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Beta ionone increases Lasioderma serricorne F. (Coleoptera: Anobidae) captures in sex pheromone-baited traps

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Abstract:	The cigarette beetle, Lasioderma serricorne F. (Coleoptera: Anobiidae), is an important stored product pest increasingly impacting museums and herbaria. Monitoring methods make use of pheromone traps which can be implemented using chili fruit powder as food attractant. Further laboratory studies evidenced that the main cues involved in this attraction are the terpenoids α -ionone and β -ionone. In this study a trap bioassay was carried out in a bread industry with pheromone traps implemented with α -ionone or β -ionone at different doses to evaluate the possible enhance of captures determined by such odorants in comparison with traps loaded with the synthetic pheromone alone. Furthermore, in order to optimize the type of device used, the chemical that elicited the highest performance was tested using two types of dispenser: a polyethylene and a silicone one. The results indicated that pheromone traps with the addition of β -ionone at the dose of 10 mg captured the highest amount of L. serricorne adults and significantly more than traps loaded with pheromone alone or with pheromone plus α -ionone. Differently, captures of pheromone traps supplemented with α -ionone didn’t differ statistically from those baited solely with the synthetic pheromone. Moreover, the traps baited with β -ionone loaded in polyethylene dispenser allowed a higher number of catches of the adults of the beetle in comparison with those obtained using silicone dispenser. This data indicate that such co-attractant can be positively exploited for trapping L. serricorne adults representing a highly-sensitive monitoring device and an important tool that can find possible application for mass trapping purposes.
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Declaration of interests

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Highlights

- The cigarette beetle, *Lasioderma serricorne* F. (Coleoptera: Anobiidae), is an important stored product pest commonly monitored by pheromone traps;
- Objective of this work was to enhance pheromone traps performance using two chemical volatiles that have already demonstrated to attract *L. serricorne* in laboratory: α-ionone and β-ionone;
- Attractant were tested using different doses and two different dispenser type;
- β-ionone at the dose of 10 mg determined the highest level of adults captured;
- Polyethylene dispenser determined the best performance in terms of captures;
- These results suggest the implementation of pheromone traps with such attractant for a more sensitive device tool and possible mass trapping purposes;

Beta ionone increases *Lasioderma serricorne* F. (Coleoptera: Anobidae) captures in sex pheromone-baited traps

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Abstract:

The cigarette beetle, *Lasioderma serricorne* F. (Coleoptera: Anobiidae), is an important stored product pest increasingly impacting museums and herbaria. Monitoring methods make use of pheromone traps which can be implemented using chili fruit powder as food attractant. Further laboratory studies evidenced that the main cues involved in this attraction are the terpenoids *α*-ionone and *β*-ionone. In this study a trap bioassay was carried out in a bread industry with pheromone traps implemented with *α*-ionone or *β*-ionone at different doses to evaluate the possible enhance of captures determined by such odorants in comparison with traps loaded with the synthetic pheromone alone. Furthermore, in order to optimize the type of device used, the chemical that elicited the highest performance was tested using two types of dispenser: a polyethylene and a silicone one. The results indicated that pheromone traps with the addition of *β*-ionone at the dose of 10 mg captured the highest amount of *L. serricorne* adults and significantly more than traps loaded with pheromone alone or with pheromone plus *α*-ionone. Differently, captures of pheromone traps supplemented with *α*-ionone didn't differ statistically from those baited solely with the synthetic pheromone. Moreover, the traps baited with *β*-ionone loaded in polyethylene dispenser allowed a higher number of catches of the adults of the beetle in comparison with those obtained using silicone dispenser. This data indicate that such co-attractant can be positively exploited for trapping *L. serricorne* adults representing a highly-sensitive monitoring device and an important tool that can find possible application for mass trapping purposes.

Keywords: *α*-ionone; cigarette beetle; serricornin; semiochemicals; polyethylene dispenser;

1. Introduction

The anobid beetle *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae), also known as cigarette beetle, is a serious pest of food storage, tobacco industry, and commodities stored in museums and herbaria (Chaudhari et al., 2020; Edde, 2019; Guarino et al., 2020). This species is particularly hazardous for its ability to penetrate actively the majority of the common packaging materials used for food or other commodities (Lü and Ma, 2015; Riudavets et al., 2007). The larval feeding activity determine a direct loss of the infested product and, the presence of dead insects, frass, exuviae, and gnawed particles indirectly negatively affect the market value of the products (Edde, 2019; Linnie, 1994). The control of *L. serricorne* populations is usually carried out with the use of fumigants such as phosphine, in particular on tobacco (Athanassiou et al., 2018). However, a few studies evidenced an increasing resistance to phosphine by this pest (Rajendran and Narasimhan, 1994; Sağlam et al., 2015). Moreover, restrictions to the use of chemicals are increasing in several countries; therefore, alternative tools for the management (Schöller et al., 2018) and the prompt detection of this pest are highly recommended.

Integrated pest management (IPM) strategies for *L. serricorne* rely on the use of monitoring methods based on sticky traps baited with sex pheromone (Athanassiou et al., 2018; Chaitanya and Swamy, 2018; Papadopoulou and Buchelos, 2002), whose main component is (4S,6S,7S)-4,6-dimethyl-7-hydroxynonan-3-one, commonly named serricornin, that strongly attracts male beetles (Chuman et al., 1985, 1979). Also in consideration of the high costs of serricornin, the use of pheromone traps for mass trapping this pest had so far limited application (Buchelos and Levinson, 1993). However, several studies have showed that plant-derived volatiles have a synergistic action in increasing the efficiency of pheromone-baited traps towards stored-product pests, as these compounds mimic food and oviposition sites, giving the opportunity to exploit this tool also for mass trapping technique (Trematerra, 2012). In particular, in the case of the cigarette beetle, it was reported that females respond more sensitively to plant volatiles than males, which may be due to their necessity to locate food sources and oviposition sites (Hori et al., 2011; Mahroof and Phillips, 2007).

Among possible attraction sources for *L. serricorne* adults, few studies have highlighted that the use of dried fruit powder of red chili, *Capsicum annuum* L. is particularly encouraging (Guarino et al., 2020; Mahroof and Phillips, 2008). More recently, Zhao et al., (2020) showed that, among several compounds from different plant tested, the hexane extract of the red chili elicited, in olfactometer studies, the highest attraction toward *L. serricorne* adults. In addition, laboratory experiments carried out from Guarino et al., (2021) evidenced that among the volatiles emitted by dried fruit powder of *C. annuum*, only their polar fraction determine attraction for adults. The chemical analysis of such fraction, exhibited the presence of few predominant compounds as α-ionone and β-ionone and further bioassays shown that both these molecules determine attraction for *L. serricorne* adults in olfactometer (Guarino et al., 2021). In field experiments, these compounds have evidenced the possibility to enhance the number of trap captures as reported by Faria and Zanella (2015) in their studies on *Euglossa mandibularis* Friese (Hymenoptera: Apidae).

In this context, this research aims to investigate the synergistic effect of α-ionone and β-ionone added to the sex pheromone in order to develop a sensitive attraction system for field trapping cigarette beetles. The improvement of the trapping system actually for this pest can have important consequences not only in the optimization of monitoring sensitivity but also in perspective to encourage the mass trapping of *L. serricorne*, particularly desired for those operators that work in organic farming conditions (Cox, 2004; Savoldelli and Trematerra, 2011). Furthermore, to optimize the efficacy of monitoring trap and in consideration that, several factors (e.g. dispenser type, field aging of the dispenser, the attractant dose in the releaser, etc.) can affect trap attractiveness (Anshelevich et al., 1994; Athanassiou et al., 2004), we evaluated also different attractant doses loaded in two different releasers, estimating the rate of emission of the chemicals during the weeks of utilization.

2. Materials and Methods

2.1. Trap bioassay

Experiments were carried out in an industrial bakery located in central Sicily (Italy) in two different time period of the year, i.e. from 10th September to 12th November 2020 (Trial 1) and from 17th March to 15th May 2021 (Trial 2). The *L. serricorne* response to α-ionone and β-ionone was tested using commercially available anobid traps (GEA srl, Settimo Milanese, Milan, Italy) that were distributed inside the bakery facilities as subsequently described. In both trials (see below) polyethylene pheromone dispenser (0.5 mL) loaded with 4mg of (4*S*,6*S*,7*S*)-4,6-dimethyl-7-hydroxynonan-3-one (afterward named serricornin) (purity grade 97.9%, Bedoukian, Danbury, USA) were used to bait the traps. Traps were inspected weekly for eight weeks, and the number of adults captured scored. The position of each treatment was randomized within each block and the trap position clock-wise 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.

2.1.1. Trial 1

In this first set of experiments, the pheromone traps were supplemented with candidate co-attractants α-ionone and β-ionone (both ≥95% purity, furnished from Sigma Aldrich, Milan, Italy) at the dose of 5 or 10 mg applied singly in a polyethylene 0.5 mL dispenser of the same type used for the pheromone. Moreover, α-ionone and β-ionone were also tested together, at the dose of 5+5 mg in the same dispenser, in traps without the pheromone. The different lure combinations are reported in Table 1. All the treatments and control were divided in 5 blocks each containing seven traps, one for each treatment, (n=35) at a distance of approximatively 8 m from each other.

The emission rate of the two compounds tested was estimated in laboratory through dynamic headspace collection and GC-MS analysis. In detail, the amount of α-ionone or β-ionone emitted from polyethylene tubes was collected by placing the dispensers in an air entertainment cylindrical glass chamber (25 mL volume) where charcoal-filtered air was passing through at 300 mL/min. A glass tube containing a plug of 100 mg of Porapak Q (80–100 mesh; Sigma-Aldrich) was used to collect α-ionone or β-ionone emitted after one hour from the dispenser loading and afterward every seven days until day 28 from their load. After collecting for 30 min, the collectors were eluted with 0.4 mL of hexane. Extracts were stored at 4°C in glass vials with Teflon cap liners until used for gas chromatography-mass spectrometry (GC/MS) analyses. The experiment was carried out in temperature-controlled room (25 ± 1°C). The dispensers after each sampling were stored in separate temperature-controlled room at the same conditions. Three replications were carried out for each compound at each dose (5 and 10 mg). GC-MS analyses were performed on an Agilent 6890 GC system interfaced with an MS5973 quadruple mass spectrometer. One μL of extract was injected onto a DB5-MS column in splitless mode. Injector and detector temperatures were 260°C and 280°C respectively. Helium was used as the carrier gas. The GC oven temperature was set at 40°C for 5 min, and then increased by 10°C/min to 250°C. Electron impact ionization spectra were obtained at 70 eV, recording mass spectra from 40 to 550 amu. In order to estimate the quantitative amounts of ionone acetate emitted from polyethylene tube, the integrated GC peaks were compared with a calibration curve carried out with standard solutions. Linearity was determined for this compound injecting in the GC concentrations of 6, 12, 25, 50 and 100 ng μL^{-1}, determining a calibration curve that had regression coefficients (R^2) of 0.997.

2.1.2. Trial 2

A further evaluation of the response of *L. serricorne* adults to the attractants was carried out on the basis of the results obtained in Trial 1. In this case, the pheromone traps were supplemented with 10 mg of β-ionone comparing if, two different types of dispenser, i.e. the polyethylene one, used in trial 1 and a silicone-based dispenser, could affect their attractive capacity. The combination lure used in this trial is reported in Table 1. As in Trial 1, all the treatments and control were divided in 5 blocks containing a replication for each treatment (n=25) at a distance of approximatively 10 m from each other. Finally, the β-ionone emission rate from both the used polyethylene and silicone dispensers was determined using the same methodology described for Trial 1.

Table 1. Different combinations of lures tested for trapping efficacy in the two field trials carried out in an Italian industrial bakery.

Trial n. 1		Trial n. 2	
(αI5+P)	α-ionone 5 mg + pheromone	[P(βI+P)]	polyethylene dispenser with β-ionone + pheromone
(αI10+P)	α-ionone 10 mg + pheromone	[P(P)]	polyethylene dispenser with pheromone
(βI5+P)	β-ionone 5 mg + pheromone	[S(βI+P)]	silicone dispenser with β-ionone + pheromone
(βI10+P)	β-ionone 10 mg + pheromone	[S(P)]	silicone dispenser with pheromone
(αI5+βI5)	α-ionone 5 mg + β-ionone 5 mg	(ctrl)	empty trap
(P)	pheromone		
(ctrl)	empty trap		

2.2. Statistics

The number of captured adults per trap per week was analyzed by using a one-way ANOVA and mean comparisons were performed according to the Tukey test. The emission for each chemical tested at different doses and using the two different type of dispenser was analyzed by multi factorial ANOVA, followed by Tukey test. All the statistical analyses were performed using Statistica 7.0 for Window (Statsoft 2001, Vigonza, PD, Italy).

3. Results

3.1. Trial 1

The results of Trial 1 are reported in Fig 1. Overall, the results evidenced strong statistical differences among the treatments and the control ($F_{6,273} = 19.14$; $P < 0.001$; ANOVA), with the pheromone traps supplemented with β-ionone determining the highest level of adult captured. In detail, βI10+P determined the highest level of captures, with a mean (±SE) of 31.57 ± 3.87 trapped individuals per trap per week, statistically higher than all the other treatments ($P < 0.001$; ANOVA followed by Tukey test), except from βI5+P (P = NS) that captured 27.62 ± 3.45 adults per trap per week. The latter determined higher captures than control, P and αI5+βI5 ($P < 0.001$), and similar to αI5+P and αI10+P. Traps baited with pheromone and α-ionone (αI5+P and αI10+P) captured a similar number of individuals than those baited with pheromone (P = NS). Finally, the combination of α and β ionone (αI5+βI5) determined the lesser attractive effect, comparable to those obtained using the unbaited traps (ctrl).

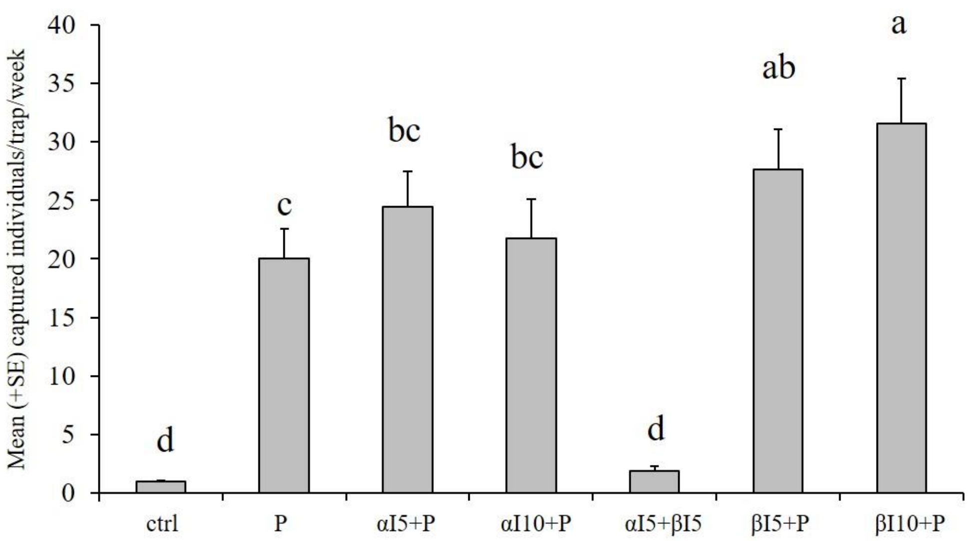

Fig. 1. Mean (+SE) captured adults of *Lasioderma serricorne* per trap per week in traps in the different treatments: α-ionone 5 mg + pheromone (αI5+P), α-ionone 10 mg + pheromone (αI10+P), β-ionone 5 mg + pheromone (βI5+P), β-ionone 10 mg + pheromone (βI10+P), α-ionone 5 mg + β-

ionone 5 mg (αI5+βI5), only pheromone (P), empty traps (ctrl). No letter in common indicate significant differences for $P < 0.05$ (ANOVA, followed by Tukey's test)

The emission from the polyethylene dispenser loaded with α-ionone or β-ionone at 5 or 10 mg is reported in Fig. 2. Overall, chemical emission was influenced by the time from dispenser loading and dose tested ($F_{12,40} = 4.00$; $P < 0.001$). The emission of the chemical from the dispenser was highest at the releaser opening using 10 mg for both α-ionone and β-ionone ($P < 0.001$; ANOVA). After seven days of exposure the emission was still greater in the dispenser loaded with 10 mg of α-ionone or β-ionone compared to the lower dose ($P < 0.001$; ANOVA), while from the 14th day onward the emission rate of α-ionone and β-ionone was not significantly different for both the measured doses.

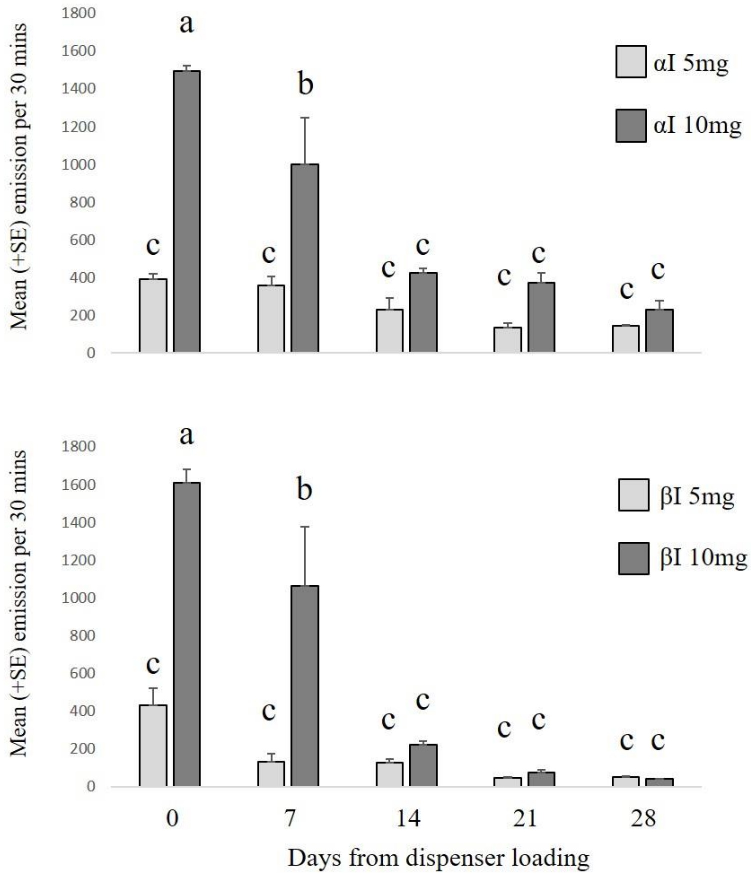

Fig. 2. Mean (+SE) release rates of chemicals from lures measured by collecting the volatiles by using dynamic head-space method at 25 C°. For variables with the same letter, the difference is not statistically significant for $P < 0.05$ (ANOVA)

3.2. Trial 2

The results of Trial 2 are reported in Fig 3. A different mean number of adult captures was observed among the treatments ($F_{5,195}$= 39.49; P < 0.001; ANOVA). Overall pheromone traps supplemented with β-ionone determined a high level of adults captured in comparison with pheromone traps using both the type of dispenser. In detail, [P(βI+P)] evidenced a number of captures statistically greater than all other treatments and control (P < 0.001). [S(βI+P)] higher than to the other treatments and the control (P < 0.001). The captures in [P(P)] and [S(P)] were similar to the control (P = NS).

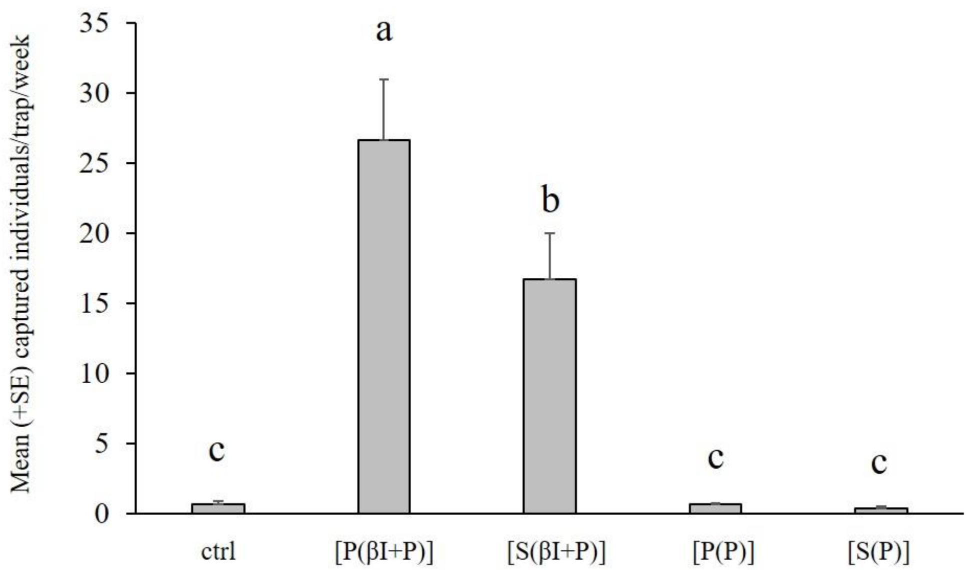

Fig. 3. Mean (+SE) captured adults of *Lasioderma serricorne* per trap per week in traps baited in the different treatments: polyethylene dispenser filled with β-ionone + pheromone [P(βI+P)], polyethylene dispenser filled with pheromone [P(P)], silicone dispenser filled with β-ionone + pheromone [S(βI+P)], silicone dispenser filled with pheromone [S(P)], empty trap (ctrl). For variables with the same letter, the difference is not statistically significant for P < 0.05 (ANOVA, followed by Tukey's test)

The estimation of the amount of β-ionone released by the polyethylene and silicone dispenser is reported in figure 4. Overall, the highest emission was observed in polyethylene 30 minutes after the dispenser loading ($P < 0.05$; ANOVA). The chemical emission from silicone dispenser was higher 30 minutes after the dispenser loading and after seven days of exposure compared to 28 days of exposure ($P < 0.05$; ANOVA).

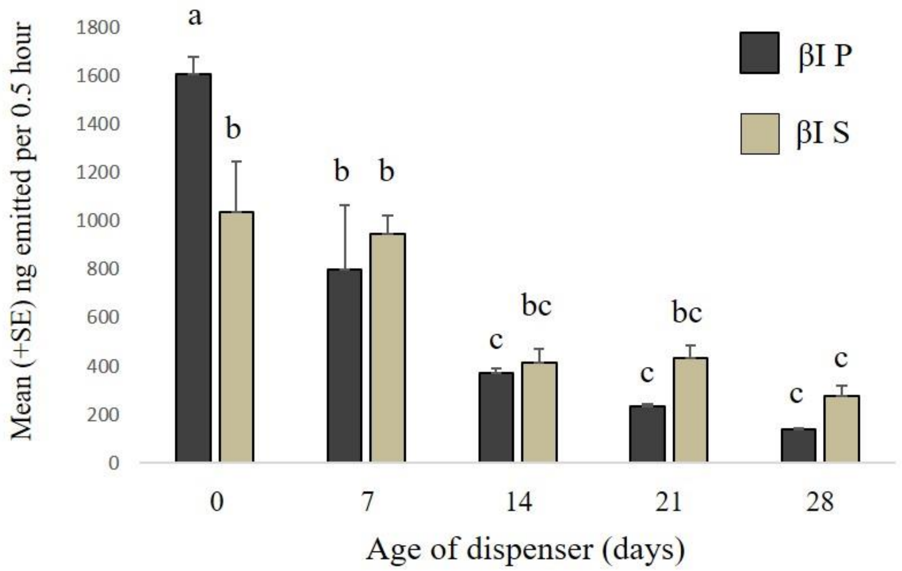

Fig. 4. Mean (+SE) release rates of chemicals from lures measured by collecting the volatiles by using dynamic head-space method at 25 C°. For variables with the same letter, the difference is not statistically significant for $P < 0.05$ (ANOVA)

4. Discussion

The results obtained in this study indicate that the β-ionone-added pheromone traps works better than the commercially available pheromone traps strongly increasing the captures of *L. serricorne* and, that the type of dispenser device used can significantly influence this number. Overall, the data obtained suggest that β-ionone, can be considered and exploited as co-attractant in *L. serricorne* pheromone traps and that the dose of 10 mg performed optimally.

In the experiment conducted during late summer-early fall 2020, we observed that the use of β-ionone enhanced the number of individuals captured, regardless of the dose used, in comparison with traps baited solely with serricornin. In particular, the pheromone baited traps supplemented with the dose β-ionone (10 mg) determined the highest number of captures, increasing the trapped individuals one and half fold in comparison with traps loaded only with pheromone. Differently, the use of α-ionone added to pheromone traps did not statistically elicit an increase of adults trapped in comparison with traps loaded only with pheromone. The data obtained from the estimation of the emission of α-ionone and β-ionone after the different weeks of exposure evidenced a similar emission of the two compounds during the different times of exposure considered. Moreover, the dispensers loaded with the higher dose (10 mg) confirmed a higher emission rate compared to the lower dose (5 mg) during the first seven days from the field exposition.

β-Ionone is a VOC that might determine different behavioral response in phytophagous insects. In some cases, β-ionone produced from plants protect themselves from herbivorous insects (Cáceres et al., 2016; Paparella et al., 2021) by deterring them as in the case of the cabbage butterfly, *Pieris rapae* L. (Lepidoptera: Pieridae) (Ômura et al., 2000), or the crucifer flea beetle, *Phyllotreta cruciferae* (Goeze) (Coleoptera: Crhysomelidae) (Gruber et al., 2009). In other cases, β-ionone, showed attractant properties as for the coleopteran scarabeids *Oxythyrea testaceoguttata* Blanchard and *Anomala transvaalensis* Arrow (Donaldson et al., 1990). According to the results obtained in this study it seems that β-ionone acts as important key mediator for *L. serricorne* by mimicking food and oviposition sources (Guarino et al., 2020). The first report of possible response of *L. serricorne* to β-ionone was speculated by Phoonan et al., (2014) who evidenced that this compound was one of the main volatiles of the mulberry tea leaves, with a strong attractiveness to the cigarette beetle. However, the result obtained in our study clearly indicate that the use of β-ionone enhances cigarette beetle captures only when added to pheromone and not *per se*. In fact, in our experiments, the use of β-ionone and α-ionone alone without pheromone determined a number of captures lower than the use of the pheromone alone. These results are in accordance with a previous study from Papadopoulou and Buchelos, (2002) who evidenced a higher number of captures determined by pheromone traps rather in traps loaded with an undisclosed food attractant produced by Fuji Flavor Co. Ltd (Tokyo, Japan).

In trial 2, conducted during spring 2021, it was confirmed that β-ionone (10 mg) increased the *L. serricorne* adult captures in comparison with pheromone traps but also that the loading of this chemical in polyethylene dispenser improved the number of captures more strongly than it was possible to observe using the silicone dispensers. This result can be linked with the different emission of the two dispensers, with a higher emission of β-ionone in polyethylene during the first phase that might have determined the higher number of captures. Few studies have evidence that the dispenser type used can affect the number of insect captures in the field (Athanassiou et al., 2004; Zhang et al., 2013).

During this second trial and differently from the first one, the number of captured individuals in pheromone traps was very low and not statistically different from that obtained in the unbaited traps. We can speculate that these results can be influenced by a different sensitivity to the pheromone along the year of this species, and that the implementation with a synthetic food attractant as *β*-ionone is necessary to elicit attraction response. In other systems the overwintering (diapausing) individuals may need a lag period in their development of pheromone sensitivity after a period of insensitivity (Birch, 1974) as observed to occur *Ips pini* (Say) (Coleoptera: Curculionidae) (Steed and Wagner, 2008).

In consideration that the cigarette beetle population inside the bread industry, where the experiment was conducted, was present at a constant population density, we can assume that this species can respond with a different sensitivity to the pheromone along the year, and consequently a co-attractant able to elicit a response could be critical. In fact, the presence of β-ionone as co-attractant strongly enhanced the trap activity, determining high level of captures also in a period of the year when the sensitivity of *L. serricorne* to the pheromone is low. This aspect should be taken in account not only in the perspective to optimize monitoring trap for *L. serricorne*, as improved trap efficacy is crucial for implementing control methods for the cigarette beetle. To conclude, the use of β-ionone at the dose of 10 mg loaded in polyethylene dispensers and used in combination with the commercial synthetic sexual pheromone, is able to strongly enhance the level of captures of the cigarette beetles. The obtained results represent a very important aspect to be considered also in the perspective of planning the mass trapping technique against this pest, particularly recommended in those contexts where chemical control has to be reduced or avoided at all (e.g. cigarette or organic factories).

Author contribution: Conceptualization: SG, EP, PS; Data curation: PR, PS, SG, SB; Investigation SB, SG, PS, PR; Methodology: SG, EP, PS; Roles/Writing - original draft: SG, EP, PS; Writing - review & editing: SB.

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