Development of pull and push–pull systems for management of lesser mealworm, *Alphitobius diaperinus*, in poultry houses using alarm and aggregation pheromones

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Abstract

BACKGROUND: The lesser mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae), is the most important insect pest affecting poultry production around the world, with all life stages being susceptible to infection by bacteria, viruses and fungi. Control of *A. diaperinus* in poultry houses using intensive insecticide application is not effective due to the cryp-tic behaviour of this pest. Here, we evaluated the potential of recently identified *A. diaperinus* alarm (1,4-benzoquinone, 2-methyl-1,4-benzoquinone and 2-ethyl-1,4-benzoquinone) and aggregation [(*R*)-limonene, 2-nonanone, (*E*)-ocimene, (*S*)-linalool, (*R*)-daucene and (*E*,*E*)- -farnesene] pheromones as tools for the management of this pest in poultry houses in Brazil.

RESULTS: Laboratory arena assays with synthetic alarm pheromone confirmed *A. diaperinus* repellency. In an initial field assay, traps baited with synthetic aggregation pheromone captured significantly more insects than control traps. In further field assays that compared a pull (aggregation pheromone) and a push–pull (simultaneous alarm/aggregation pheromone deployment) system, a higher number of *A. diaperinus* were captured in aggregation pheromone-baited traps in the push–pull system.

CONCLUSION: Our results suggest that alarm and aggregation pheromones can be deployed in poultry houses to trap significant numbers of adult *A. diaperinus*. Studies are underway to determine the potential for using these components as part of an integrated *A. diaperinus* management strategy.

Supporting information may be found in the online version of this article.

Keywords: poultry production; aggregation pheromone; pheromone traps; attractant and repellent

1 INTRODUCTION

The lesser mealworm, *Alphitobius diaperinus* Panzer 1797 (Coleoptera: Tenebrionidae), is the most important insect pest affecting poultry production around the world.[1 – 3] Modern broiler facilities offer suitable environmental conditions for *A. diaperinus* proliferation, including high temperatures, dark and sheltered sites, moisture and food availability. As a consequence, poultry houses contain high densities of larvae and adults that aggre-gate predominantly under feeders and along house edges.[4 – 6] In addition, all *A. diaperinus* life stages are susceptible to infection by bacteria, viruses and fungi, characterizing these insects as poultry pathogen vectors.[7 – 10] Control of *A. diaperinus* in poul-try houses is currently undertaken using intensive insecticide application, causing potential contamination of poultry and aviaries, and threatening the delivery of safe food.[11] Addition-ally, due to the cryptic behaviour of this pest, insecticide control is usually not effective.[12,13] In recent years, several alternative methods for lesser mealworm control have been proposed, with the aim of minimizing the use of insecticides and enhancing the quality of the food that is being produced.[14 –16] Semiochemicals, mainly pheromones, have been suggested for use in monitoring and controlling *A. diaperinus* in poultry houses.[17 –21] Recently, we identified three benzoquinones, i.e. 1,4-benzoquinone, 2-methyl-1,4-benzoquinone and 2-ethyl-1,4-benzoquinone, as alarm pheromone components from the abdominal glands of

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Brazilian male and female *A. diaperinus*.[20] Furthermore, we also recently identified a male-produced aggregation pheromone for Brazilian *A. diaperinus* as a six-component blend comprising (*R*)-limonene, 2-nonanone, (*E*)-ocimene, (*S*)-linalool, (*R*)-daucene and (*E*,*E*)- -farnesene.[21] Identification of a six-component blend was a surprising discovery, because earlier work had shown that the aggregation pheromone for North American *A. diaperinus* comprised only five of these six compounds, with (*E*,*E*)- -farnesene being absent from the pheromone blend.[17] Moreover, we reported that all six pheromone components were needed for attraction of Brazilian *A. diaperinus*, whereas for the North American popula-tion, only three of the components, (*E*)-ocimene, 2-nonanone and (*R*)-daucene, were needed to attract both sexes.[19]

Semiochemicals have great versatility and potential to be used in insect pest management. They can be applied, for example, in push –pull systems combining attractant and repellent semio-chemicals, to manipulate the distribution and abundance of pests.[22,23] The aim of this study was to evaluate the potential of aggregation and alarm pheromones produced by Brazilian *A. diaperinus* as tools for management of this pest in poultry houses in tropical and subtropical environments. Two different approaches were evaluated: (i) a pull system using a semiochem-ical blend to attract insects to traps, i.e. traps baited with the synthetic aggregation pheromone (mass-trapping); and (ii) a push –pull system using two semiochemical blends simultane-ously, i.e. the synthetic alarm pheromone and traps baited with the synthetic aggregation pheromone, with the alarm pheromone being deployed to displace insects from their hiding spots and the aggregation pheromone-baited traps to attract displaced insects into traps. In addition, the pheromone traps were evaluated in poultry houses with different population levels, i.e. with new (low population level) and used (high population level) poultry litter. Experiments were conducted in two different locations, in the centre – west (tropical, with temperatures of 25 to 35 °C) and south (sub-tropical, with temperatures of 5 to 25 °C of Brazil), to evaluate whether different weather temperatures could interfere with insect capture. These areas were chosen because of their importance to the Brazilian poultry industry.

2 MATERIAL AND METHODS

2.1 Chemicals

Hexane (HPLC grade, ≥ 97%) and diethyl ether were purchased from Sigma-Aldrich (Steinheim, Germany) and re-distilled before use. 1,4-Benzoquinone (98%) and 2-methyl-1,4-benzoquinone (98%) were also purchased from Sigma-Aldrich. (*R*)-Limonene (95%) was purchased from TCI-America (Portland, OR, USA). 2-Nonanone (99%) was provided by Jeffrey R. Aldrich Consult-ing LLC (Santa Cruz, CA, USA). 2-Ethyl-1,4-benzoquinone (98%), (*E*)-ocimene (98%), (*S*)-linalool (98%), (*R*)-daucene (87%) and (*E*,*E*)- -farnesene (> 95%) were synthesized in the laboratory as described previously.[20,21]

2.2 Laboratory experiments – alarm pheromone

To evaluate the potential of the *A. diaperinus* alarm pheromone as the push component of a push – pull system, laboratory arena tests were conducted. Because of the cryptic behaviour of *A. diaperinus*, the bioassay was conducted under photophase conditions for 24 h to force insects to find hiding places. The arena consisted of open plastic boxes (40 × 60 × 10 cm), in which two polyvinyl chloride (PVC) tubes (3 cm diameter × and 20 cm length) were placed close to the horizontal edges of the arena. Two treatments were evaluated: (i) the alarm pheromone, which consisted of a solution of 1,4-benzoquinone (1 μg), 2-methyl-1,4-benzoquinone (249 μg) and 2-ethyl-1,4-benzoquinone (750 μg) in hexane (400 μL); and (ii) a solvent control, which consisted of hexane (400 μL). Treatments were placed on folded pieces of filter paper (5 × 2 cm, 80 g m^{-2}, J. Prolab, PR-Brazil), which were placed into the PVC tubes. Filter papers were replaced every 24 h. The following experiments were conducted: (i) both PVC tubes treated with hexane (negative control), (ii) one PVC tube treated with solvent and the other treated with alarm pheromone, and (iii) both PVC tubes treated with alarm pheromone (positive control). For each experiment, 100 insects (50 males and 50 females) were released at the centre of arena. After 24 h, the numbers of insects inside the PVC tubes and in the centre of the arena were counted. Each experiment was replicated 10 times.

2.3 Pitfall traps

Pitfall traps designed and used in field experiments comprised cylindrical plastic boxes (10 cm height × 14 cm diameter). For each trap, a 6-cm diameter hole was drilled into the lid, through which a rubber septum impregnated with synthetic aggrega-tion pheromone could be placed inside the trap environment. Each septum was suspended by a wire that was attached to a round cardboard cover, which was attached to the plastic lid using screws (Fig. 1). The cardboard was attached such that a 2-cm gap between it and the lid of the plastic box could be maintained, sufficient to allow insects to move into the trap. Each trap was buried in poultry litter up to the level of the lid, taking care not to cover the lid completely, leaving space for the pheromonal plume to disperse above and through the poultry litter. Prior to their use in in poultry houses, traps were tested in the lab-oratory to determine whether insects would be able to escape after falling into them. For this, 1000 insects were placed inside a trap buried in a plastic box (20 × 40 cm) containing wood shav-ings (n = 10). After 24 h, the insects were counted and none had escaped.

2.4 Pheromone lures

Rubber septa (10 mm, Sigma-Aldrich) were cleaned by Soxhlet extraction (×2) with hexane for 4 h, followed by drying at 40 °C overnight in a gravity convection oven (Precision, Chicago, IL, USA). If working with a greater number of septa or larger septa, it is preferable to remove the excess of hexane in a fume hood rather than by heating, for safety reasons. For formulation of the syn-thetic aggregation pheromone, the six pheromone components were added to one rubber septum in the same ratio produced by males, with the total combined amount of the six compounds being 1 mg.[17] A solution with (*R*)-limonene 230 μg, (*E*)-ocimene 160 μg, 2-nonanone 40 μg, (*S*)-linalool 260 μg, (*R*)-daucene 80 μg and (*E*,*E*)- -farnesene 230 μg in hexane (200 μL) was prepared and used. The alarm pheromone was formulated in the same propor-tions produced by *A. diaperinus* in their abdominal glands,[20] i.e. 1 μg 1,4-benzoquinone, 249 μg 2-methyl-1,4-benzoquinone and 750 μg 2-ethyl-1,4-benzoquinone were diluted in diethyl ether (200 μL) and added to a septum. After septa were impregnated with either aggregation or alarm pheromone components, they were left in a laminar flow hood at room temperature to allow solvent evaporation for 8 h. Pheromone-baited septa were then stored in a sealed aluminium storage bags (Mitsubishi Gas Chem-icals Co., Inc., Tokyo, Japan) at – 20 °C until required for use in release rate experiments.

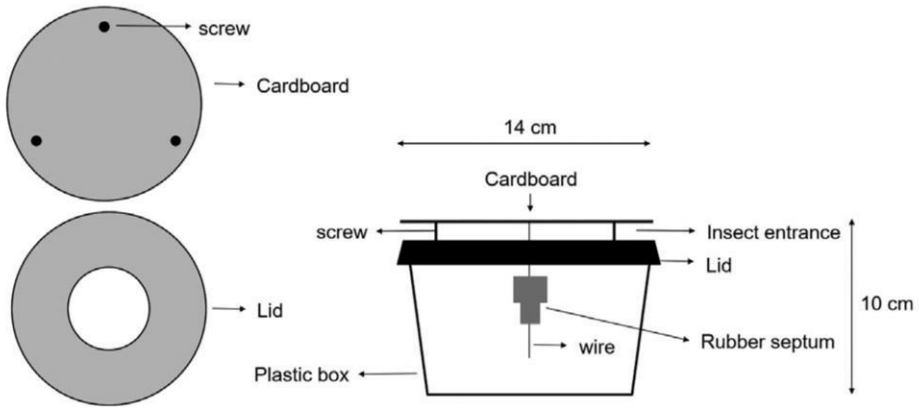

Figure 1. Schematic drawing of pitfall trap.

2.5 Pheromone release rate

To verify the volatile emission ratio from rubber septa impregnated with aggregation pheromone (*N* = 4) and alarm pheromone (*N* = 4) components, each septum was placed individually into a glass syringe (30 mL). One end of the syringe was connected to an activated charcoal filter (4 – 20 mesh) and the other end was con-nected to a glass tube containing the adsorbent Tenax GR (100 mg, Restek Corporation, Bellefonte, PA, USA). The tube containing the adsorbent was connected to a vacuum pump and purified air was drawn through the tube at 300 mL min^{-1}. The volatiles were col-lected every 24 h for 3 consecutive days. The trapped volatiles were eluted from the adsorbent with hexane (1 mL), and an internal standard (1 μL of 1 mg mL^{-1} (*E*)-caryophyllene) was added to the sample. After addition of the IS, the samples were concentrated under a gentle flow of nitrogen to a final volume of 50 μL. The con-centrated extracts were kept at −20 °C until required for chemical analysis.

2.6 Chemical analyses

Gas chromatography (GC) analyses of volatile extracts collected from impregnated septa were performed using a gas chromatograph (Agilent 7890A) equipped with a DB-5MS column (30 m length, 0.25 mm i.d., 0.25 μm film thickness; Supelco, Bellefonte, PA, USA) and a split –splitless injector. The carrier gas was helium. The oven temperature programme started at 50 °C for 2 min, increased at a rate of 15 °C min^{-1} to 250 °C, with a final hold time of 20 min. The column effluent was analysed using a flame ioniza-tion detector (FID) at 270 °C. One microlitre of each selected sam-ple was injected in splitless mode; the injector temperature was 250 °C. Compounds were quantified by comparing GC peak areas with the peak area of the internal standard, (*E*)-caryophyllene, pre-pared at a final concentration of 20 μg mL^{-1}.[20]

2.7 Aggregation pheromone field tests

Field experiments with *A. diaperinus* aggregation pheromone were performed in commercial poultry farms in Brasília, Distrito Fed-eral (15°59′40.6″S, 47°37′23.4″W) and Concórdia, Santa Catarina (27°18′36.2″S, 51°59′53.1″W), Brazil. The poultry houses used in the current study were 120 m long, 10 m wide and 3 m high. The houses were an open-sided design, with 35-cm high side-walls closed by a wire screen (7 cm mesh diameter) up to the roof and covered by a yellow plastic curtain to provide shelter. This design prevented other birds from getting into the poul-try houses. The yellow curtain was either opened or closed to keep the internal temperature consistent and provide necessary protection from excessive heat. During the experiments, the curtains remained open.[24] A single replicate consisted of one commercial poultry production building (10 m width × 120 m length) with 20 pitfall traps (Fig. 1) containing the treatments installed every 10 m in two rows (Fig. 2, pull aviary). The population den-sity of *A. diaperinus* is distributed as strong aggregations in poul-try houses,[2,4] and, therefore, to avoid bias due the uneven pop-ulation distribution, the experiment had a paired design. Two treatments were tested in the poultry houses: (i) traps contain-ing the aggregation pheromone septa (*N* = 10), and (ii) traps with control (hexane) septa (*N* = 10). To evaluate the infestation level of each building, 20 manual samplings (1000 cm^3) of poultry lit-ter were collected between the trap positions (Fig. 2). Samples were transferred to the laboratory, and adults were separated from the litter and counted. The experiments were conducted dur-ing the time between flocks, after hens had been moved out. The traps remained in position for 48 h and were then taken to the laboratory for insect counting. Random samples of 50 bee-tles from each trap and treatment were dissected to determine the sex ratio. The experiments were conducted under two differ-ent conditions: (i) in new poultry litter, where the building was cleaned, and all the litter material was replaced with fresh material before the next chicken flock arrived; and (ii) in used poultry litter, where the poultry material was exposed to two or more chicken flocks. In addition, the experiments were performed under differ-ent climate conditions, i.e. in central – west Brazil, Distrito Federal, where the average outdoor temperature during the experimen-tal time (November – February) was 25.80 ± 3.45 °C (mean ± SD) and in Santa Catarina, where the average outdoor temperature during the experimental time (June – August) was 10.27 ± 1.94 °C. For each poultry litter and location, experiments were repeated 10 times.

2.8 Pull and push–pull field tests

The experimental unit consisted of two poultry houses with a sim-ilar infestation level (evaluated previously by manual sampling). One building, described as the 'pull' aviary, contained 10 aggre-gation pheromone traps and 10 control traps as described above (Fig. 2, pull aviary). In the other building, a push –pull system was set up, comprising of 10 aggregation pheromone traps, 10 con-trol traps and 17 alarm pheromone releasers that were distributed as follows: 12 along the edges and five in the centre line next to the central pillars (Fig. 2, push – pull aviary). The releasers were allo-cated in wire cages (5 cm height × 3 cm diameter) and left above

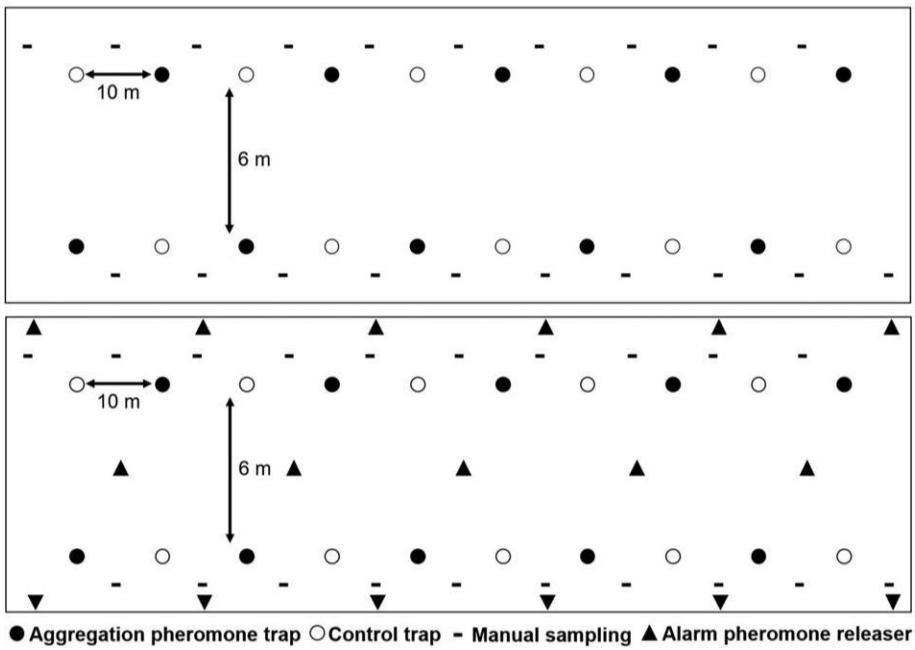

Figure 2. Schematic drawing of trap distribution in poultry houses.

the litter. Six replicates were performed per treatment and climate condition. To evaluate the infestation level of each building, 20 manual samplings (1000 cm^3) of poultry litter were collected between the trap positions (Fig. 2). The experiments were conducted during the time between flocks, i.e. after the hens had been moved out. Traps remained in position for 24 h, and were then taken to the laboratory for insect counting.

2.9 Statistical analyses

The quantity of each pheromone component released from rub-ber septa during the first 3 days following impregnation was transformed to proportion-released data and analysed by analy-sis of variance (ANOVA) with repeated measures. Male and female captures in aggregation pheromone traps and control traps were evaluated using a chi-square test. The mean of insects captured in each treatment in the arena test as well as in all field tests were analysed using generalized linear model (GLM) and deviance anal-yses with Poisson error distribution with logarithm link function and contrast analyses when necessary. All the statistical analyses were conducted using the statistical program R 2.14.0, and signifi-cance was accepted at the ≤ 0.05 level.[25]

3 RESULTS

3.1 Alarm pheromone – laboratory assays

In arena assays using synthetic alarm pheromone, when both PVC tubes were treated with solvent control (hexane), significantly more *A. diaperinus* were found in PVC tubes compared with the arena (^{2} = 9.19, df = 2, P = 0.010), but there was no difference between PVC tubes (Fig. 3a). When one PVC tube was treated with the alarm pheromone and the other was treated with solvent, significantly fewer *A. diaperinus* were found in the tube treated with the alarm pheromone compared with the solvent-treated PVC tube or arena (^{2} = 88.31, df = 2, P < 0.001) (Fig. 3b). Furthermore, when both PVC tubes were treated with the alarm pheromone, a significantly higher number of insects remained outside the PVC tubes (^{2} = 311.41, df = 2, P < 0.001) (Fig. 3c).

3.2 Pheromone release from formulations

GC analysis of air entrainment extracts collected from aggregation pheromone-impregnated rubber septa showed that all compounds were released, but the mean proportion of components released from septa differed from the proportion initially added to the septa (Table S1). The component (*E*,*E*)- -farnesene was released in lower amounts compared with the original ratio loaded. This could occur due to either competition with the other components, the different vapour pressure of the components or the higher affinity of this sesquiterpene with the rubber septa material. Possible degradation of this component was discarded, because when septa were washed with hexane, this component was recovered almost completely (data not shown). For the alarm pheromone, the compound 1,4-benzoquinone was not detected via GC –FID due to the low quantity added. The other two compo-nents were detected and released in a similar ratio to that of the original loading ratio (Table S2).

3.3 Aggregation pheromone field tests

The field experiments demonstrated an overall significant treat-ment effect. Traps baited with synthetic aggregation pheromone captured 2.8 times more adult *A. diaperinus* than control traps (^{2} = 10.02, df = 1, P = 0.001) (Fig. S1). Traps baited with aggrega-tion pheromone captured more insects under both clean and used poultry litter conditions in tropical (Distrito Federal: new poultry litter, ^{2} = 5.881, df = 1, P = 0.015, 3.9 times more catches and used poultry litter, ^{2} = 6.037, df = 1, P = 0.014, 2.8 times more catches) and subtropical environments (Santa Catarina: new poul-try litter, ^{2} = 3.853, df = 1, P = 0.049, 3.1 times more catches and used poultry litter: ^{2} = 3.941, df = 1, P = 0.047, 2.5 times more catches) (Fig. 4). Furthermore, the behaviour of immature indi-viduals was not affected by the aggregation pheromone (Dis-trito Federal: new poultry litter, ^{2} = 2.789, df = 1, P = 0.094 and used poultry litter, ^{2} = 1.280, df = 1, P = 0.257; Santa Catarina: new poultry litter, ^{2} = 0.452, df = 1, P = 0.501 and used poul-try litter: ^{2} = 3.144, df = 1, P = 0.080) (Fig. S2). Additionally,

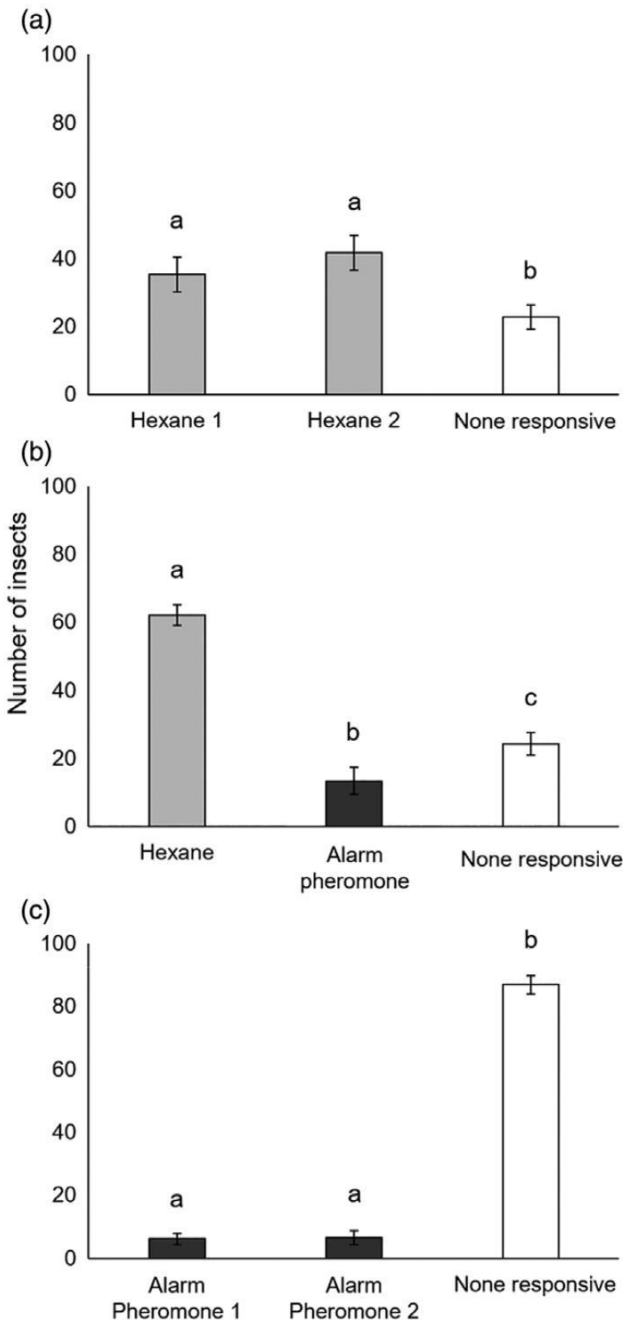

Figure 3. Mean (± SE) number of adult *Alphitobius diaperinus* present in polyvinyl chloride (PVC) tubes and in the arena 24 h post treatment. (a) Hexane × hexane, (b) hexane × alarm pheromone and (c) alarm pheromone × alarm pheromone. Analyses were carried out using generalized linear model (GLM) and deviance analysis with Poisson error distribution and log-arithm link function and contrast analysis. Different letters indicate signifi-cant differences within the treatments tested (≤ 0.05).

there were no differences in the capture of males and females between treatments in new poultry litter (Distrito Federal: control traps, ^{2} = 1.28, df = 1, *P* = 0.257 and pheromone traps, ^{2} = 0.72, df = 1, *P* = 0.396; Santa Catarina: control traps, ^{2} = 2.88, df = 1, P = 0.091 and pheromone traps, ^{2} = 2.00, df = 1, *P* = 0.157) and used poultry litter (Distrito Federal: control traps, ^{2} = 2.000, df = 1, *P* = 0.157 and pheromone traps, ^{2} = 2.88, df = 1, *P* = 0.091; Santa Catarina: control traps, ^{2} = 2.000, df = 1, *P* = 0.157 and pheromone traps, ^{2} = 1.28, df = 1, *P* = 0.257) (Fig. S3).

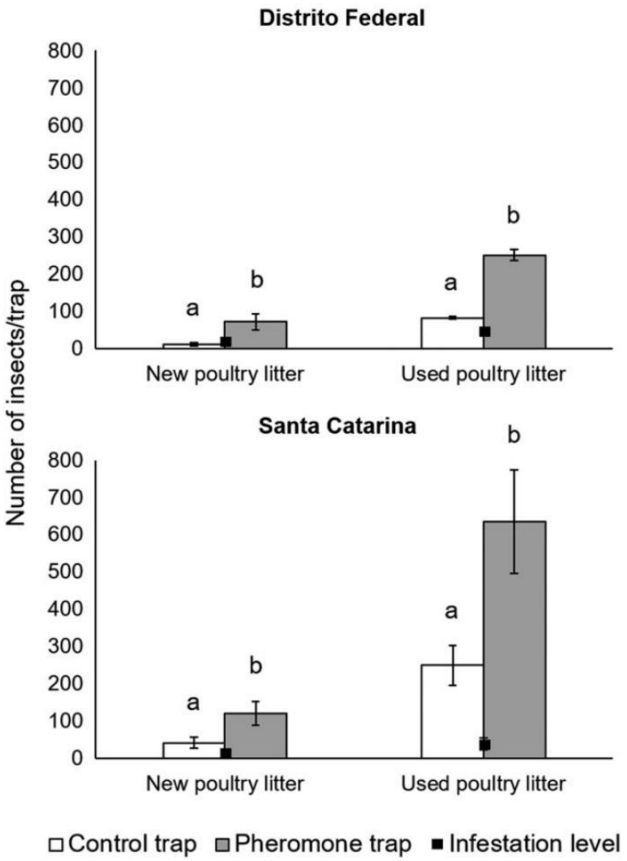

Figure 4. Mean (± SE) number of adult *Alphitobius diaperinus* captured in control and pheromone traps (bars) and infestation level (squares) for each poultry litter (new and used) and location (Distrito Federal and Santa Catarina) (number of adults per 1000 cm^3 of litter). Analyses were carried out using generalized linear model (GLM) and deviance analysis with Poisson error distribution and logarithm link function. Different letters in the same type of litter indicate significant differences (≤ 0.05).

3.4 Pull and push–pull field experiments

For these experiments, paired poultry houses with similar levels of *A. diaperinus* infestation were selected for pull and push –pull experiments (Distrito Federal: ^{2} = 3.154, df = 1, *P* = 0.076; Santa Catarina: ^{2} = 0.010, df = 1, *P* = 0.917) (Fig. 5). Significantly greater numbers of insects were captured in aggregation pheromone traps, for both pull and push –pull experiments, when compared with control traps (Distrito Federal: pull aviary, ^{2} = 55.284, df = 1, *P* < 0.001, 2.8 times more catches and push –pull aviary, ^{2} = 5.535, df = 1, *P* = 0.018, 2.4 times more catches; Santa Cata-rina: pull aviary, ^{2} = 42.253, df = 1, *P* < 0.001, 2.3 times more catches and push –pull aviary, ^{2} = 38.418, df = 1, *P* < 0.001, 3.1 times more catches). (Fig. 5). Significantly more insects were captured in the push –pull aviary than in the pull aviary (Dis-trito Federal: control traps, ^{2} = 21.646, df = 1, *P* < 0.001, 5.2 times more catches and pheromone traps, ^{2} = 32.870, df = 1, *P* < 0.001, 4.1 times more catches; Santa Catarina: control traps, ^{2} = 24.752, df = 1, *P* < 0.001, 8.1 times more catches and pheromone traps, ^{2} = 34.160, df = 1, *P* < 0.001, 9.4 times more catches) (Fig. 5).

4 DISCUSSION

In all field experiments, the number of adult *A. diaperinus* caught in aggregation pheromone-baited traps was higher than in either

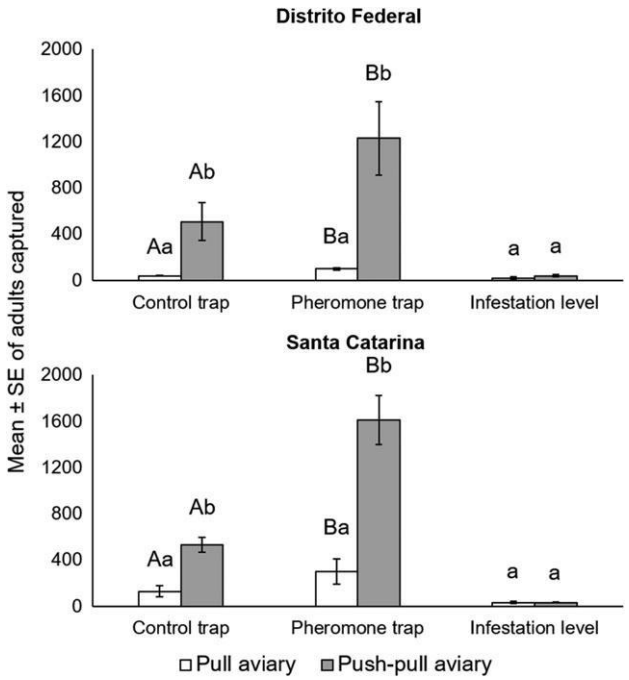

Figure 5. Mean (± SE) number of adult *Alphitobius diaperinus* captured in traps (control and pheromone), and infestation level (number of adults per 1000 cm^3 of litter) in a pull and push – pull aviary in Distrito Federal and Santa Catarina. Analyses were carried out using generalized linear model (GLM) and deviance analysis with Poisson error distribution and logarithm link function. Different letters indicate significant differences in the same type of trap between treatments (lowercase) and between control and pheromone traps in the same treatment (uppercase) (≤ 0.05).

control traps or poultry litter samplings. This indicates the poten-tial for using the aggregation pheromone as a pull component to improve the control of *A. diaperinus*. Although the ratio of aggrega-tion pheromone components emitted from impregnated rubber septa differed from the ratio added, this divergence did not affect attraction to traps, suggesting that the olfactory system of adult *A. diaperinus* possesses plasticity with regard to the relative amounts of the aggregation pheromone components. Future studies will be undertaken to evaluate different ratios between the components in field conditions, to obtain the most efficient formulation.

Pheromones have been used successfully to manage coleopteran pests, including weevils such as the boll weevil, *Anthonomus grandis* Boheman, the palm weevil, *Rhynchophorus palmarum* L. and the rice weevil, *Sitophilus oryzae* L., and tene-brionids including the confused flour beetle, *Tribolium confusum* du Val and the red flour beetle, *Tribolium castaneum* Herbst.[26–30] In our experiments under new poultry litter conditions, a lower number of *A. diaperinus* was, in general, captured compared with experiments under used poultry litter conditions. Used poultry litter provides better conditions for *A. diaperinus* development and population growth, and when it is replaced, significant num-bers of *A. diaperinus* are removed, decreasing the population level in poultry houses. However, replacement of used litter is not sufficient to remove all insects, with some remaining either hidden at the edges or buried in the soil. In our study, we con-firmed that, although new poultry litter contains fewer insects than used poultry litter, pheromone-baited traps still capture a significant number of insects. In addition, the higher number of insects captured in the push –pull experiment compared with the pull experiment supports the hypothesis that insects remain hidden in the facilities in the pull experiment. In the push –pull experiment, the alarm pheromone disturbs the insects from their hiding places, favouring higher capture in the aggregation pheromone traps. Brazilian poultry production is located in both tropical and subtropical regions, with 70% of Brazilian poultry production being concentrated in the south of the country where low temperatures can be reached during winter.[31] Our results in tropical and subtropical environments shows that attraction of *A. diaperinus* does not appear to be influenced by temperature and other climatic conditions.

Studies on the aggregation pheromone from a US population of *A. diaperinus* comprising five components, i.e. (*R*)-limonene, (*E*)-ocimene, 2-nonanone, (*S*)-linalool and (*R*)-daucene, reported that (*R*)-limonene and (*S*)-linalool were not necessary for attraction,[18,19] and that higher numbers of larvae were caught in aggregation pheromone-baited traps.[18] By contrast, Brazil-ian populations of *A. diaperinus* require all six compounds [(*R*)-limonene, (*E*)-ocimene, 2-nonanone, (*S*)-linalool, (*R*)-daucene and (*E*,*E*)- -farnesene] for effective attraction,[21] and in this study, our data showed that immature stages of *A. diaperinus* were not caught in baited traps. The capture of immature forms of holometabolan species in pheromone traps has been described for other insects, such as *T. castaneum*, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) and the codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae).[32 – 34] *A. diaperinus* larvae feed on different sources of food, such as wood, paper and chicken faeces. For the US population of *A. diaperinus*, it was shown that adults and larvae of *A. diaperinus* are attracted to poultry litter odour, and that the combination of aggregation pheromone and chicken faeces is more attractive to larvae than the aggregation pheromone alone.[18] Larvae might be more responsive to odour from food sources than to odour of conspecific adults, i.e. the aggregation pheromone. This hypothesis could be tested by eval-uating larvae responses to conspecific aggregation pheromone. The absence of trapped larvae in our experiments might also be related to our trap design, i.e. the opening of the pitfall trap is on the top of the cap, above the poultry litter, and the external wall of the plastic traps is smooth, which makes it difficult for larvae to move along. Furthermore, during the experiments, larvae were hardly ever seen on the top of the litter, which would be required prior to trap entry.

Deployment of the alarm pheromone for *A. diaperinus* as well as the aggregation pheromone in the push –pull experiment signifi-cantly increased trap catches compared with the pull experiment. This suggests that the alarm pheromone can displace *A. diaperi-nus* from its hiding places and allows more insects to be captured in the aggregation pheromone-baited traps. The efficiency of the displacing effect caused by the push –pull strategy compared with the pull strategy can be correlated with the increased number of insects captured also in the control traps in the push –pull system compared with the pull system. Aggregation pheromone traps could potentially lose their efficacy under conditions with high *A. diaperinus* population levels, due to competition with the natural pheromone released by insects in the control area. However, in our study, the use of the alarm pheromone to disturb the insects from their hiding places promoted higher mobility and conse-quently increased the likelihood of insects being caught in traps. This illustrates the potential of semiochemical-based push –pull systems for pest management, and how the combination of two different classes of semiochemicals can enhance their ability to manage a pest. The most successful push –pull system currently in use for pest management is for cereal stemborers in Eastern

Africa, involving the use of repellent intercrops and attractive trap plants alongside cereal crops to regulate the population of the targeted pest and their natural enemies.[23,35–38] Push–pull systems have also been tested for the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae), showing promising results for management of this pest,[39] and for the Douglas fir beetle, *D. pseudotsugae* Hopkins (Coleoptera: Curculionidae), where the use of anti-aggregation and aggregation pheromone reduced the pest population by ~ 80%.[40]

Although benzoquinones are considered potentially toxic compounds, the quantities used in this study are equivalent to the amounts stored in the abdominal glands of ~ 200 *A. diaperinus*, and the quantity released by 10 000 insects when disturbed.[20] Because it is well known that *A. diaperinus* populations can reach several thousand in poultry houses, the quantity of the alarm pheromone deployed in our experiments already exists in poul-try houses under natural conditions. However, because *A. diaper-inus* is completely adapted to poultry houses conditions, where there is no competition for food and reproductive partners, it is possible that the lower amounts used in our experiments were sufficiently effective to cause disaggregation in the areas where alarm pheromone was released. In the long term, insect popu-lation reduction will provide a safer environment for birds and humans. More experiments are needed to show if the push–pull strategy can reduce *A. diaperinus* populations over time and main-tain low insect numbers.

A. diaperinus populations are difficult to manage because their cryptic behaviour reduces contact with control agents such as insecticides. Furthermore, control agents can only be applied at times when flocks are not present, to avoid side effects. Reduction of the population level of *A. diaperinus* in commercial poultry houses could involve a long-term experiment (6 months to 1 year experiment) using semiochemicals combined with sani-tary measures such as periodic changes of poultry litter, the use of brickwork poultry houses, and cleaning the surrounding areas of the poultry houses to restrict the movement of insects between the facilities. A pull or push–pull strategy that uses aggregation pheromones and alarm/aggregation pheromones respectively could be used, with aggregation pheromone-baited traps containing a control agent such as a biopesticide to cap-ture, infect and kill insects. Traps combined with biological con-trol agents could also be placed in the external areas of the poultry houses to capture migrating insects. The risk of toxic effects of the semiochemicals upon birds and humans would be minimized because the pheromone lures are enclosed in traps, there is no physical contact with the emitted chemicals, and the pheromones are released at physiologically relevant lev-els, i.e. levels released by the insects. An attract-and-kill strat-egy that combines the use of entomopathogenic fungi with attractant pheromones has been described previously for other insects, e.g. the grain borer, *Prostephanus truncates* (Coleoptera: Bostrichidae), the western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae), and the banana weevil, *Cosmopolites sordidus* (Coleoptera: Curculionidae).[41–45] We believe that this combined approach has the potential to be used for *A. diaperinus* management. However, fungi could be applied either as a powder or as a fat formulation in pheromone traps, with the pathogen being protected from the litter fungicide, and the insects being brought into contact with the pathogen in the pheromone traps.[46–50]

In conclusion, our results suggest that alarm and aggregation pheromones can be deployed under poultry house conditions to trap significant numbers of adult *A. diaperinus*. Further long-term studies are underway to determine whether pheromone deployment can be combined with suitable entomopathogenic fungi to maintain *A. diaperinus* populations at low levels over time, and to evaluate the potential for using these components as part of an integrated *A. diaperinus* management strategy.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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