Journal of Stored Products Research 106 (2024) 102296

Contents lists available at ScienceDirect

Journal of Stored Products Research

journal homepage: www.elsevier.com/locate/jspr

Evaluation of pheromone co-attractants for capturing *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae)

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ARTICLE INFO

Handling Editor: Dr Raul Guedes

Keywords:
α-ionone
β-ionone
Kairomones
Serricornin

ABSTRACT

The cigarette beetle, *Lasioderma serricorne* (F.), poses a significant threat to food storage, tobacco industries, and herbaria. The development of effective trapping methods with efficient lures is crucial for promptly monitoring this species and exploring potential mass trapping programs. The study aims to examine the potential synergistic effect of combining the synthetic sex pheromone (serricornin) with a blend of α-ionone and β-ionone as an attractant for the cigarette beetle, with the purpose of determining the capture potential of female individuals and exploring an increase in male response. Both laboratory dual-choice arena bioassays and field trap tests were conducted to assess the effectiveness of this attractant combination. The bioassays were performed in Plexiglas cages with traps baited with serricornin, α-ionone, and β-ionone. Field trap tests were conducted in a bakery plant, comparing pheromone traps with a mixture of α-ionone and β-ionone to pheromone traps lured with β-ionone alone. On the one hand, the results of the laboratory bioassays showed that traps with serricornin supplemented with a combination of α-ionone and β-ionone captured a significantly higher number of beetles compared to traps with serricornin alone. The same trend was observed for both males and females. On the other hand, field trap tests, while capturing more individuals with the combined attractant, did not show statistical significance compared to traps with only β-ionone. The study concludes that the combination of serricornin with α-ionone and β-ionone shows promise as an attractant for *L. serricorne*, as evidenced by significant captures in laboratory bioassays. However, the transition to field conditions reveals a need for further exploration and optimization to fill the observed gap between controlled and real-world settings.

1. Introduction

The cigarette beetle, *Lasioderma serricorne* F. (Coleoptera: Anobiidae), ranks among the most destructive pests in the global food storage and tobacco industries (Mueller et al., 1990; Shinoda and Fujisaki, 2001; Cabrera, 2002; Trematerra, 2020). Its significance extends beyond these sectors, as it has increasingly become a concern at heritage sites, including museums (Gilberg and Roach, 1991; Aiello et al., 2010), and herbaria (Phillips and Throne, 2010; Guarino et al., 2020). Adult cigarette beetles possess the unsettling ability to infiltrate packaged goods, leaving behind distinct round entry holes. This characteristic places them in the category of "true penetrators" of food packaging (Riudavets et al., 2007; Athanassiou et al., 2011). However, the bulk of the damage inflicted by this species stems from the feeding activities of their larvae that not only cause direct losses by consuming the product but also contribute to a decrease in the market value of affected items due to the residual presence of deceased insects, frass, exuviae, and the fragments of their gnawed feasts (Linnie, 1994; Edde, 2019).

Effective monitoring and control methods are essential to manage this pest. Integrated pest management (IPM) strategies for *L. serricorne* primarily hinge upon the use of monitoring methods that employ sticky traps baited with sex pheromone, as highlighted by previous studies (Athanassiou et al., 2018; Chaitanya and Swamy, 2018; Papadopoulou and Buchelos, 2002a). The primary component of this sex pheromone is (4*S*,6*S*,7*S*)-4,6-dimethyl-7-hydroxynonan-3-one, (afterward named serricornin), which has demonstrated a strong attraction towards male beetles (Chuman et al., 1979, 1985). Such tools can be implemented by the incorporation of food attractants to enhance their efficacy. Studies,

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https://doi.org/10.1016/j.jspr.2024.102296
Received 11 December 2023; Received in revised form 17 January 2024; Accepted 18 March 2024
Available online 23 March 2024
0022-474X/

for instance, have pointed to the promising utilization of dried red chili fruit powder from *Capsicum annuum* L. to increase trap catches of adult individuals (Mahroof and Phillips, 2008; Guarino et al., 2020). Additional laboratory investigations have elucidated the role of specific terpenoids, found among the main volatiles of *C. annuum* fruit powder, serving as key attractants (kairomones) for *L. serricorne* adults, particularly α-ionone and β-ionone (Guarino et al., 2021). In a recent development, Guarino et al. (2022) conducted a field experiment, demonstrating that the incorporation of the sex pheromone along with β-ionone, loaded into a polyethylene dispenser, resulted in an approximately 1.5-fold increase in the capture of *L. serricorne* individuals compared to the use of pheromone alone. These findings have opened up new possibilities for optimizing pheromone-based traps to improve their performance in capturing these stored product pests. However, these studies have not provided evidence on whether the inclusion of these chemicals in pheromone traps could enhance the capture of female individuals, a factor that holds significant promise and could make this tool suitable for mass trapping applications (Trematerra, 2012). Additionally, there is no evidence yet regarding the potential increased effectiveness of a pheromone trap supplemented with both terpenoids, α-ionone and β-ionone, as opposed to solely β-ionone, in improving capture efficacy.

In this context, our study aims to investigate the potential synergistic effect of combining the synthetic pheromone with a blend of α-ionone and β-ionone in laboratory and field settings. By assessing the attractiveness of this novel combination, this research was carried out to determine whether it can achieve higher efficacy in capturing *L. serricorne* adults. Furthermore, the authors recognize the importance of assessing the potential catch of female adults in light of its impact on population control strategies. Hence, in this study it was examined the catch rates of both male and female *L. serricorne* adults to gain a comprehensive understanding of the lure's effectiveness. In addition to laboratory evaluations, field trials were conducted to assess the practical applicability of this combined lure in real-world settings. These trials can provide insights into the potential of this tool for use in monitoring and mass trapping efforts aimed at mitigating the impact of *L. serricorne* in a vast array of environments.

Such investigation can offer a deeper exploration of the potential benefits of combining pheromone attractants with α-ionone and β-ionone, not only seeking to enhance the sensitivity of monitoring tools but also exploring the possibility of more effective population control measures, providing valuable insights for pest management strategies.

2. Materials and methods

2.1. Laboratory trial

2.1.1. Insects

A colony of *L. serricorne* was obtained from infested chamomile tea substrates and reared in an environmentally controlled room (24 ± 2 °C, 70 ± 10% RH, photoperiod 16L:8D) in plastic cages 7 * 18 * 6 cm with metal net at the top for ventilation. Each plastic cage contained a rearing substrate made of a mixture consisting of chamomile powder (100 g), 00 flour (95 g), 0 flour (60 g), bran (40 g), and brewer's yeast (5 g). Periodically one-hundred adults were collected and placed in a new cage to renew the rearing. To establish the sex ratio of the tested adults, an aliquot of 50 individuals was randomly collected from the rearing cages and dissected under microscope, revealing a percentage of about 55% of males and 45% of females.

2.1.2. Dual-choice arena bioassays

Bioassays were conducted comparing the attractiveness of the sex pheromone added with both α-ionone and β-ionone (Pαβ) versus the sex pheromone alone (P) and versus the combination of sex pheromone added with β-ionone (Pβ). The dual choice arena was a Plexiglas cage measuring 50 cm in width, 10 cm in height, and 50 cm in depth, with mesh-covered ventilation on the top side. The interior of the cage housed two traps, positioned 20 cm apart, containing the attractants being tested. The traps consisted of 9 cm diameter petri dishes filled with water and covered by a black plastic "Rufy trap waterproof" model (GEA srl, Settimo Milanese, Milan, Italy). Polyethylene dispensers (0.5 mL) (GEA srl, Settimo Milanese, Milan, Italy), contained 4 mg of pheromone and its isomers (purity grade 97.9%, Bedoukian, Danbury, USA), were suspended 2 cm above the water.

The pheromone traps were supplemented with 10 mg of α-ionone and 10 mg of β-ionone (both ≥95% purity, furnished from Sigma Aldrich, Milan, Italy). Similarly to the procedure carried out by Guarino et al. (2022), the compounds were dissolved individually in hexane at 10% in volume and the solution applied singly on another polyethylene dispenser of the same type used for the pheromone. After the compounds application, the dispensers were left under the vacuum cabin for 2 h for solvent evaporation and then sealed until their use for the experiment. For the bioassays, each trap was then provided with two dispensers: one containing the sex pheromone and the other housing the ionone(s).

The adult beetles (age 3–5 days) were randomly selected from the rearing cage, individually isolated in 15 mL vials, and acclimatized in the dual choice arena room for 1 h before the behavioral bioassays. Groups of twelve unsexed adult beetles were then released into the arena and exposed to the attractive effect of the baited traps; the number of the captured beetles in Pαβ, P and Pβ traps was recorded after 24h of exposition. Adults attracted were then dissected using micro-scissors and forceps under microscope, to observe the genitalia and score the number of males and females captured. Non-responsive insects that remained in the arena without entering a trap were removed and not included in the statistics, but were also dissected to evaluate their overall sex ratio.

A total of 36 replicates was conducted for the experiment and the position of the different traps was alternated between replicates. After each replicate, the Plexiglas cage was thoroughly cleaned and dried. The bioassays were performed under laboratory conditions at 25 ± 3 °C and 50 ± 15% U.R.

2.2. Field trials

2.2.1. Estimation of attractant efficacy

This experiment, carried out starting from March 2023 in a bakery plant located in central Sicily (Italy), was aimed at evaluate the total efficacy in terms of adults captured by supplementing pheromone traps with α-ionone and β-ionone (Pαβ) or with β-ionone alone (Pβ). To test the response of *L. serricorne* adults, the commercially available anobid traps with the polyethylene dispensers as those employed in the laboratory bioassays (GEA srl, Settimo Milanese, Milan, Italy), were used. The traps were examined weekly for a duration of eight weeks and the number of captured adult beetles was recorded.

Fourteen pheromone traps were used in total, seven supplemented with a mixture of α-ionone and β-ionone (10 mg + 10 mg) (Pαβ) and seven supplemented with β-ionone (10 mg) alone (Pβ). These trials were conducted within a single area covering approximately 4000 m^2, where the bakery's packaged food products were stored prior to shipment and, in its central zone the traps were placed at a distance of 8–10 m apart from each other, suspended at 1.50 m above the floor. The trap positions were clockwise rotated after each inspection. At the end of the 4th week, all the traps and lures were replaced, and the experiment was repeated for four weeks more.

2.2.2. Sex ratio assessment of trapped adults

To determine whether the co-attractants employed in trap baiting significantly influenced the attraction of both male and female beetles in field conditions, a trial was carried out in another food storage area within the same bakery plant, spanning approximately 1000 m^2.

Considering that the specimens trapped in the sticky traps are often difficult to submit to any morphological examination process, there was the necessity to collect undamaged adults for the estimation of the sex ratio. To do this, plastic bucket traps filled whit a mixture of distilled water and propylene glycol (ratio 1:3) were utilized. Also in this case, the traps were baited with (Pαβ) or (Pβ) at the same doses of the previous field experiment. Four traps for each bait combination were positioned on the floor, spaced 10–15 m apart from each other, and subjected to weekly inspections over a four-week period. In total, 500 adults were collected for each lure combination. These individuals were then transported to the laboratory and their sex ratio was determined by segregating females from males, following the methodology outlined in Papadopoulou and Buchelos (2002b).

This method is based on the solvent (70% alcohol in distilled water) treatment of *L. serricorne* adults collected within the bucket traps, that permits the urosternite transparency, rendering the apodeme that is V-shaped in females and U-shaped in males (Howe, 1957; Levinson and Levinson, 1995) clearly visible by simple observation of the abdomen under a stereomicroscope (Leica EZ4D).

2.3. Statistical analysis

The data of the dual-choice arena bioassays, i.e. captured beetles in control and test traps was analyzed by *t*-test for dependent samples in order to compare total number of captures, males' captures and females' captures. The number of captured insects alongside the trap test were analyzed using a one-way ANOVA. The data were analyzed using Statistica 10.0 for Window (Statsoft 2001, Vigonza, PD, Italy).

3. Results

3.1. Dual-choice arena bioassays

The results of the dual-choice arena bioassays are summarized in Figs. 1 and 2. The combination Pαβ determined a higher number of captured beetles when compared to the traps baited with pheromone alone (P) ($t = 3.34$; $N = 36$; $p < 0.01$) (Fig. 1). Specifically, the attractiveness of Pαβ was higher for both males ($t = 2.67$; $N = 36$; $p = 0.01$) (Fig. 1) and females ($t = 2.18$; $N = 36$; $p < 0.05$) (Fig. 1). The non-responder insects were 57% females and 43% males.

The traps of the Pαβ combination determined also a higher number of captured adults in comparison with the Pβ traps ($t = 3.85$; $N = 36$; $p < 0.01$) (Fig. 2) and, in details this effect, was recorded for both males ($t = 3.38$; $N = 36$; $p < 0.01$) (Fig. 2) and females ($t = 2.00$; $N = 36$; $p < 0.05$) (Fig. 2). The non-responder insects were 55% females and 45% males.

3.2. Field trials

3.2.1. Estimation of attractant efficacy

The results of the trial are reported in Fig. 3. Overall, 3681 adults were captured along the experimental test in all the traps. In detail, the number of captured individuals per trap per week was 34.53 ± 3.15 in Pαβ traps while was 31.19 ± 2.91 in Pβ traps. Such results did not indicate statistical differences among the level of captured adults in the two treatments ($t = 1.07$; $N = 56$; $p = 0.28$).

3.2.2. Sex ratio assessment of trapped adults

The apodeme morphological analysis of the sampled individuals ($N = 500$) captured in Pαβ traps revealed that 98% of the examined specimens exhibited the characteristic male's U-shaped apodeme. Similar results were achieved examining the specimens sampled from the bucket traps lured with the Pβ combination, were the 97% of the total adults subjected to examination ($N = 500$) exhibited the U-shaped apodeme, indicator of male identity.

4. Discussion

The presented study aimed to investigate the efficacy of pheromone supplemented with the combination of both α-ionone and β-ionone as an attractant for cigarette beetles, with promising results observed in laboratory experiments. However, the translation of these findings into the field setting revealed only partial confirmation of the laboratory results.

In detail, laboratory experiments carried out in dual choices arena showed that adults (both males and females) of *L. serricorne* were more strongly attracted by traps loaded with the combination of the pheromone with α-ionone and β-ionone in comparison with the traps loaded with pheromone alone. Similarly, the combination of the pheromone with α-ionone and β-ionone was also more attractive for the beetles than the combination of the pheromone with β-ionone. These data obtained in laboratory showed and increasing attraction activity determined by the tested substance not only toward males, but also toward females.

Nevertheless, the observations from field trap experiments only partially confirmed such results given that, although more individuals were captured in pheromone traps with both α-ionone and β-ionone compared to traps with only β-ionone, data did not show statistical significance.

This incongruence leads us to explore the reasons contributing to the observed differences between laboratory and field results. Firstly, the field environment introduces a myriad of complexities absent in laboratory settings. It is a well-known fact that factors such as uncontrolled environment conditions, competing odors, habitat, landscape, trap

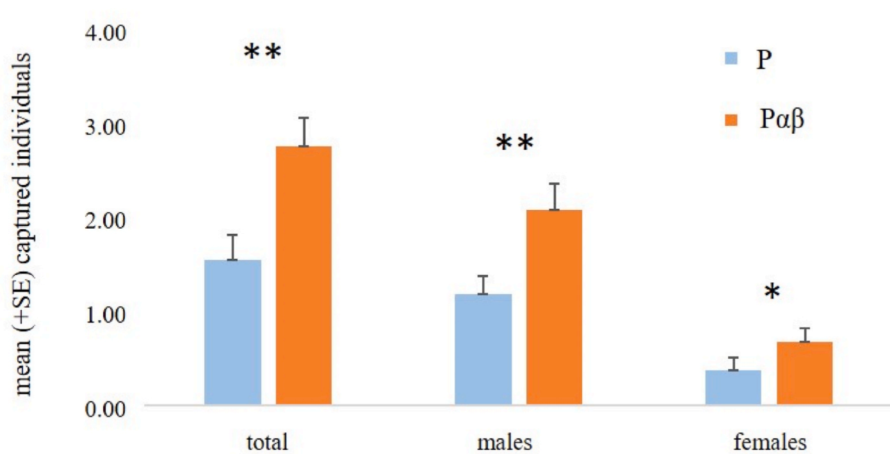

Fig. 1. Captures of total adults, males and females of *Lasioderma serricorne* to pheromone alone (P) or pheromone supplemented with the combination of α-ionone and β-ionone (Pαβ) after 24h in dual-choice arena bioassays. **p < 0.01; *p < 0.05.

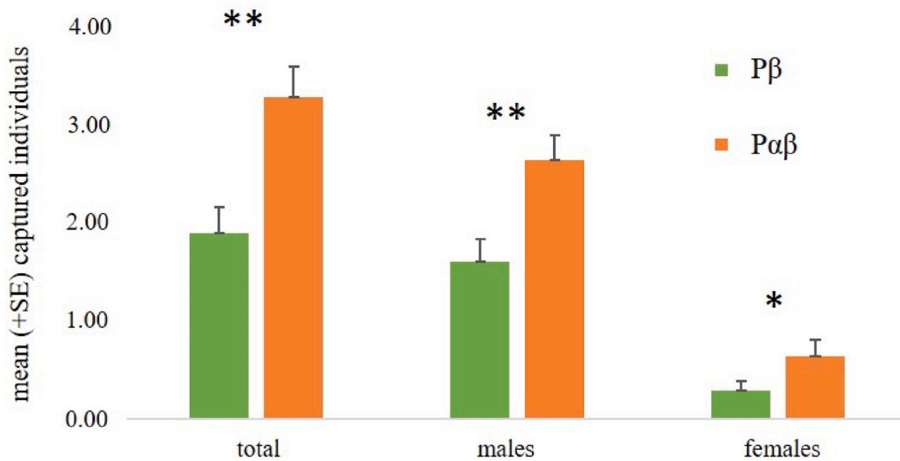

Fig. 2. Captures of total adults, males and females of *Lasioderma serricorne* to pheromone supplemented with β-ionone (Pβ) or pheromone supplemented with the combination of α-ionone and β-ionone (Pαβ) after 24h in dual-choice arena bioassays. $^{**}p \leq 0.01$; $^{*}p \leq 0.05$.

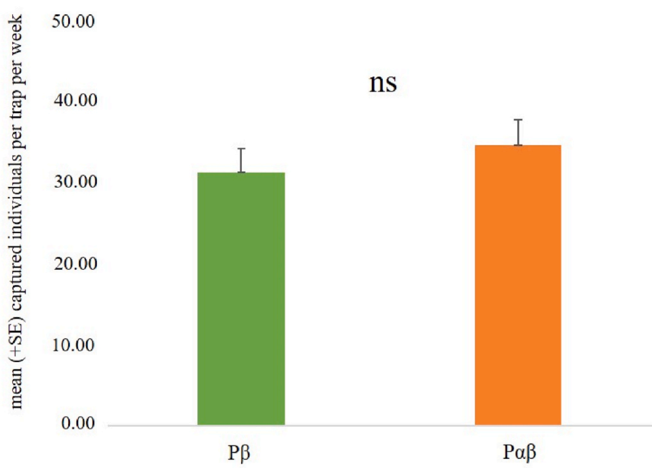

Fig. 3. Mean (+SE) captured adults of *Lasioderma serricorne* per trap per week in traps baited in the different treatments: polyethylene dispenser filled with pheromone + β-ionone (Pβ) or with pheromone + α-ionone + β-ionone (Pαβ).

placement and the presence of natural predators can affect the response of beetles to attractants (Campbell et al., 2002; Edde et al., 2005). Furthemore, fluctuations in population densities and human activities linked to the products handling, drying and cooling all have the potential to affect insect response to a lure (Cox and Collins, 2002).

Such intricate interplay of these variables may have dampened the efficacy of the attractants in the field, contributing to the observed differences. In addition, laboratory experiments often involved 24h exposure periods, whereas field conditions expose beetles to attractants over more extended periods. The beetles may exhibit behavioral adaptations over time, influencing their responsiveness to specific attractant combinations (Drukker et al., 2000). Moreover, beetles in real-world condition settings may demonstrate distinct sensitivities to attractants when compared with their counterparts in laboratory conditions (Salamanca et al., 2017). Variables such as air movement and direction have the potential to affect the dispersal and efficacy of the attractants, thereby influencing the overall rates of capture (Cardé, 2014). Finally, the same factors mentioned earlier could be assumed to account for the outcomes observed in the experiments conducted to evaluate the sex ratio of trapped adults, with only very few adult females being attracted to the bucket traps exposed in field conditions, regardless of the lures system adopted. Overall, the data obtained in field trap test confirm that, baiting the pheromone traps with β-ionone, independently by the further addiction of α-ionone, determined a clear synergistic effect on the response of *L. serricorne* to pheromone-baited traps, increasing the number of captured beetles. That said, from a practical point of view, using only β-ionone as co-attractant is economically convenient. The number of individuals captured by the trap containing such combination on a weekly basis was very similar to what was observed in in the past experiment by Guarino et al. (2022). Such considerable number of captures in the field, despite lacking statistical significance determined by the combination of the two terpenoids in comparison with the use of pheromone and β-ionone, may provide important indications for further exploration of such kairomones in implementing pheromone traps (e.g. by evaluating different doses and ratios). The integration of insect pheromone lures with such kind of molecules that act as kairomones in pest management practices presents a number of significant advantages. These compounds replicate environmental cues that pests do associate with their essential activities, such as feeding or oviposition. As a result, traps utilizing kairomones can become substantially more alluring to pests, leading to significantly improved capture rates (Guarino et al., 2011; Vacas et al., 2017). In recent years, there has been a growing interest in kairomone compounds that can attract *L. serricorne* adults: recent studies conducted by other research groups have demonstrated the potential of volatiles emitted by microorganisms (Ponce et al., 2023) and/or other substrate borne semiochemicals (Gries et al., 2022; Ren et al., 2022). Most of these experiments have primarily evaluated the attractant potential of a few number of volatiles in laboratory settings as olfactometers. In details, a combination of sex pheromone + *β*-ionone also implemented with 2,3,5,6-tetramethylpyrazine, citronellal and paeonol showed the best attractant properties in terms of dwell time of the adults in test arm of the olfactometer (Ren et al., 2022). In their future research, the authors plan to incorporate these promising volatiles for more extensive field studies in order to further improve trapping efficiency and develop tools that could contribute to reducing the reliance on insecticides to manage *L. serricorne* infestations.

CRediT authorship contribution statement

Salvatore Guarino: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. **Pietro Ranno:** Investigation, Methodology, Writing – review & editing. **Ezio Peri:** Conceptualization, Data curation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Pompeo Suma:** Conceptualization, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Nothing to declare If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The activity of this work was carried out within a project funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.3 - Call for tender No. 341 of 15 March 2022 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU; Award Number: Project code PE00000003, Concession Decree No. 1550 of 11 October 2022 adopted by the Italian Ministry of University and Research, CUP D93C22000890001, Project title "ON Foods - Research and innovation network on food and nutrition Sustainability, Safety and Security – Working ON Foods. The authors also would express their gratitude to Dr. Michele Tarsilla for his valuable comments in revising the English style of this manuscript.

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