

Evaluation of Potential Attractants for *Liposcelis bostrychophila* (Psocoptera: Liposcelidae)

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ABSTRACT The psocid, *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelidae), can cause significant damage to stored commodities, and its pest status in the United States has been increasing over the last decade. Because *L. bostrychophila* is difficult to control with conventional methods, it is critical to explore alternative approaches such as the use of attractants that can be incorporated into integrated pest management programs for monitoring psocids. The orientation response of several *L. bostrychophila* life stages (first and second instars, third and fourth instars, 0- to 7-d-old adults, 21- to 28-d-old adults, and adults of mixed ages) to a range of potential attractants (including whole and cracked grains, grain-based oils, wheat germ, brewer's yeast, and commercially available kairomone lures) was studied using a two-choice pitfall test to identify candidates for further development as lures in traps. Among the potential attractants evaluated, the strongest response by all stages of *L. bostrychophila* was to brewer's yeast. Other materials for which there was consistently a strong response were psocid diet, wheat germ, and wheat germ oil. These results show the potential for developing monitoring tools for integrated pest management programs for *L. bostrychophila* and other psocid species.

RESUMEN El psocido *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelidae) puede causar daño significativo a diversos productos almacenados y su estatus como plaga en los Estados Unidos ha incrementado durante la última década. Debido a que *L. bostrychophila* es difícil de controlar con métodos convencionales es necesario explorar diferentes estrategias, como el uso de atrayentes, para que puedan ser incorporadas en un programa de manejo integrado para monitorear psocidos. Se investigó el comportamiento de diferentes estadios de *L. bostrychophila* (ninfas de 1^{er} y 2^{do} instar, ninfas de 3^{er} y 4^{to} instar, adultos de 0–7 días de edad, adultos de 21–28 días de edad y adultos de diversas edades) a varios atrayentes potenciales (incluyendo granos enteros y triturados, aceites de diferentes granos, germen de trigo, levadura de cerveza y cebos con kairomonas disponibles comercialmente) por medio de experimentos de libre selección para identificar atrayentes para ser utilizados en trampas. De todos los potenciales atrayentes evaluados, la respuesta mas fuerte de todos los estadios de *L. bostrychophila* fue a la levadura de cerveza. El aceite de germen de trigo, el germen de trigo y la dieta para los psocidos fueron los siguientes con una fuerte respuesta. Estos resultados parecen ser potenciales para desarrollar técnicas de monitoreo para programas de manejo integrado de *L. bostrychophila* y otras especies de psocidos.

KEY WORDS booklice, monitoring, attractant, grain, stored product

Psocids in the genus *Liposcelis* have become important global pests of stored products. Despite their small size, psocids can cause significant reductions in grain

weight and quality (McFarlane 1982, Rees and Walker 1990, Kučerová 2002), contaminate packaged foods, and infest structures where food is processed and stored (Turner 1994, Athanassiou et al. 2010b). *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelidae) is one of the most commonly found species (Mills et al. 1992, Turner 1994), and it has become an important pest in Australia (Rees and Walker 1990, Rees 1994), Asia (Leong and Ho 1994; Rajendran 1994; Rees 1994; Wang et al. 1999, 2000b), Europe (Turner 1994, 1998), Canada (Mills et al. 1992), and the United States (Throne 2010, Phillips and Throne 2010).

A number of factors contribute to the pest status of *L. bostrychophila*. This species has a rapid development from egg to adult (18 d at 32.5°C), a high lifetime reproductive capacity (Wang et al. 2000a), and par-

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thenogenetic reproduction (Mockford 1971) that lead to rapid increases in population size on stored grains and other diets (Mills et al. 1992, Ding et al. 2002, Kučerová 2002, Gautam et al. 2013). *L. bostrychophila* can persist when food is not present, surviving without food for up to 60 d (Turner and Maude-Roxby 1988), and can feed on alternate food sources such as fungi (Mills et al. 1992). Control of psocids with insecticides is often problematic, as many products used to control other stored-product insects are not effective against psocids (Athanasios et al. 2009). Populations of *L. bostrychophila* have shown resistance to fumigants (Ho and Winks 1995; Nayak et al. 1998, 2003; Nayak and Collins 2008), contact insecticides (Büchi 1994; Nayak et al. 1998, 2002, 2005; Ding et al. 2002; Dou et al. 2006; Guedes et al. 2008; Athanasios et al. 2009, 2010a; Opit et al. 2012), and entomopathogenic fungi (Howard and Lord 2003, Lord and Howard 2004).

Effective monitoring is a critical component of integrated pest management programs, but limited information is available on how to most effectively attract and capture psocids. Psocids can be captured using commercially available pitfall-type traps and corrugated cardboard refuges (Opit et al. 2009), but attraction by psocids to commercially available attractants or capture efficiency of traps has not been evaluated. Yeast-baited traps were used to monitor psocids in domestic household surveys in the United Kingdom (Turner and Maude-Roxby 1989, Turner and Bishop 1998), with *L. bostrychophila* being the predominant species captured. Hu et al. (2009) reported that adults of five *Liposcelis* species, including *L. bostrychophila*, have sensillae with likely olfactory functions, but their response to specific stimuli was not evaluated. *L. bostrychophila* has been demonstrated to select substrates with fungal extracts over control substrates (Green 2008), preferred yellow millet to other grains (Green and Turner 2005), and increased their response to buckwheat and yellow millet when brewer's yeast was added. However, in all of these studies, the psocids were allowed to come in contact with the materials, so it is difficult to determine if response was attraction or arrestment, or if psocids were responding to any of the volatile cues associated with the materials. There has not been a systematic evaluation of psocid behavioral response to volatile cues that might be used to attract insects to traps. As part of a broader project to identify attractants for use in traps for monitoring psocids, the response of *L. bostrychophila* to volatiles from different grains, grain-based oils, brewer's yeast, and commercially available food baits was assessed.

Materials and Methods

Insect Culture. *L. bostrychophila* populations (voucher specimen No. 202 deposited at the Kansas State University Museum of Entomological and Prairie Arthropod Research) used in this study were from cultures that were started in 2006 with psocids collected from a grain elevator in Manhattan, KS. Psocids were reared on a diet of 95% cracked hard red winter

wheat (*Triticum aestivum* L. '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.

Five *L. bostrychophila* age categories or life stages were evaluated in the experiments: 0- to 7-d-old nymphs (first and second instars), 8- to 12-d-old nymphs (third and fourth instars), 0- to 7-d-old adults, 21- to 28-d-old adults, and mixed age adults (between 0 and 90 d; adult longevity is 89.4 d at 30°C [Wang et al. 2000a]) from a 3- to 6-mo-old culture. Age ranges of nymphal stages used in this study were determined according to studies at 30°C by Wang et al. (2000a). To obtain psocids at these five stages, containers with psocid diet were set up with 400 adults at regular intervals. Adults were allowed to lay eggs for 7 d, and then removed. The progeny were then collected for the experiments during the appropriate time intervals needed for the experiments. Although *L. bostrychophila* is generally parthenogenetic (Mockford 1971), males have recently been reported (Mockford and Krushelnycky 2008). Our colonies only contain females.

Potential Attractants Evaluated. Two groups of potential attractants were evaluated. The first group consisted primarily of whole and cracked grains of wheat, corn (*Zea mays* L., 'Golden Harvest'), rice (*Oryza sativa* L., mixed variety long grain rough rice from commercial source in Arkansas), oats (*Avena sativa* L., whole organic oats, Living Whole Foods Inc., West Springville, UT), and yellow millet (*Panicum miliaceum* L., organic yellow millet, Eden Organic Foods Inc., Clinton, MI). In addition, we tested cracked carob pods (*Ceratonia siliqua* L., carob raw, Mountain Rose Herbs, Eugene, OR), brewer's yeast, brewer's yeast suspension in water (10% suspension applied to 3-cm-diameter filter paper by placing paper in suspension, removing it, and letting it dry), and psocid diet (described above). For all materials, except the brewer's yeast suspension, 1 g was used in experiments. Carob pods were included because other stored-product pests have shown preference for this material (Dobie 1978). Brewer's yeast was included because of reports in the literature (Turner and Maude-Roxby 1989, Turner and Bishop 1998) and preliminary experiments that indicated a strong preference by *L. bostrychophila* and other psocid species.

The second group of potential attractants was represented primarily by oils and commercially available kairomone lures. The following oils (0.5 ml) were evaluated: 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 preliminary experiments indicated that fewer *L. bostrychophila* individuals selected clove bud

oil compared with control petri dishes. Three commercially available food attractants used in traps intended primarily to attract stored-product beetles were also included: 1) kairomone food oil (0.5 ml) from the Dome trap (Trécé Inc., Adair, OK), 2) multi-species food attractant pellet from Xlure MST trap (Russell IPM, Deeside, Flintshire, United Kingdom), and 3) cigarette beetle kairomone lure (CB/K 3171, Trécé Inc.). Wheat germ (1 g) and cracked wheat (1 g) were included. In addition, cracked wheat (1 g) infested with 10 *L. bostrychophila* adults for 1, 2, 3, or 4 wk before the experiment were also included to determine if *L. bostrychophila* responded to grain infested with living conspecifics. In addition, brewer's yeast (1 g) was included for comparison because it elicited a strong response in the first group.

Experimental Bioassay. The response of *L. bostrychophila* to potential attractants was evaluated in an arena that consisted of a polystyrene petri dish (14 cm in diameter by 1.4 cm in height). The bottom half of this petri dish had 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). A small polystyrene petri dish (3.5 cm in diameter by 1.0 cm in height) was placed below each hole. The interior walls of all petri dishes were coated with polytetrafluoroethylene (60 wt. % dispersion in water, Sigma-Aldrich Co., St. Louis, MO) to prevent psocid climbing and avoid escapes. The potential attractant material was placed in one of the small petri dishes and the other small petri dish contained no materials and served as a blank control in a paired choice test. A control treatment with both small petri dishes left blank was also performed.

Fifteen psocid individuals (nymphs or adults) were collected and placed in a petri dish (3.5 cm in diameter). Subsequently, this petri dish was turned over carefully at the center of the arena, and lights were turned off. After a 1-h acclimation period, the small petri dish was removed, and psocids were allowed to move freely in the large dish arena. The experiments were conducted in a walk-in growth chamber under dark conditions 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. The number of psocids in the treatments of cracked wheat infested with psocids was estimated by subtracting the total number of psocids used ($n = 15$) by the numbers of psocids found outside (in the arena) and inside the control treatment. The petri dishes in each experiment were taken into the walk-in growth chamber at least 1 h before starting the experiment, and this seemed to remove static electricity observed when relative humidity was low in the laboratory.

Experimental Design and Analysis. Within each test group and *L. bostrychophila* age or life stage, all the different potential attractants were evaluated at the same time. 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 stage or age-group. Post hoc multiple comparisons were conducted using Tukey's studentized range test (SAS Institute 2008).

Results

First Group of Potential Attractants. There were no significant differences in the response of *L. bostrychophila* first-second instars to the potential attractants evaluated ($F = 1.5$; $df = 14, 126$; $P = 0.125$; Fig. 1A), and ≤ 3 nymphs responded to potential attractants. The strongest response by third-fourth instars was to the brewer's yeast ($F = 7.5$; $df = 14, 126$; $P < 0.001$), with all other tested potential attractants not different from the control (Fig. 1B), although overall response was low, with ≤ 4 nymphs responded to any of the potential attractants. *L. bostrychophila* 0- to 7-d-old ($F = 8.7$; $df = 14, 126$; $P < 0.001$; Fig. 1C) and 21- to 28-d-old adults ($F = 9.7$; $df = 14, 126$; $P < 0.001$; Fig. 1D) both had a stronger response to brewer's yeast, compared with any of the other treatments except psocid diet (that also contains brewer's yeast). *L. bostrychophila* mixed-age adults had a stronger response to brewer's yeast than to all other potential attractants ($F = 6.7$; $df = 14, 126$; $P < 0.001$; Fig. 1E). Although adult response to some of the potential attractants was significantly greater than the blank control, none of the materials other than brewer's yeast exhibited a consistently strong response. Overall, the average response of adults to brewer's yeast was >11 adults ($>70\%$).

Second Group of Potential Attractants. *L. bostrychophila* first-second instars had relatively weak responses (≤ 3 nymphs) to all of the potential attractants. Brewer's yeast had the highest average response; however, this response was not statistically different from most of the potential attractants tested ($F = 4.1$; $df = 14, 126$; $P < 0.001$; Fig. 2A). Similarly, third-fourth instars had a higher response to brewer's yeast than to the blank control, but it was not statistically greater than several of the other potential attractants tested ($F = 8.9$; $df = 14, 126$; $P < 0.001$; Fig. 2B). The 0- to 7-d-old adults ($F = 23.0$; $df = 14, 126$; $P < 0.001$; Fig. 2C), 21- to 28-d-old adults ($F = 21.0$; $df = 14, 126$; $P < 0.001$; Fig. 2D), and mixed-age adults ($F = 20.1$; $df = 14, 126$; $P < 0.001$; Fig. 2E) all had the strongest numerical response to brewer's yeast, and this response was significantly greater than to the blank control. However, response to brewer's yeast was not significantly different from that to wheat germ oil or wheat germ for any of the adult age-groups. For the 21- to 28-d-old adults, response to brewer's yeast was also not different from that to the kairomone food oil from the Dome trap, and cracked wheat infested with psocids 1 and 4 wk before the experiments. Again, the average adult response to brewer's yeast was >11 adults ($>70\%$). For all adult ages, the response to clove bud oil was significantly less than the response to the blank control and to all other materials (Fig. 2C-E).

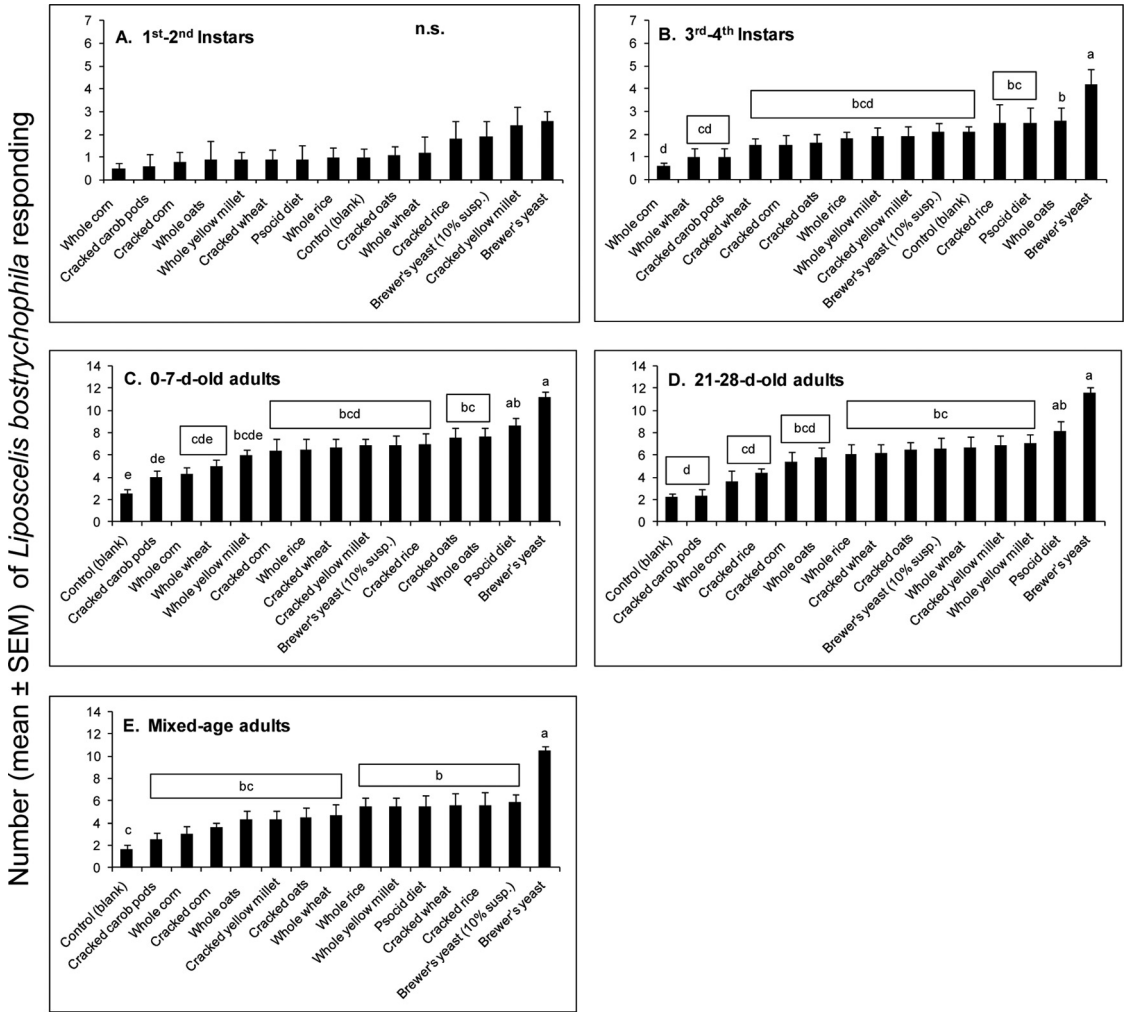


Fig. 1. Number (mean ± SEM) of *L. bostrychophila* selecting a potential attractant when 15 individuals were given a choice between a treatment dish with different grains or brewer's yeast and a blank control dish. Scale bars with different letters are significantly different ($P < 0.05$; Tukey's studentized range test). Groups of letters within a box indicate that they are the same for two or more treatments. Ten replications per each *L. bostrychophila* stage were evaluated.

Discussion

Brewer's yeast elicited the most consistent strong response (i.e., relatively high number of individuals in the dish with potential attractant) for all *L. bostrychophila* life stages and ages tested and in both groups of potential attractants, although the response to other materials also may have been as significantly strong depending on the life stage or age tested. The percentage of adults that response to brewer's yeast in the three different adult ages evaluated ranged from 70 to 77% and 76 to 80% in the first and second groups of potential attractants, respectively. In the first group, *L. bostrychophila* adults also exhibited a strong response to psocid diet, but response only ranged from 37 to 58%. In the second group, wheat germ and wheat germ oil also elicited a strong response by adults, but percentage response ranged from 67 to 72% and 64 to 75%, respectively. A strong response by psocid adults to

brewer's yeast was also observed in a choice study by Green and Turner (2005) where *L. bostrychophila* adults significantly preferred ground buckwheat or ground yellow millet with yeast over the same materials without yeast. Green and Turner (2005) also tested the preference of *L. bostrychophila* adults using pairs of five materials (whole buckwheat, whole pot barley, whole yellow millet, ground brown rice flour, and wheat germ). The percentages of adults that significantly preferred yellow millet over wheat germ, pot barley, and buckwheat were 62, 60, and 58%, respectively. In contrast, in our study the percentage of *L. bostrychophila* adults preferring wheat germ (67–72%) was higher than for whole (37–47%) or cracked (29–46%) yellow millet; however, we did not perform choice tests between multiple attractants. These earlier studies allowed the psocids to contact the food materials, and thus selection may have been triggered

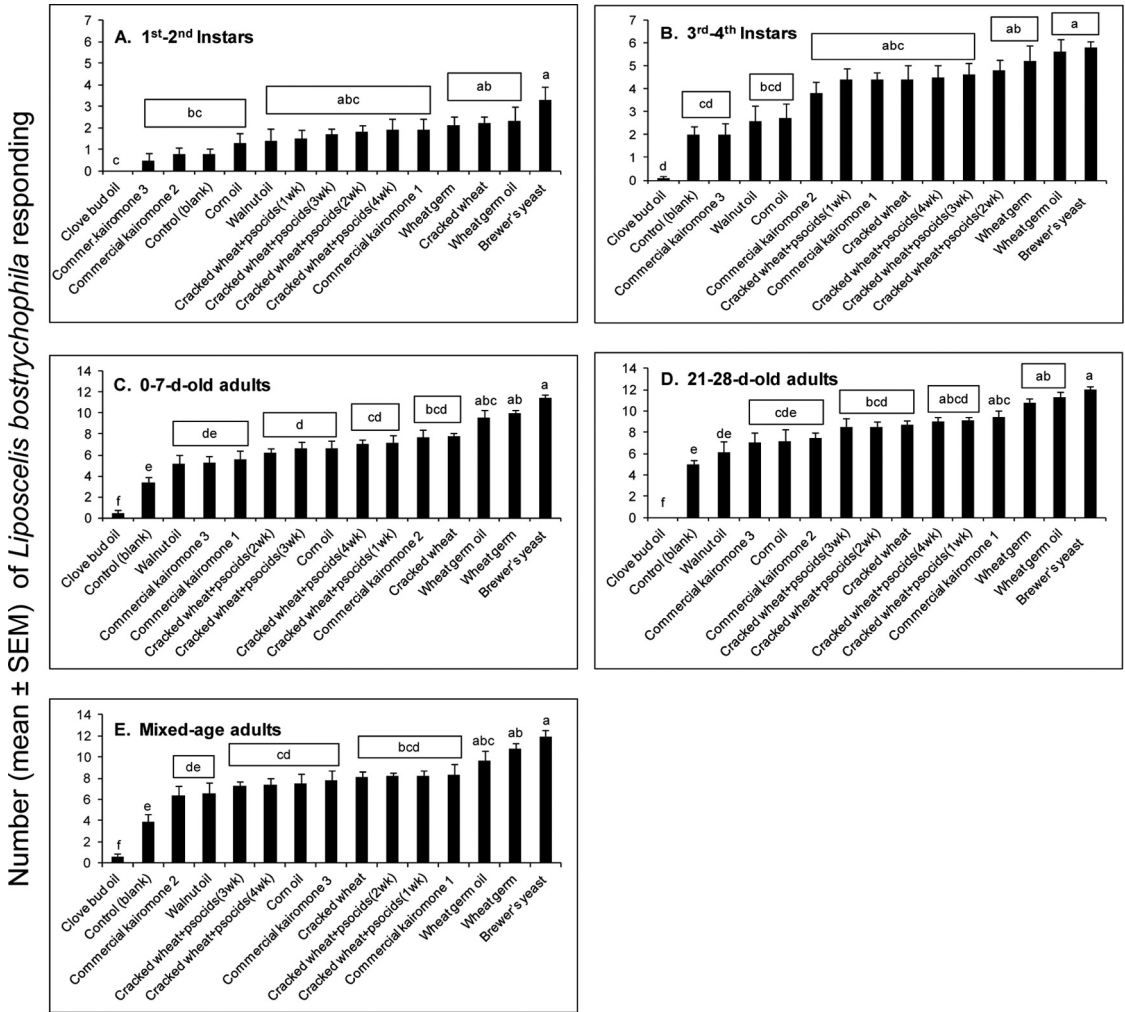


Fig. 2. Number (mean ± SEM) of *L. bostrychophila* selecting a potential attractant when 15 individuals were given a choice between a treatment dish with different oils, commercial lures, wheat germ, cracked wheat, brewer's yeast, or cracked wheat infested with 10 *L. bostrychophila* adults 1, 2, 3, and 4 wk before the experiment and a blank control dish. Scale bars with different letters are significantly different ($P < 0.05$; Tukey's studentized range test). Groups of letters within a box indicate that they are the same for two or more treatments. Ten replications per each *L. bostrychophila* stage were evaluated. Commercial kairomones tested were 1) kairomone food oil from Dome trap (Trécé Inc., Adair, OK); 2) Multi-Species food attractant pellet from Xlure MST trap (Russell IPM, Deeside, Flintshire, United Kingdom); and 3) cigarette beetle kairomone lure (CB/K 3171, Trécé Inc.).

by a variety of tactual and chemosensory mechanisms. The design of the current study was to detect the response primarily to volatile cues.

We did not detect an increased or decreased response by *L. bostrychophila* to cracked wheat infested with living conspecifics, which, for example, might occur if a pheromone was present. Shao et al. (2006) demonstrated the presence of a sex pheromone in *Liposcelis entomophila* (Enderlein), but there are no reports of pheromones in *L. bostrychophila*. Green (2005) found that *L. bostrychophila* extracts from a blend of nymphs and adults significantly (at different concentrations 10,000, 1000, 100, and 10 ppm) triggered avoidance by *L. bostrychophila* adults at the two highest concentrations, and preference at the lowest

concentrations did not differ from controls. Furthermore, Green (2011) studied individual compounds from these extracts and found that adults were able to distinguish between four fatty acids in two-way choice tests in which psocids selected stearic acid followed by oleic acid; when these four fatty acids were presented at the same time, *L. bostrychophila* adults chose stearic acid over oleic, linoleic, or linolenic acids. These results suggest the potential for a *L. bostrychophila* pheromone. In our study, we included treatments with cracked wheat that was previously infested with 10 *L. bostrychophila* adults 1, 2, 3, and 4 wk before the experiments. In all of the experiments, the number of *L. bostrychophila* attracted to these four treatments was not significantly different from the number of

psocids found on the cracked wheat only treatment. Our results seem to indicate that nymphs and adults of *L. bostrychophila* are neither attracted nor repelled by living conspecifics, or that the response to living conspecifics is no stronger than to cracked wheat alone. It could be that this insect only releases pheromones or other attractive substances in specific situations that were not present in our colonies or experiments. It is also possible that the response in the earlier study was associated with contact cues rather than volatile cues.

Clove bud oil has been reported to be an effective repellent against insects such as mosquitoes (Barnard 1999, Trongtokit et al. 2005, Kang et al. 2009), bean bug, *Riptortus clavatus* (Thunberg) (Yang et al. 2009), and the potato psyllid, *Bactericera cockerelli* (Sulc) (Diaz-Montano and Trumble 2013). Although our primary objective was to identify potential attractants for *L. bostrychophila*, we included clove bud oil as a negative control and also to evaluate its potential as a repellent for psocids. In all of the experiments performed, we observed the lowest response to clove bud oil (0–4% response) relative to the other materials, and, in many cases, the response was significantly lower than to the blank control, suggesting a strong repellency effect. Wang et al. (2001) tested the repellency of several essential oils to *L. bostrychophila* adults and found a strong repellency of the oils of the Chinese weeping cypress, *Cupressus funebris* Endlicher, and the Scots pine, *Pinus sylvestris* L.

In general, the grains and other materials tested in this study, many of which are foods that can be infested by *L. bostrychophila*, elicited a low to intermediate response by all *L. bostrychophila* stages. Overall, the trend was for psocids to be more attracted to cracked grains than to whole grains of the same kind, but there were no statistical differences. To our knowledge this is the first evaluation of psocid nymphs in attractant studies. Response by nymphs to the potential attractants was low in comparison with adults. For example, brewer's yeast elicited the response of ca. 3 nymphs (first-second instars), 5 nymphs (third-fourth instars), and 12 adults. This could be owing to less mobility, limited olfactory capability, or both, in nymphs compared with adults. There are differences in the antennal segments present in nymphs and adults. *Liposcelis* species (females) first instars have 9 segments and second instars have 15 antennal segments that are partly joined (Kučerová et al. 2009). Third and fourth instars also have 15 segments, but these segments are completely separated which is similar to adults. This presumably means fewer sensilla are present in nymphs. Hu et al. (2009) described sensilla types in adults of five *Liposcelis* species (including *L. bostrychophila*) and suggested that Böhm bristles and microtrichial sensillae present may have mechanoreception functions, and chaetal sensillae and basiconic sensillae may have olfactory functions. The commercially available kairomone lures tested were not specifically developed for psocids and tended to have a low to intermediate attractant response by *L. bostrychophila*. This finding highlights the

need to have more effective attractants included in traps for the monitoring of psocids.

In this research, we presented a simple and effective two-choice pitfall bioassay to test the responses of psocids to volatiles produced by different potential attractants. This approach does not however provide information on the specific behavioral mechanism involved in the response, so it is not possible to determine if these materials will attract psocids from a distance. Using this approach, we demonstrated that brewer's yeast, and to a lesser extent wheat germ and wheat germ oil, are likely candidates for further development as attractants for *L. bostrychophila*. Additional studies will need to be conducted to determine if these materials are psocid attractants and over what distances they might influence behavior. The strong response to brewer's yeast along with the efficient use of traps containing brewer's yeast in household surveys in the United Kingdom (Turner and Maude-Roxby 1989, Turner and Bishop 1998) suggest that there is high potential for incorporating this attractant into a psocid monitoring program. The relatively weak response to grains in this study and the finding that brewer's yeast improved response to grains (Green and Turner 2005) suggests that brewer's yeast might be an effective attractant even in food facilities with competing food odors. Because food facilities often contain multiple psocid species, future research should include similar studies with other psocid species to determine if the same attractant can be used for multiple species, or if specific attractants will be needed for specific psocid species. In addition, further research is needed to evaluate the behavioral response of psocids to traps baited with these attractants under larger and more realistic spatial scales.

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