiple species, or if specific attractants will be needed for specific psocid species. The objective of this study was to evaluate the response of

adults of six additional psocid species that are important pests of grain and grain-based products. In the first group of tests of potential attractants, we evaluated the response of *L. entomophila* and *Liposcelis paeta* Pearman to volatiles from different grains, grain-based oils, brewer's yeast, and commercially available food baits. Because the responses were similar for *L. bostrychophila* (Diaz-Montano et al. 2014) and for the two species in the first group of tests, a reduced set of potential attractants was used to evaluate the response of *Liposcelis brunnea* Motschulsky, *L. decolor*, *Liposcelis corrodens* (Heymons), and *Lepinotus reticulatus* Enderlein (Psocoptera: Trogiidae).

Materials and Methods

Insect Cultures. The psocids used in this study, *Lep. reticulatus*, *L. entomophila*, *L. brunnea*, *L. corrodens*, *L. decolor*, and *L. paeta* (voucher specimens number 181, 182, 203, 204, 205, and 207, respectively, in the Kansas State University Museum of Entomological and Prairie Arthropod Research), were taken from colonies maintained at the U.S. Department of Agriculture, Center for Grain and Animal Health Research in Manhattan, KS. Insects were reared on a diet of 95% cracked hard red winter wheat 'Santa Fe', 2% wheat germ (Natural Raw Wheat Germ, Bob's Red Mill Natural Foods Inc., Milwaukie, OR), 2% brewer's yeast (MP Biomedicals, Solon, OH), and 1% crisped rice cereal (Rice Krispies, Kellogg's Company, Battle Creek, MI) at 30°C, 65% relative humidity (RH), and a photoperiod of 16:8 (L:D) h.

Two *L. entomophila* and two *L. paeta* age categories were evaluated in the experiments: 0–7-d-old and 21–28-d-old adults. To obtain psocids at these two stages, acrylic vials (3 cm in diameter by 8.5 cm in height) containing 1 g of psocid diet were set up with 10 female and 5 male adults at regular intervals. Adults were allowed to lay eggs for 10 d, and then removed. Development from egg to adult takes 24 and 22 d at 30°C for *L. entomophila* (Leong and Ho 1995, Wang et al. 1998) and *L. paeta* (Wang et al. 2009), respectively. The progeny were then collected for the experiments during the appropriate time intervals needed for the experiments. For the other psocid species only one age category was evaluated: mixed-age adults taken from 3–6-mo-old cultures. Females and males were evaluated separately for all the psocid species, except for *Lep. reticulatus*, which is parthenogenetic and therefore only females were evaluated for this species.

Potential Attractants Evaluated. Two series of experiments were conducted. In the first series of experiments, *L. entomophila* and *L. paeta* were evaluated. Nineteen potential attractants were tested and were represented primarily by grain kernels, oils, and commercially available kairomone lures. The grains (1 g) included were whole and cracked wheat kernels, cracked corn (*Zea mays* L., 'Golden Harvest'), cracked rice (mixed variety long grain rough rice from commercial source in Arkansas), cracked oats (*Avena sativa* L., whole organic oats, Living Whole Foods Inc., West

Springville, UT), cracked yellow millet (*Panicum miliaceum* L., organic yellow millet, Eden Organic Foods Inc., Clinton, MI), and cracked carob pods (*Ceratonia siliqua* L., carob raw, Mountain Rose Herbs, Eugene, OR). The oils (0.5 ml) evaluated were corn (Kroger Co., Cincinnati, OH), wheat germ (Viobin, McShares, Inc., Monticello, IL), walnut (*Juglans regia* L., Spectrum Organic Products, LLC., Melville, NY), and clove bud (*Syzygium aromaticum* L., Plant Therapy Essential oils, Twin Falls, ID). Clove bud oil was included as a negative control, as this essential oil has been reported to be repellent to other insects and experiments with *L. bostrychophila* indicated that fewer individuals selected clove bud oil dishes compared with control dishes (Diaz-Montano et al. 2014). Three commercially available food attractants used in traps intended primarily to capture stored-product beetles were also included: 1) kairomone food oil (0.5 ml) from the Dome trap (Trécé Inc., Adair, OK), 2) multispecies food attractant pellet from Xlure MST trap (Russell IPM, Deeside, Flintshire, UK), and 3) cigarette beetle kairomone lure (CB/K 3171, Trécé Inc., Adair, OK). In addition, brewer's yeast (1 g), wheat germ (1 g), psocid diet (1 g, described above), and cracked wheat (1 g) infested with five female and five male adults for 1 or 4 wk before the experiment were also included to determine if *L. entomophila* or *L. paeta* responded to grain infested with living conspecifics.

In the second series of experiments, response by *Lep. reticulatus*, *L. brunnea*, *L. decolor*, and *L. corrodens* to a reduced set of potential attractants was evaluated. Potential attractants with a low to intermediate behavioral response by *L. entomophila* and *L. paeta* in this study and by *L. bostrychophila* (Diaz-Montano et al. 2014) were dropped from consideration when testing these additional species. Only the three most promising potential attractants (brewer's yeast, wheat germ, and wheat germ oil) and clove bud oil (included as a negative control) were included in these evaluations.

Bioassay. The responses of the six psocid species to different potential attractants in this study were evaluated using the same protocol as in Diaz-Montano et al. (2014). A polystyrene Petri dish (14 cm in diameter by 1.4 cm in height) was used as the arena. At the base of the Petri dish two equidistant holes (2 mm in diameter, 2 cm from the edge of the Petri dish, and 10 cm apart along the diameter mid-line of the dish) were made. The larger arena Petri dish was placed on top of two small polystyrene Petri dishes (3.5 cm in diameter by 1.0 cm in height), with a small dish centered under each hole of the larger dish. One of the small Petri dishes contained a potential attractant material, and the other small Petri dish served as a blank control in a paired choice test. In addition, a control treatment was included with the two small Petri dishes left blank. To avoid psocid escapes, the interior walls of all Petri dishes were coated with polytetrafluoroethylene (60 wt. % dispersion in water, Sigma-Aldrich Co., St. Louis, MO), which makes the walls slippery so that psocids are unable to climb and so that no additional sealing between the small and large dishes was needed.

A total of 15 psocid adults were collected and placed in a small polystyrene Petri dish (3.5 cm in diameter). This Petri dish was turned over at the center of the arena, and lights were turned off. After a 1-h acclimation period, the small Petri dish was removed, and then psocids were able to move freely in the arena. The experiments were conducted in the dark at 25°C and 65% RH. After 24 h, the number of psocids outside (in the arena) and inside each of the small Petri dishes was counted. For treatments of cracked wheat previously infested with psocids, the number selecting the treatment small dish could not be directly counted, so the number was estimated by taking the total number of psocids used ($n = 15$) and subtracting the number of psocids found outside (in the arena) and inside the control dish.

Experimental Design and Analysis. All the different potential attractants were evaluated at the same time for all psocid species. One replicate of each treatment was set up per day with a total of 10 replications per test group. New Petri dishes were used for each replication. Analysis of variance (ANOVA) was conducted to compare the numbers of psocids captured in the dish containing the potential attractant among the different materials within a psocid age group. Post hoc multiple comparisons were conducted using Tukey's studentized range test (SAS Institute 2008).

Results

Liposcelis entomophila. For both sexes and age groups, response to brewer's yeast was greater than or equal to that for all other treatments (Fig. 1). For the 0–7-d-old females ($F = 9.1$; $df = 19, 171$; $P < 0.001$; Fig. 1A) and males ($F = 6.7$; $df = 19, 171$; $P < 0.001$; Fig. 1B), the response to brewer's yeast was not significantly different from that to wheat germ oil, wheat germ, or psocid diet. For the females, response to brewer's yeast was also not different from that to the whole wheat (Fig. 1A). And for the males, this response was not different from that to kairomone food oil from the Dome trap, cigarette beetle kairomone lure, cracked oats, cracked carob pods, cracked wheat, and cracked wheat infested with psocids 1 wk before the experiments (Fig. 1B). *L. entomophila* 21–28-d-old females ($F = 8.9$; $df = 19, 171$; $P < 0.001$) (Fig. 1C) and males ($F = 13.9$; $df = 19, 171$; $P < 0.001$; Fig. 1D) had a stronger response to brewer's yeast compared with any of the other potential attractants. The average response to brewer's yeast was between 9.3–11 adults (62–73%). For all ages, the response to clove bud oil was significantly less than the response to the blank control and to all other materials (Fig. 1A–D).

Liposcelis paeta. For both sexes and age groups, response to wheat germ oil was numerically highest, but in all cases it was not significantly different from a range of materials including wheat germ and brewer's yeast (Fig. 2). The 0–7-d-old females ($F = 10.5$; $df = 19, 171$; $P < 0.001$; Fig. 2A) and the males ($F = 16.3$; $df = 19, 171$; $P < 0.001$; Fig. 2B) had the greatest response to wheat germ oil, but this response

was not significantly greater than to brewer's yeast, wheat germ, kairomone food oil from the Dome trap, psocid diet, cracked grains of yellow millet, oats, and corn, and cracked wheat infested with psocids 1 wk before the experiments. In addition, for females, response to wheat germ oil was not different from that to cracked wheat (Fig. 2A), and, for males, this response was not different from that to the corn oil (Fig. 2B). *L. paeta* 21–28-d-old females ($F = 16.7$; $df = 19, 171$; $P < 0.001$; Fig. 2C) and males ($F = 24.0$; $df = 19, 171$; $P < 0.001$; Fig. 2D) also had the strongest response to wheat germ oil, but this response was not significantly different from that to brewer's yeast, wheat germ, psocid diet, kairomone food oil from the Dome trap, or cracked grains of yellow millet, corn, wheat, and oats. For the females, response to wheat germ oil also was not different from that to the cracked wheat infested with psocids 1 wk before the experiments (Fig. 2C), and, for the males, this response also was not different from that to the cracked rice, corn oil, and walnut oil (Fig. 2D). The average response to wheat germ oil was between 8.3–9.6 adults (55–64%), and the response to brewer's yeast was between 6.3–8.6 adults (42–57%). The lowest response always was to clove bud oil, and this response was not significantly different from that to cigarette beetle kairomone lure (Fig. 2A–D).

***Liposcelis brunnea*.** Both mixed-age females ($F = 55.7$; $df = 4, 36$; $P < 0.001$; Fig. 3A) and males ($F = 163.8$; $df = 4, 36$; $P < 0.001$; Fig. 3B) had a strong response for brewer's yeast, but this response was not significantly different from that to wheat germ oil, and, for males, response to brewer's yeast also was not different from that to wheat germ. The average response to brewer's yeast was between 9.6–10.2 adults (64–68%). For males and females, the response to the clove bud oil was significantly less than the response to all other materials, except for the blank control (Fig. 3A–B).

***Liposcelis decolor*.** For both mixed-age females ($F = 31.7$; $df = 4, 36$; $P < 0.001$; Fig. 3C) and males ($F = 32.4$; $df = 4, 36$; $P < 0.001$; Fig. 3D), response to brewer's yeast was greater than for all other treatments. The average response to brewer's yeast was between 10.9–11.7 adults (73–78%). For males and females, the response to the clove bud oil was significantly less than the response to all other materials, except for the blank control (Fig. 3C–D).

***Liposcelis corrodens*.** For both sexes and age groups, there was a very low response (≤ 3 adults responded) to the potential attractants. The response of mixed-age females did not differ with the materials evaluated ($F = 2.5$; $df = 4, 36$; $P = 0.063$; Fig. 4A). The mixed-age males had the strongest response ($F = 6.2$; $df = 4, 36$; $P < 0.001$) to brewer's yeast, wheat germ oil, and wheat germ (Fig. 4B). The clove bud oil was the least preferred, but was not significantly different from the blank control and wheat germ (Fig. 4B). The average response to brewer's yeast was between 2.3–2.9 adults (15–19%).

***Lepinotus reticulatus*.** The mixed-age females had the strongest response to brewer's yeast, wheat germ

oil, and wheat germ (Fig. 4C). The average response to brewer's yeast was 6.0 adults (40%). The response to the clove bud oil was significantly ($F = 36.3$; $df = 4, 36$; $P < 0.001$) less than the response to all other materials, but not different from the blank control (Fig. 4C).

Discussion

Among the potential attractants evaluated, brewer's yeast was the material that most consistently elicited the strongest response for the psocid species evaluated. However, this response frequently was not significantly different from that to wheat germ and wheat germ oil. This outcome is similar to the response by *L. bostrychophila* for brewer's yeast in our previous research (Diaz-Montano et al. 2014). In the present study, the percentage response to brewer's yeast varied considerably among the psocid species tested: *L. decolor* (73–78%), *L. entomophila* (62–73%), *L. brunnea* (64–68%), *L. paeta* (42–57%), *Lep. reticulatus* (40%), and *L. corrodens* (15–19%). Two psocid species (*L. corrodens* and *Lep. reticulatus*) had low responses to all the potential attractants evaluated. This low response could be due to lower mobility compared with other psocid species, preference for other volatile cues, or an overall low response to volatile food cues. Observations from other experiments in which the walking surface was paper indicated that these two species appeared to move similarly to the other psocid species. Further experiments will need to be performed to evaluate the reasons for the low response to these volatile cues.

In our previous study of evaluation of potential attractants for *L. bostrychophila* (Diaz-Montano et al. 2014), we proposed that psocids may release pheromones or other attractive substances under specific conditions that were not present in our colonies or experiments at that time. Shao et al. (2006) determined the presence of a sex pheromone in *L. entomophila*, but there are no reports of pheromones in any of the other psocid species. Extracts from *L. bostrychophila* (blend of adults and nymphs) elicited avoidance at the highest concentrations and preference at the two lowest concentrations (Green 2005). In another study, individual compounds from these extracts were tested, and *L. bostrychophila* adults congregated on stearic acid rather than on oleic, linoleic, or linolenic acids (Green 2011). Subsequently, Green (2014) tested three volatile chemical compounds collected from *L. bostrychophila* extracts (isobutanoic acid, butanoic acid, and hexanoic acid); when psocids were given multiple choices of these three compounds for 1, 2, 4, and 24 h, the proportion of insects responding did not vary among the volatiles. Hexanoic acid was initially avoided, but this effect gradually decreased by 24 h. These studies suggest the potential presence of psocid pheromones because psocids congregated on extracts and chemical compounds from conspecifics. However, in our previous study with *L. bostrychophila* (Diaz-Montano et al. 2014) and in the present study, one of the potential attractants included was cracked wheat that was previously infested with living conspecifics, *L. entomophila* and *L. paeta*, for one and four weeks before the

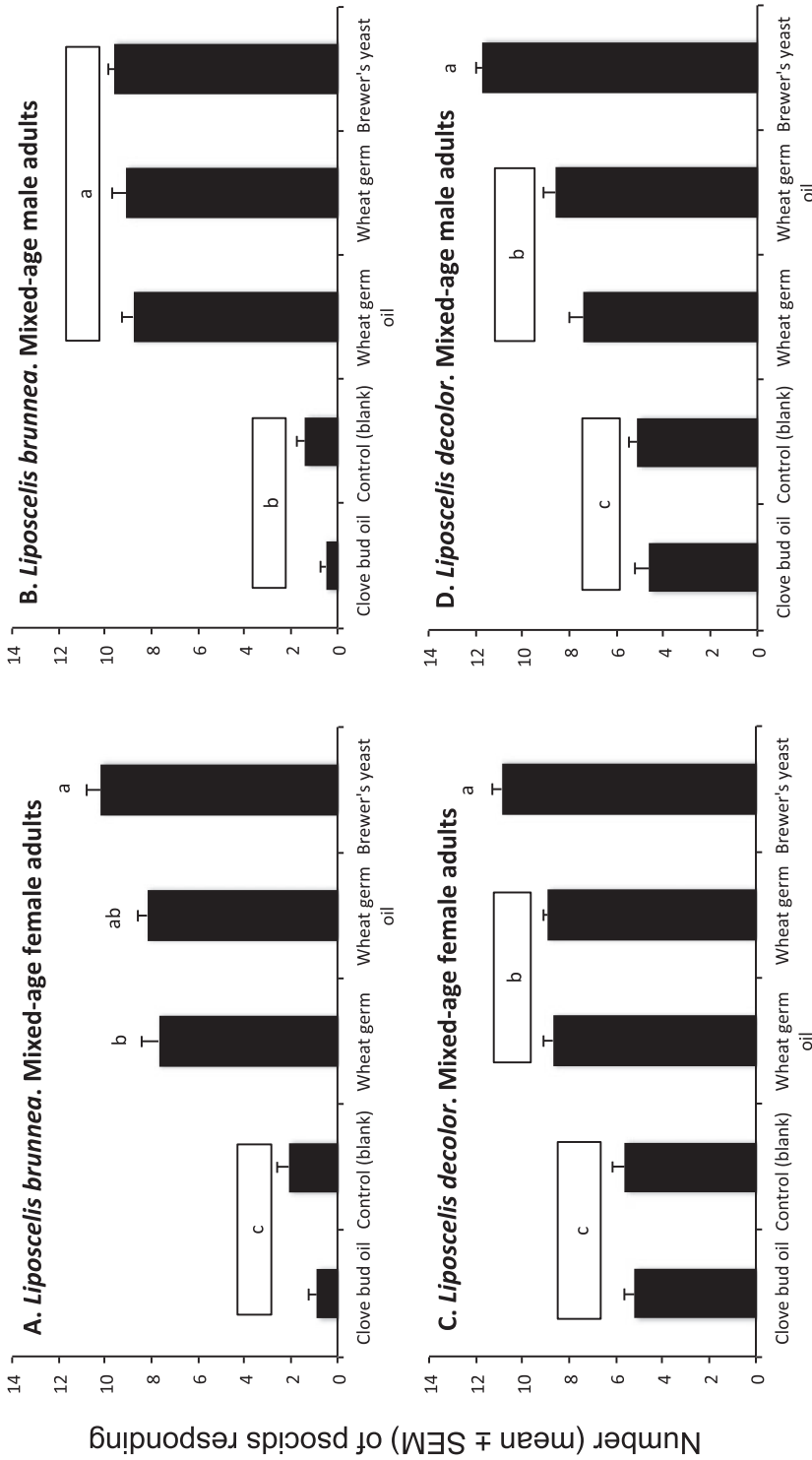


Fig. 3. Number (mean \pm SEM) out of 15 individuals of (A) *L. brunnea* mixed-age female adults, (B) *L. brunnea* mixed-age male adults, (C) *L. decolor* mixed-age female adults, and (D) *L. decolor* mixed-age male adults that selected the treatment dish when given a choice between a potential attractant treatment dish and a blank control dish. Bars with different letters are significantly different ($P < 0.05$; Tukey's studentized range test). Groups of letters within a box indicate that two or more treatments were not significantly different from each other.

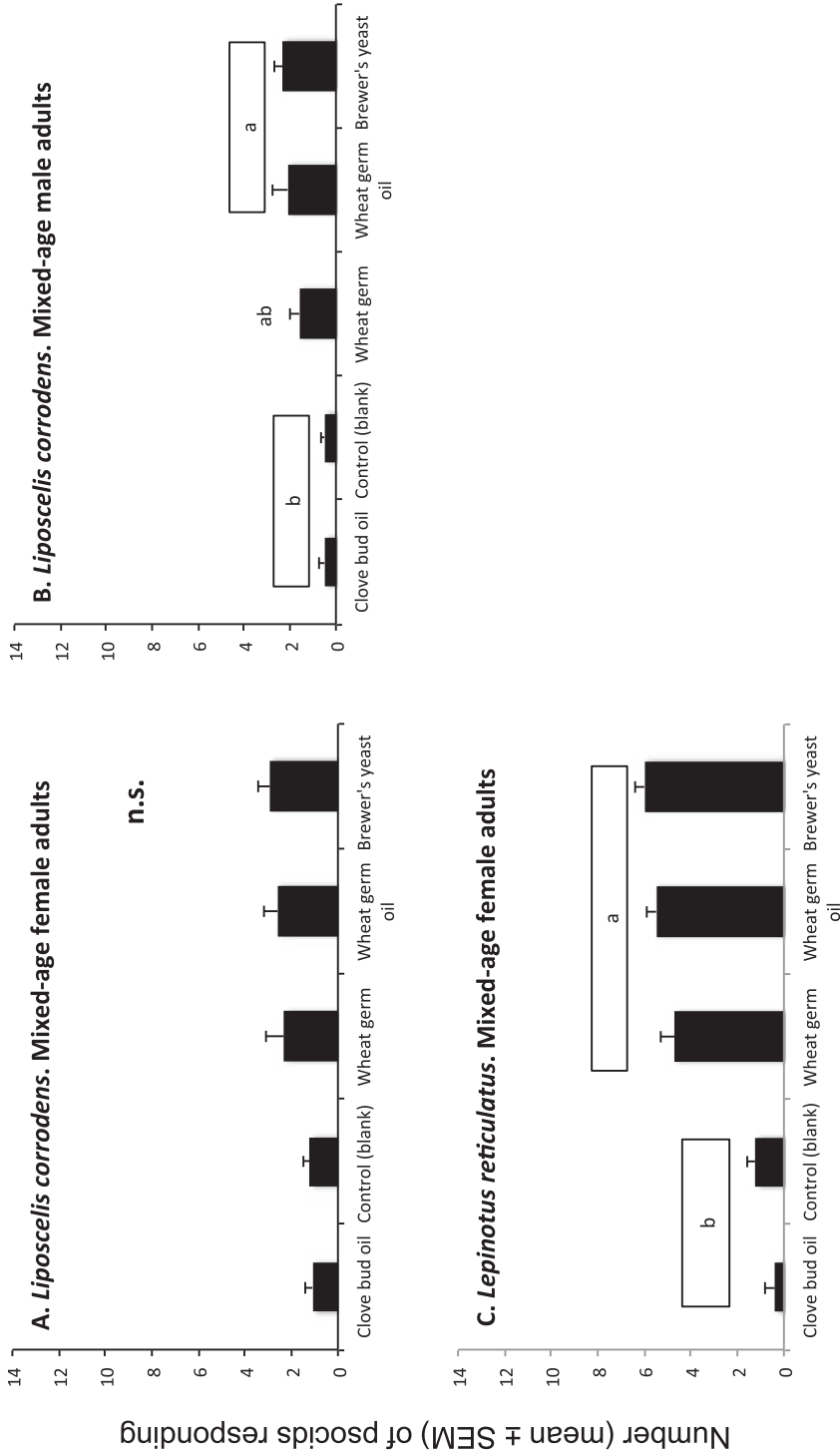


Fig. 4. Number (mean ± SEM) out of 15 individuals of (A) *L. corrodens* mixed-age female adults, (B) *L. corrodens* mixed-age male adults, and (C) *Lep. reticulatus* mixed-age female adults that selected the treatment dish when given a choice between a potential attractant treatment dish and a blank control dish. Bars with different letters are significantly different ($P < 0.05$; Tukey's studentized range test). Groups of letters within a box indicate that two or more treatments were not significantly different from each other.

experiment, and we found that this did not increase or decrease the response of the two species tested, compared with cracked wheat alone.

Most psocid species evaluated here had a very low response to clove bud oil (1.3–8%), often significantly lower than to the blank control, but *L. decolor* had a relatively high response to clove bud oil (31–35%). However, this response was significantly lower than to the other materials evaluated, but not different from response to the blank control. Clove bud oil is known to be a strong repellent to multiple insect pests, including mosquitoes (Barnard 1999, Trongtokit et al. 2005, Kang et al. 2009), bean bug, *Riptortus clavatus* (Thunberg) (Yang et al. 2009), the potato psyllid, *Bactericera cockerelli* (Sulc) (Diaz-Montano and Trumble 2013), and *L. bostrychophila* (Diaz-Montano et al. 2014) among others. The main focus of this investigation was to identify potential attractants to psocids, but given the strong repellent effect of the clove bud oil on *L. bostrychophila* and other insect species, as described above, we considered it important to explore the potential repellent effect of this oil on the psocid species evaluated in this study. Other oils have been found to elicit a strong repellent effect on *L. bostrychophila*, such as Chinese weeping cypress, *Cupressus funebris* Endl., Scots pine, *Pinus sylvestris* L. (Wang et al. 2001), and the essential oil of *Kaempferia galanga* L. rhizomes (Liu et al. 2014).

In general, the other potential attractants evaluated using *L. entomophila* and *L. paeta* elicited only a low to intermediate response by adults of both ages evaluated. The commercial kairomone 3 (cigarette beetle kairomone lure) had a similar repellent effect to clove bud oil on *L. paeta* adults in both sexes and ages tested. This kairomone elicited an intermediate response by *L. entomophila* in this study and low to intermediate response by *L. bostrychophila* (Diaz-Montano et al. 2014). The other two commercial kairomones tested produced an intermediate response by *L. entomophila* and *L. paeta*. Thus, there is potential to improve the effectiveness of currently available traps by adding attractants specific for psocids that generate a stronger response than currently available attractants.

To study the responses of six psocid species to different potential attractants, we used a two-choice pitfall bioassay that was efficiently used first in complementary experiments with *L. bostrychophila* (Diaz-Montano et al. 2014). In the present study, we were able to identify some materials as prospective candidates for development as attractants for psocid species. These candidates included brewer's yeast and in some cases wheat germ and wheat germ oil, which elicited the strongest responses by psocids. However, additional studies will need to be conducted to determine if psocids can orient to these materials over longer distances. The strong responses to brewer's yeast by most of the psocid species evaluated in this study and by *L. bostrychophila* (Diaz-Montano et al. 2014) suggests that incorporating this attractant into traps for a psocid-monitoring program in food storage facilities could result in improved detection of a wide range of psocid species.

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