Factors affecting field performance of pheromone traps for tobacco beetle, *Lasioderma serricorne* and tobacco moth, *Ephestia elutella*

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Abstract Tobacco beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae), is one of the most serious insect pests of stored tobacco and traps baited with the female-produced sex pheromone, serricornin, are used for monitoring the pest. In two trapping experiments carried out in tobacco warehouses in Greece, two commercially-available trap and lure systems for *L. serricorne* were found to be equally effective in terms of numbers of beetles trapped. In contrast to previous reports, anhydroserricornin was unattractive and lures containing serricornin and anhydroserricornin were less attractive than lures containing serricornin only. The sex pheromone of the other main insect pest of tobacco, *Ephestia elutella* (Hübner) (Lepidoptera: Pyralidae), could be added to the lures without affecting the attractiveness of either pheromones to their respective species. Lures remained attractive for at least four weeks under field conditions, and, in laboratory tests, release of pheromone

could still be detected after 30 days at 27°C. The stereoisomeric composition of the sericornin in the two commercial lures was similar with high proportions of the attractive (4*S*,6*S*,7*S*)-isomer. The proportion of the (4*S*,6*S*,7*R*)-isomer was low and this is known to reduce the attractiveness.

Keywords Sericornin • 4,6-Dimethyl-7-hydroxynonan-3-one • Anhydrosericornin • 2,6-Diethyl-3,5-dimethyl-3,4-dihydro-2H-pyran • *Ephestia elutella* • (*Z*,*E*)-9,12-Tetradecadienyl acetate • (*Z*,*E*)-9,12-Tetradecadien-1-ol • Stored product pests

Key message

- Two commercially-available trap and lure systems for the main insect pest of tobacco, *Lasioderma sericorne* were found equally effective in terms of numbers of beetles trapped.
- In contrast to previous reports, anhydrosericornin was unattractive and lures containing sericornin and anhydrosericornin were less attractive than lures containing sericornin only.
- The sex pheromone of the other main insect pest of tobacco, *Ephestia elutella*, could be added to the lures without affecting the attractiveness of either pheromone to their respective species.

Introduction

The cigarette or tobacco beetle, *Lasioderma sericorne* (F.) (Coleoptera: Anobiidae) is one of the most important insect pests of stored products globally (Ashworth 1993b; Buchelos and Levinson 1993). It has a wide variety of food preferences, ranging from amylaceous materials, such as flour and cereals, to dried plants, such as herbs and dried fruit (Ashworth

1993b; Mahroof and Phillips 2008a, 2008b, 2011). This species is particularly abundant in various types of storage and processing facilities such as flour mills, feed mills, retail stores and tobacco warehouses (Buchelos 1981; Levinson and Buchelos 1988; Mahroof and Phillips 2011), but it is also common outdoors on weeds such as thistles (Buchelos 1989). Mahroof and Phillips (2008a) investigated the effects of eight different food sources on the development patterns of *L. serricorne,* and found that wheat was more suitable than herbs or tobacco. Nevertheless, *L. serricorne* is considered to be the most serious insect pest of tobacco, followed by the tobacco moth, *Ephestia elutella* (Hübner) (Lepidoptera: Pyralidae) (Ashworth 1993a). This is because *L. serricorne* is one of the few insect species that is able to develop in tobacco which is toxic for most other species. Hence, *L. serricorne* can develop on raw and processed tobacco without competition from other species and build up high population densities (Buchelos and Levinson 1993; Buchelos and Trematerra 1998; Mahroof and Phillips 2008a).

Control of *L. serricorne* is typically based on the use of fumigants such as phosphine, especially on tobacco. In order to monitor the seasonal activity of this species and to time the application of control measures, pheromone-baited traps are used. These traps are baited with serricornin (4,6-dimethyl-7-hydroxynonan-3-one) and/or anhydroserricornin (2,6-diethyl-3,5-dimethyl-3,4-dihydro-2H-pyran) which were identified as components of the sex pheromone produced by female *L. serricorne* attracting male beetles (Chuman et al., 1985 and references therein). Serricornin has eight stereoisomers – four pairs of enantiomers – and commercially available material is a racemic mixture of stereoisomers. (4*S*,6*S*,7*S*)-Serricornin was shown to be produced by female *L. serricorne* and is the isomer most attractive to male beetles (Mori et al. 1982). The addition of (4*S*,6*S*,7*R*)-serricornin was reported to reduce greatly the attractiveness of both (4*S*,6*S*,7*S*)-serricornin (Mori et al. 1986) and anhydroserricornin (Levinson and Levinson 1986a, 1986b).

Traps baited with lures containing anhydroserricornin were used in early studies on monitoring (Levinson et al. 1981; Buchelos and Levinson 1985; Levinson and Buchelos 1988) and mass trapping (Buchelos and Levinson 1993) of *L. serricorne* in tobacco storage facilities in Greece. However, newer surveys have used traps baited with serricornin (Arbogast et al. 2003; Mahroof and Phillips 2008b, 2011) while Papadopoulou and Buchelos (2002) used a lure containing 80% serricornin and 20% anhydroserricornin. Currently, some commercial traps also contain additional compounds, such as food attractants, that enhance trapping efficacy, mostly through increasing the capture of adult females (Papadopoulou and Buchelos 2002; Mahroof and Phillips 2008b, 2011).

The performance of pheromone traps in commercial facilities is also influenced by biotic (e.g. insect behaviour) or abiotic factors (e.g. temperature). Storage and processing facilities are generally enclosed, warm environments, such that insect activity is likely to occur throughout the year under a wide range of temperature levels (Buchelos and Trematerra 1998). Changes in temperature are expected to affect the release rate of pheromones and also possibly the stability (Howse et al. 1998). The effects of temperature could therefore impact on the attractiveness and longevity of the respective lures, and, as a result, trapping efficacy. However, the effects of temperature on the longevity of commercial lures for *L. serricorne* have not been investigated previously.

This study compared the performance of two commercially-available traps and lures for trapping *L. serricorne* under field conditions. One of the lures also contained the sex pheromone of *E. elutella*, and the effects of combining the two pheromones on attraction of the two species were determined. The influence of the isomeric composition of serricornin in the lures as well as the effect of anhydroserricornin on attractiveness were investigated. Finally, the longevity of the lures at different temperatures was determined under laboratory conditions.

Materials and methods

Traps and lures

Two commercial brands of traps and lures available for trapping *L. serricorne* were used in the tests. The MoBe Combo MK2 trap (Barrettine Environmental Health, Bristol, UK), is a folded cardboard trap (195 (l) x 100 (w) x 20 (h) mm) with a dry adhesive liner. The MoBe lure is a polymer sheet impregnated with serricornin and also (*Z*,*E*)-9,12-tetradecadienyl acetate (ZETA) and the corresponding alcohol (ZETOH), which are attractants for several pyralid pests of stored products (Levinson and Buchelos 1981; Trematerra et al. 2011, 2013). The Serrico trap (Fuji Flavor Co., Tokyo, Japan) is a similar, folded cardboard adhesive trap (190 (l) x 80 (w) x 25 (h) mm) baited with separate tablets containing serricornin and a food attractant. White delta traps (12 cm side; made by the University of Thessaly) were also used in the tests, as a "control" trap design. Traps were positioned approximately 1.80 m from the floor and at least 5 m apart. Replicates were at least 20 m apart.

Field trial 2011

This trial was carried out in a tobacco processing facility in central Greece on a single, ground floor (7,000 m^2) filled with pallets of cardboard boxes of unprocessed tobacco of several types and varieties, with corridors to allow machinery movement. Catches with five different lure/trap combinations were compared with four replicates: (A) MoBe trap with standard MoBe lure containing serricornin, ZETA and ZETOL; (B) MoBe trap with MoBelure containing anhydroserricornin, ZETA and ZETOL; (C) MoBe trap with MoBe lure containing serricornin only; (D) MoBe trap with MoBe lure with anhydroserricornin added in equal amount to the serricornin; (E) Serrico trap with standard Serrico lure of pheromone and food attractant. The custom MoBe lures were provided by Barrettine Environmental Health.

Traps were installed on 23 June 2011, and were checked at weekly intervals until 8 September 2011. At each inspection, the traps were replaced with new ones, while the lures were changed every four weeks. After each check, the treatments were rotated one position clockwise to eliminate the influence of the trapping location. The traps were then returned to the Laboratory of Entomology and Agricultural Zoology for counting and identification of insects captured. Apart from *L. serricorne*, numbers of *E. elutella* and the Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) that were captured were also recorded.

Field trial 2012

Following up the results from the trials in 2011, the trials in 2012 were carried out in a commercial tobacco facility in Northern Greece with two floors (5,000 m^3 each) full of cardboard boxes with unprocessed tobacco, as above. Catches with six different lure/trap combinations were compared with four replicates, two on each floor. Treatments were (A) Serrico trap with standard Serrico lure; (B) Serrico trap with standard MoBe lure; (C) MoBe trap with Serrico lure; (D) MoBe trap with MoBe lure; (E) delta trap with MoBe lure; (F) delta trap with MoBe lure containing purified serricornin (details below).

Traps were installed on 16 August 2012 when *L. serricorne* captures were high, and the traps were monitored for captured adult beetles at weekly intervals for a period of four consecutive weeks. As above, after each trap inspection, the traps were replaced with new ones, without changing the lures, and the treatments were rotated one position. Catches of *L. serricorne*, *E. elutella* and *P. interpunctella* were recorded, as above. For both years (2011 and 2012), the temperatures in the tested facilities during the monitoring period, ranged between 20.5 and 33.5 ^{o}C.

Determination of isomeric composition of serricornin

In order to quantify the isomeric composition of serricornin, we followed a method developed at the Natural Resources Institute (Hall, in preparation). After acetylation of serricornin with acetic anhydride and pyridine at room temperature overnight, analyses by gas chromatography (GC) using conditions described below showed three peaks on both polar and non-polar GC columns. The configurations of these were assigned by comparison of the ^{13}C Nuclear Magnetic Resonance spectra of purified fractions (see below) with those reported by Chuman et al. (1985) as (4*S**,6*S**,7*S** + 4*R**,6*S**,7*S**), 4*S**,6*R**,7*S**, and 4*S**,6*S**,7*R** on the polar GC column, and 4*S**,6*S**,7*S**, (4*R**,6*S**,7*S** + 4*S**,6*R**,7*S**), and 4*S**,6*S**,7*R** on the non-polar column, where the * denotes the racemic mixture of enantiomers which were not separated under these conditions. Thus, analysis of the mixture of acetates on the two GC columns allows determination of the proportions of the 4*S**,6*S**,7*S**, 4*S**,6*R**,7*S**, and 4*S**,6*S**,7*R** isomers directly and that of 4*R**,6*S**,7*S** by difference.

The three broad peaks observed on GC analysis of the serricornin before acetylation on both GC columns were assigned to the 4*S**,6*R**,7*S**, (4*S**,6*S**,7*S** + 4*S**,6*S**,7*R**), and 4*R**,6*S**,7*S**, respectively.

Release rate measurements

MoBe and Serrico lures were maintained in a wind tunnel (8 km/h windspeed) in a temperature controlled room at 27 ± 1°C in order to mimic average temperatures of tobacco storage normally found in warmer parts of the world.

At intervals, volatiles were collected from individual lures contained within a glass chamber (10 x 3 cm). Air was drawn in (2 l/min.) through a filter of activated charcoal (20 x 2 cm; 10-18 mesh; Fisher Scientific, Loughborough, UK) and out through a collection filter consisting of a Pasteur pipette (4 mm i.d.) containing Porapak Q (200 mg; 50-80 mesh;

Supelco, Gillingham, Dorset, UK) held between plugs of silanised glass wool. The Porapak was purified by Soxhlet extraction with chloroform for 8 h, followed by washing with dichloromethane (2 ml) and drying in a stream of nitrogen immediately before use. Collections were made from the same lures for 1.5 – 3 h at one of three temperatures: 27 ± 1°C in the wind tunnel room, 37 ± 0.5°C in an incubator and 22 ± 1°C in the laboratory.

Volatiles were eluted from the Porapak collection filter with dichloromethane (1 ml; Pesticide Residue Grade) and decyl acetate (5 µg) added as internal standard. Solutions were analysed by gas chromatography (GC) with flame ionisation detection (FID) using HP6850 (Agilent, Manchester, UK) machines. GC columns (30 m x 0.32 mm i.d. x 0.25 µ film thickness) were coated with polar DBWax (Supelco) or non-polar HP5 (Agilent) and the carrier gas was helium (2.4 ml/min). Injection was splitless (220°C) and the oven temperature was programmed at 50°C for 2 min then at 10°C/min to 250°C. Data were captured and processed with EZChrom Elite (Agilent). Serricornin eluted as three broad peaks on both columns and quantification was by comparison of the total peak area with that of the internal standard. Results are the means of measurements on two lures.

Lures were also extracted with diethyl ether (5 ml) for 24 h at room temperature and the serricornin and anhydroserricornin quantified by GC as above against decyl acetate as internal standard.

Purification of serricornin and synthesis of anhydroserricornin

Serricornin (Bedoukian, Danbury, CT06810, USA) was purified by chromatography on silica gel eluted with petroleum spirit containing increasing amounts of diethyl ether as described by Mori and Watanabe (1985). Fractions were monitored by GC analysis as above and combined to give three main fractions. The middle fraction contained the 4*S**,6*R**,7*S**,

4*S**,6*S**,7*S**, 4*S**,6*S**,7*R**, and 4*R**,6*S**,7*S** isomers in 0 : 72: 7 : 20 ratio, compared with 3 : 67 : 1 : 28 in the original material.

Anhydroserricornin was synthesised by refluxing serricornin in benzene with a catalytic amount of *p*-toluenesulphonic acid for 2 h and purification by chromatography on silica gel eluted with 2% diethyl ether in petroleum spirit and kugelrohr distillation (bp 110°C/15 mm). Spectral data were consistent with those reported by Chuman et al. (1985).

Data analysis

Insect distributions were over-dispersed between traps and catch data were transformed to log (x+1) prior to entry as the dependent variable in a general linear model (glm) in the R statistical package (R Core Team, 2014). Trap check date and trap and lure combination were the response variables and tested separately for each species and year. Significance of both factors and the interaction in the ANOVA were assessed through *F*-tests on sequential sums of squares. Where a significant interaction was found, Tukey's tests ($P < 0.05$) were used to compare least-squared means of insects captured by each trap type at each trap check date (Lenth 2016). Where a significant effect of trap type, but no interaction with trap check date, was found, Tukey's tests compared numbers caught by each trap and lure combination across the entire experiment.

The number of *E. elutella* caught in 2012 was too low for analysis by glm. Instead, numbers caught by each trap type were summed across the experiment, and compared using a χ^2-test in R. Standardized residuals greater than 2 identified trap types which caught more *E. elutella* than expected under the χ^2-squared distribution (Agresti 2007).

Results

Field trial 2011

A significant interaction was found between trap check date and trap and lure combination ($F_{40,165}$ = 7.5, P < 0.001) on the number of *L. serricorne* adults captured. Date of capture ($F_{10,209}$ = 78.4, P < 0.001) and trap and lure combination ($F_{4,205}$ = 273.4, P < 0.001) were also significant in the model. For the first two weeks of the experiment captures were low in all traps, with difference between lure and trap combinations in numbers of insects captured more pronounced in August than July. Correcting for differences in captures between weeks, the MoBe trap with MoBe lure (MoBeTrap+lure), MoBe trap with serricornin only (MoBeTrap+Serri) and the Serrico trap with Serrico lure (SerricoTrap+lure) all captured significantly more *L. serricorne* than the MoBe trap with MoBe lure with added anhydroserricornin (MoBeTrap+lure+anhydro), which in turn captured more *L. serricorne* than the MoBe trap baited with anhydroserricornin only (MoBeTrap+anhydro) (Fig. 1)

In the same experiment, a significant interaction was also found between trap check date and trap and lure combination on number of *E. elutella* adults captured ($F_{40,165}$= 4.3, P < 0.001), with date of capture ($F_{10,209}$ = 22.0, P < 0.001) and trap and lure combination ($F_{4,205}$ = 481.0, P < 0.001) also significant in the model. As for *L. serricorne*, numbers of *E. elutella* captured increased over the first five weeks of the experiment. As expected, the traps with lures including ZETA and ZETOL, i.e. MoBeTrap+lure, MoBeTrap+anhydro and MoBeTrap+lure+anhydro, caught significantly more *E .elutella* than MoBeTrap+Serri and the SerricoTrap+lure (Fig 2). Captures of *E. elutella* in the MoBeTrap+Serri and the SerricoTrap+lure were negligible throughout the experiment (Fig 2).

Only 7 *P. interpunctella* were captured throughout the experiment in 2011 and the data were not analysed.

Field trial 2012

Significant effects of capture date ($F_{3,92}$ = 6.7, P < 0.001) and trap type ($F_{5,87}$ = 7.9, P < 0.001) were found on numbers of *L. serricorne* captured in 2012. However, as there was no significant interaction between the two factors on captures ($F_{15,72}$ = 0.9, P = 0.52), post-hoc comparisons between trap and lure combinations were made across the entire experiment. Overall, significantly more *L. serricorne* were captured in the Serrico traps baited with the MoBe lure (SerricoTrap+MoBelure) than delta traps baited with the MoBe lure with purified serricornin (DeltaTrap+MoBelure†) (Fig. 2). Captures in other trap types were intermediate between these two trap and lure combinations.

In total 23 *E. elutella* were captured across the experiment in 2012, all in traps baited with the MoBe lures containing ZETA and ZETOL: three in the SerricoTrap+MoBelure, five in the MoBe trap with the MoBe lure (MoBeTrap+MoBelure), three in the delta trap with the MoBe lure (DeltaTrap+MoBelure), and 13 in the DeltaTrap+MoBelure†. A significant difference was found between trap and lure combinations in total *E. elutella* captured (χ^2= 29, df = 5, P < 0.001), with the DeltaTrap+MoBelure† capturing significantly more *E. elutella* than expected under the χ^2-squared distribution (standardized residual = 4.9).

Nine *P. interpuctella* were captured in total in 2012: 2 in the SericcoTrap+Serricolure, 3 in the MoBeTrap+MoBelure, 2 in the DeltaTrap+MoBelure and 2 in the DeltaTrap+MoBelure†).

Release rates

Measurement of release rates from samples of dispensers used in the 2011 trapping experiments confirmed release of the components as expected with increased release of anhydroserricornin from the MoBe lures with anhydroserricornin only (B) and those with anhydroserricornin and serricornin (D). The release rate of serricornin from the Serrico lures

was lower than that from the MoBe lures, and the relative release rate of anhydroserricornin was higher in the former (Table 1).

Analysis of the pheromone extracted from lures confirmed the higher proportion of anhydroserricornin in the Serrico lures (Table 2). The isomeric composition of the serricornin was similar in both Serrico and MoBe lures with the naturally-produced 4*S**,6*S**,7*S** isomer predominating. Purification of the serricornin by liquid chromatography did not greatly increase the proportion of this isomer (Table 2) although did increase the relative amount of the 4*S**,6*S**,7*R** isomer (Table 2).

Dispensers used in 2012 were maintained in a laboratory wind tunnel at 27°C and 8 km/h windspeed and release rates were measured at intervals at three different temperatures, 37°C, 27°C and 22°C (Figs. 4 and 5; Table 3). Initial release rates at 37°C and 27°C were 4-6 times and 1.7-1.9 times greater respectively than those at 22°C for the MoBe lures. For the Serrico lures the relative rates were 6-8 times and 2.3-2.5 times respectively. Release of the pheromone components was still detectable after 30 d at 27°C and 8 km/h windspeed with the release rate of serricornin 4.2-7.4 % of that at Day 1 for the MoBe lures and 3.4-6.0 % for the Serrico lures. The release rate of ZETA from the MoBe lures was still 18.5-19.7% of that at Day 1 (Table 3).

Discussion

There have been numerous reports of trapping *L. serricorne* with pheromone traps, but few have provided a comparison of commercially-available traps and lures or investigation of factors affecting their effectiveness. Some commercial traps also contain additional compounds, such as food attractants, that enhance trapping efficacy, mostly through increasing the capture of adult females (Papadopoulou and Buchelos 2002; Mahroof and Phillips 2008b, 2011).

In the 2011 experiment, there were no significant differences between catches of *L. serricorne* with the two commercial traps and lures evaluated. However, addition of anhydroserricornin to the lure significantly reduced catches, and traps baited with anhydroserricornin alone caught essentially no beetles. This was surprising in view of the results of previous studies in which traps baited with lures containing anhydroserricornin were used in monitoring (Levinson et al. 1981; Buchelos and Levinson 1985; Levinson and Buchelos 1988) and mass trapping (Buchelos and Levinson 1993) of *L. serricorne* in tobacco storage facilities in Greece. Anhydroserricornin was reported to be a minor component in the pheromone extracted from female *L. serricorne* beetles relative to the amount of serricornin (Chuman et al. 1985). It was four orders of magnitude less active in laboratory bioassays and did not stimulate an electroantennogram (EAG) response from the male beetles (Chuman et al. 1982a; 1982b). However, Levinson et al. (1981) reported anhydroserricornin to be three orders of magnitude more attractive to male *L. serricorne* beetles than serricornin in a similar laboratory bioassay, and that both compounds elicited similar EAG responses from the male beetles. The reasons for the difference in these latter results compared with those of Chuman et al. (1982a; 1982b) and those reported here are not clear, although the stereochemistry of the synthetic compounds used may be a factor. Levinson et al. (1981) did not give details on the stereochemistry of the compounds which were synthesised in their laboratory. Chuman et

316 al. (1982a) showed that only a mixture of the four *threo*-isomers of serricornin, including the
317 naturally-occurring (4*S*,6*S*,7*S*)-isomer, was active while the *erythro*-isomers and
318 anhydroserricornin had similar, much lower activities. Chuman et al. (1982b) confirmed that
319 the (4*S*,6*S*,7*S*)-isomer was significantly more active than other stereoisomers of serricornin
320 and (6*S*,7*S*)-anhydroserricornin, while Mochizuki et al. (1984) reported that, of the four
321 enantiomeric pairs of isomers of serricornin, only the (4*S**,6*S**,7*S**)-isomers showed any
322 biological activity in a laboratory bioassay or EAG test. Furthermore, addition of (4*S*,6*S*,7*R*)-
323 serricornin was reported to reduce greatly the attractiveness of (4*S*,6*S*,7*S*)-serricornin (Mori et
324 al. 1986). Thus, if the serricornin used by Levinson et al. (1981) contained only low amounts
325 of the attractive (4*S*,6*S*,7*S*)-isomer and/or high amounts of the inhibitory (4*S*,6*S*,7*R*)-
326 serricornin it may have been biologically inactive and made the anhydroserricornin appear
327 more attractive than it really is.

328 In the 2011 experiment, the Serrico lures released anhydroserricornin at
329 approximately 80% of the rate of the serricornin, although for the MoBe lures the relative
330 release rate of anhydroserricornin was much lower. These levels of anhydroserricornin did
331 not apparently decrease the attractiveness of the serricornin to *L. serricorne*, but higher rates
332 did. Anhydroserricornin is produced by dehydration of serricornin, and it is not known
333 whether the anydroserricornin in these lures was present in the original pheromone supplied
334 as a result of the manufacturing process, whether it was formed during formulation or even
335 whether it had been specifically added to the lures. Previous surveys have used traps baited
336 with serricornin only (Arbogast et al. 2003; Mahroof and Phillips 2008b, 2011) or a blend of
337 80% serricornin and 20% anhydroserricornin (Papadopoulou and Buchelos 2002).

338 The 2011 experiment also demonstrated that MoBe lures containing serricornin with
339 ZETA and ZETOL (treatments A, B and D) attracted male moths of *E. elutella*, the larvae of
340 which are the second most important insect pest of stored tobacco. Although lures with

ZETA and ZETOL only were not tested, the fact that the three lures with serricornin and/or anhydroserricornin all captured similar numbers of moths suggests there was little effect of these compounds on the catches of *E. elutella.* The presence of ZETA and ZETOL in the lures also had no effect on numbers of *L. serricorne* caught in the traps (A v C). Although this might have been expected, given the chemical dissimilarity of the moth and beetle pheromones, it should also be noted that catches of *L. serricorne* were not affected by the presence of significant numbers of the trapped moths and their hairs on the adhesive surfaces of the relatively small traps.

The presence of *P. interpunctella* in the traps was unexpected as, though polyphagous, this species is not considered as a pest on tobacco. It may have been attracted into the facility from outside as this species is known to occur, often in large numbers, outside of storage and processing facilities (Campbell et al. 2004).

The 2012 experiment showed that the two commercial lures and traps tested are interchangeable, although use of the delta trap gave lower catches. Highest catches of *L. serricorne* were obtained with the Serrico trap and MoBe lure, although the catches were not significantly greater than the catches with the traps baited with their corresponding lures. However, in this experiment catches in the MoBe trap baited with the Serrico lure were significantly lower. The MoBe and Serrico traps are quite similar in design and the isomeric composition of the serricornin in the two standard lures was essentially the same. However, the release rate of serricornin from the Serrico lures was lower than that from the MoBe lures, although the Serrico lure also included a food attractant not present with the MoBe lure. It is possible that the food attractant compensated for the lower release of pheromone in the Serrico lures. It may also be that above a certain threshold release rate the captures of *L. serricorne* are not increased. There is no data on actual release rates of pheromone from the female beetles. Chuman et al. (1979) reported isolation of 1.5 mg of serricornin from 65,00

366 female *L. serricorne* (23 ng/female) and Chuman et al. (1985) obtained 3.1 mg from 260,000
367 beetles (12 ng/female). These amounts are orders of magnitude less than the hourly release
368 rates of serricornin from the lures measured here, e.g. 7.4 μg/h and 5.2 μg/h from the MoBe
369 and Serrico lures respectively at 27°C, even allowing for the fact that the synthetic
370 pheromone contained only approximately 35% of the attractive (4*S*,6*S*,7*S*)-serricornin.
371 Increase in temperature causes significant increase in release rate with release rates of
372 serricornin from both types of lure at least six times greater at 37°C than the rates at 22°C.
373 Although release rates were measured at three different temperatures here, the lures were
374 maintained at 27°C and 8 km/h windspeed in between measurements. The lure lifetimes at
375 the higher and lower temperatures can thus be calculated relative to the lifetime at 27°C. As
376 suggested above there may be a threshold release rate required for attraction of *L. serricorne*.
377 This is not known, but release of serricornin from the lures could still be detected at
378 approximately 0.3 μg/h after 30 d at 27°C. In the 2012 experiment, the lures remained
379 attractive for at least four weeks in the tobacco stores, and, although catches were not
380 compared with those with fresh lures, there was no obvious decline in catches, suggesting the
381 lures can be used for at least four weeks under field conditions.
382 Also in the 2012 experiment, use of purified serricornin in the MoBe lures (F) gave
383 numerically lower catches than those with the standard MoBe lure in the same traps (E),
384 although the difference was not significant. In fact, the purification step did not greatly
385 improve the proportion of the (4*S*,6*S*,7*S*)-serricornin although the proportion of the
386 (4*S*,6*S*,7*R*)-isomer was enhanced. The latter is known to reduce the attractiveness of the
387 former isomer (Mori et al. 1986).
388
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Conclusions

The most important practical conclusions from this work are that the MoBe and Serrico lures and traps are interchangeable for the capture of *L. serricorne*, although use of the traps with their corresponding lures is probably to be recommended. The lures last for at least four weeks in tobacco stores in Greece and data is provided to estimate their longevity if temperatures are very high or low. There have been differing reports about the attractiveness of anhydroserricornin to *L. serricorne* in the past, but the results here showed clearly that lures containing anhydroserricornin do not attract *L. serricorne* to traps under field conditions. Lures releasing anhydroserricornin at rates less than the rate of serricornin are attractive, but this attractiveness is decreased by higher amounts of anhydroserricornin. The stereoisomeric composition of the serricornin in the two commercial lures was similar with high proportions of the (4*S*,6*S*,7*S*)-isomer, albeit only approximately 35% given the racemic nature of the material. The proportion of the (4*S*,6*S*,7*R*)-isomer was low and this is known to reduce the attractiveness. Addition of the components of the sex pheromone of *E. elutella* to lures containing serricornin resulted in captures of male *E. elutella* moths without affecting catches of *L. serricorne* beetles. These lures thus provide the option of monitoring the two pests simultaneously with one trap.

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References

Agresti A (2007) An introduction to categorical data analysis. Wiley, Hoboken, NJ

Arbogast RT, Kendra PE, Chini SR (2003) *Lasioderma serricorne* (Coleoptera: Anobiidae): spatial relationship between trap catch and distance from an infested product. Fla Entomol 86:437-444

Ashworth JR (1993a) The biology of *Ephestia elutella.* J Stored Prod Res 29:199-205

Ashworth J. R. (1993b). The biology of *Lasioderma serricorne.* J Stored Prod Res 29:291-303

Buchelos CTh (1981) Coleoptera populations at flour mills and related areas. Ann Benaki Phytopath Inst 13:6-29

Buchelos CTh, Levinson AR (1985) Population dynamics of *Ephestia elutella* (Hbn.) in tobacco stores with and without insecticidal treatments: a survey by pheromone and unbaited traps. Z Angew Entomol 100:68-78

Buchelos CTh (1989). A contribution to *Lasioderma* spp. and other Coleoptera collected from thistles in Southern Greece. Entom Hellenica 7:7-12

Buchelos CTh, Levinson AR (1993) Efficacy of multisurface traps and Lasiotraps with or without pheromone addition, for monitoring and mass-trapping of *Lasioderma serricorne* F. (Col., Anobiidae) in insecticide-free tobacco stores. J Appl Entomol 116:440-448

Buchelos CTh, Trematerra P (1998) Monitoring of stored tobacco insect pests by means of pheromones: the case of *Ephestia elutella* (Hübner) and *Lasioderma serricorne* Fabricius in South Europe, Anz Schädlingskd Pfl 71:113-116

Campbell J.F. and Mullen M.A. (2004). Distribution and dispersal behavior of *Trogoderma variabile* and *Plodia interpunctella* outside a food processing plant. J Econ Entomol 97:1455-1464

Chuman T, Mochizuki K, Mori M, Kohno M, Ono M, Onishi I, Kato K (1982a) The pheromone activity of (±)-serricornins for male cigarette beetle (*Lasioderma serricorne* F.). Agric Biol Chem 46:593-595

Chuman T, Mochizuki K, Mori M, Kohno M, Kato K, Nomi H, Mori K (1982b) Behavioural and electroantennogram responses of male cigarette beetle (*Lasioderma serricorne* F.) to optically active serricornins. Agr Biol Chem 46:3109-3112

Chuman T, Mochizuki K, Mori M, Kohno M, Kato K, Noguchi M (1985) *Lasioderma* chemistry: sex pheromone of cigarette beetle (*Lasioderma serricorne* F.). J Chem Ecol 11:417-434

Howse PE, Stevens IDR, Jones OT (1998) *Insect Pheromones and their use in Pest Management*. Chapman and Hall, London, 369 pp

Lenth RV (2016) Least-Squares Means: The R Package lsmeans. J Stat Softw 69:1-33

Levinson HZ, Levinson AR, Francke W, Mackenroth W, Heeman V (1981) The pheromone activity of anhydroserricornin and serricornin for male cigarette beetles *(Lasioderma serricorne* F.). Naturwissenschafen 68:148-149

Levinson AR, Levinson HZ (1986a) Restrained pheromone responses of male tobacco beetles *(Lasioderma serricorne* F.) to 2*S*,3*S*-anhydroserricornin in presence of 4*S*,6*S*,7*R*-serricomin. J Appl Entomol 101:282-287

Levinson AR, Levinson HZ (1986b) Antagonised pheromone responses of male tobacco beetles *(Lasioderma serricorne* F.) to erythro-diasttereoisomers of 4*S*,6*S*,7*S* serricomin. Naturwissenschafen 73:3637

Levinson HZ, Levinson AR (1987) Pheromone biology of the tobacco beetle *(Lasioderma serricorne* F., Anobiidae) with notes on the pheromone antagonism between 4*S*,6*S*,7*S* serricomin and 4*S*,6*S*,7*R* serricomin. J Appl Entomol 103:217-240

Levinson HZ, Buchelos CTh (1981) Surveillance of stored moth species (Pyralidae, Gelechiidae) in a flour mill by adhesive traps with notes on the pheromone-mediated flight behaviour of male moths. Z Angew Entomol 92:233-251

Levinson AR, Buchelos CTh (1988) Population dynamics of *Lasioderma serricorne* F. (Col., Anobiidae) in tobacco stores with and without insecticidal treatments: a three year survey by pheromone and unbaited traps. J Appl Entomol 106:201-211

Mahroof RM, Phillips TW (2008a) Life history parameters of the cigarette beetle, *Lasioderma serricorne* (F.) as influenced by food resources. J Stored Prod Res 44:219-226

Mahroof RM, Phillips TW (2008b) Responses of stored product Anobiidae to pheromone lures and plant-derived volatiles. J Appl Entomol 132:161-167

Mahroof RM, Phillips TW (2011) Mating disruption of *Lasioderma serricorne* (Coleoptera: Anobiidae) in stored product habitats using the synthetic pheromone serricornin. J Appl Entomol 138:378-386

Mochizuki K, Chuman T, mori M, Kohno M, Kato K (1984) Acticity of stereoisomers of serricornin, sex pheromone of the cigarette beetle (*Lasioderma serricorne* F.). Agric Biol Chem 48:2833-2834

Mori K, Nomi H, Chuman T, Kohno M, Kato K, Noguchi M (1982) Synthesis and absolute stereochemistry of serricornin [(4*S*,6*S*,7*S*)-7-hydroxy-4,6-dimethyl-3-nonanone], the sex pheromone of the cigarette beetle. Tetrahedron 38:3705-3711

Mori K, Watanabe H (1985) A new synthesis of serricornin [(4*S*,6*S*,7*S*)-7-hydroxy-4,6-dimethyl-3-nonanone], the sex pheromone of the cigarette beetle. Tetrahedron 41:3423

Mori M, Mochizuki K, Kohno M, Chuman T., Ohnishi A., Watanabe H, Mori K (1986). Inhibitory action of (4*S*,6*S*,7*R*)-isomer to pheromonal activity of serricornin, (4*S*,6*S*,7*S*)-7-hydroxy-4,6-dimethyl-3-nonanone. J Chem Ecol 12:83-89

Papadopoulou SCh, Buchelos CTh (2002) Comparison of trapping efficacy for *Lasioderma serricorne* (F.) adults with electric, pheromone, food attractant and control adhesive traps. J Stored Prod Res 38:375-383

R Core Team (2014) R: a language and environment for statistical computing. R foundation for statistical computing, Vienna. http://www.R-project.org/

Raina AK, Wergin WP, Murphy CA, Erbe EF (2000) Structural organization of the sex pheromone gland in *Helicoverpa zea* in relation to pheromone production and release. Arthropod Structure and Development 29:343–353

Trematerra P, Athanassiou CG, Stejskal V, Sciarretta A, Kavallieratos NG, Palyvos NE (2011). Large scale mating disruption of *Ephestia* spp. and *Plodia interpunctella* in Czech Republic, Greece and Italy. J Appl Entomol 135:749-762.

Trematerra P, Athanassiou CG, Sciarretta A, Kavallieratos NG, Buchelos CTh (2013) Efficacy of the auto-confusion system for mating disruption of *Ephestia kuehniella* (Zeller) and *Plodia interpunctella* (Hubner). J Stored Prod Res 55:90-98.

502 **Table 1** Release rates from lures after one day, measured by trapping volatiles at 27°C

	Mean release rate (ug/h; *N* = 2)			
	Anhydro-serricornin	serricornin	ZETA	ZETOH
Serrico	4.5	5.6		
MoBe standard (A)	3.4	30.1	2.21	0.43
MoBe anhydroserricornin (B)	44.7		1.67	0.28
MoBe serricornin only (C)	3.1	22.8	0.00	0.00
MoBe added anhydroserricornin (D)	24.5	27.3	1.42	0.25

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505 **Table 2** Composition of serricornin from lures as determined by GC analyses on polar and
506 non-polar columns after acetylation (anhydroserricornin expressed as % of total serricornin)

	% Composition				
	Anhydro-	Serricornin			
	serricornin	4*S**,6*R**,7*S**	4*S**,6*S**,7*S**	4*S**,6*S**,7*R**	4*R**,6*S**,7*S**
Serrico	38	8	74	0	18
MoBe	1	3	67	1	28
Purified serricornin	0	0	72	7	20

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Table 3 Release rates of pheromones from MoBe and Serrico dispensers at 37°C, 27°C and 22°C measured on Day 1 and Day 30, for dispensers maintained at 27°C and 8 km/h windspeed, and release rate at Day 30 relative to that at Day 1 (%)

	Release rates (µg/hr)					
	MoBe				Serrico	
	Anhydro-serricornin	serricornin	ZETA	ZETOL	Anhydro-serricornin	serricornin
Day 1						
37°C	25.4	26.1	4.1	0.5	35.4	12.7
27°C	10.8	7.4	1.6	0.2	10.5	5.2
22°C	6.3	4.4	0.8	0.1	4.3	2.3
Day 30						
37°C	0.38	1.10	0.80	0.02	0.06	0.43
27°C	0.15	0.32	0.29	0.01	0.03	0.21
22°C	0.07	0.32	0.15	0.00	0.02	0.14
Day 30/Day 1 (%)						
37°C	1.5	4.2	19.7	3.6	0.2	3.4
27°C	1.3	4.4	18.5	3.0	0.0	4.1
22°C	1.1	7.4	18.6	0.0	0.4	6.0

516 **Fig. 1** Mean number (± 95% confidence intervals) of *L. serricorne* captured by each trap and
517 lure combination in 2011, adjusted for differences between weeks (least-squared means).
518 Different letters indicate significant differences between trap and lure combinations types
519 across the four weeks of the experiment (Tukey's test, $P<0.05$). Analysis performed on
520 capture data transformed to log (x+1). MoBeTrap+lure: MoBe trap with standard MoBe lure
521 containing serricornin, ZETA and ZETOL; MoBeTrap+anhydro: MoBe trap with MoBelure
522 containing anhydroserricornin, ZETA and ZETOL; MoBTrap+Serri: MoBe trap with MoBe
523 lure containing serricornin only; MoBeTrap+lure+anhydro: MoBe trap with MoBe lure with
524 anhydroserricornin added in equal amount to the serricornin; SerricoTrap+lure: Serrico trap
525 with standard Serrico lure of pheromone and food attractant.
526
527 **Fig. 2** Mean number (± 95% confidence intervals) of *E. elutella* captured by each trap and
528 lure combination in 2011, adjusted for differences between weeks (least-squared means).
529 Different letters indicate significant differences between trap and lure combinations types
530 across the four weeks of the experiment (Tukey's test, $P<0.05$). Analysis performed on
531 capture data transformed to log (x+1). MoBeTrap+lure: MoBe trap with standard MoBe lure
532 containing serricornin, ZETA and ZETOL; MoBeTrap+anhydro: MoBe trap with MoBelure
533 containing anhydroserricornin, ZETA and ZETOL; MoBTrap+Serri: MoBe trap with MoBe
534 lure containing serricornin only; MoBeTrap+lure+anhydro: MoBe trap with MoBe lure with
535 anhydroserricornin added in equal amount to the serricornin; SerricoTrap+lure: Serrico trap
536 with standard Serrico lure of pheromone and food attractant.
537
538 **Fig. 3** Mean number (± 95% confidence intervals) of *Lasioderma serricorne* captured by
539 each trap and lure combination in 2012, adjusted for differences between weeks (least-
540 squared means). †: lure with increased Serriconin purity. Different letters indicate significant

differences between trap and lure combinations types across the four weeks of the experiment (Tukey's test, $P<0.05$). Analysis performed on capture data transformed to log (x+1). SerricoTrap+lure: Serrico trap with standard Serrico lure; SerricoTrap+MoBelure: Serrico trap with standard MoBe lure; MoBeTrap+Serricolure: MoBe trap with Serrico lure; MoBeTrap+MoBelure: MoBe trap with MoBe lure; DeltaTrap+MoBelure: delta trap with MoBe lure; DeltaTrap+MoBelure†: delta trap with MoBe lure containing purified serricornin.

Fig. 4 Release rates of from standard MoBe lures of anhydroserricornin (a), serricornin (b) and ZETA (c), measured at 37°C, 27°C and 22°C. Lures were maintained in a windtunnel at 27°C and 8 km/h windspeed between measurements ($N = 2$; vertical bars indicate range)

Fig. 5 Release rates from Serrico lures of anhydroserricornin (a) and serricornin (b), measured at 37°C, 27°C and 22°C. Lures were maintained in a windtunnel at 27°C and 8 km/h windspeed between measurements ($N = 2$; vertical bars indicate range)

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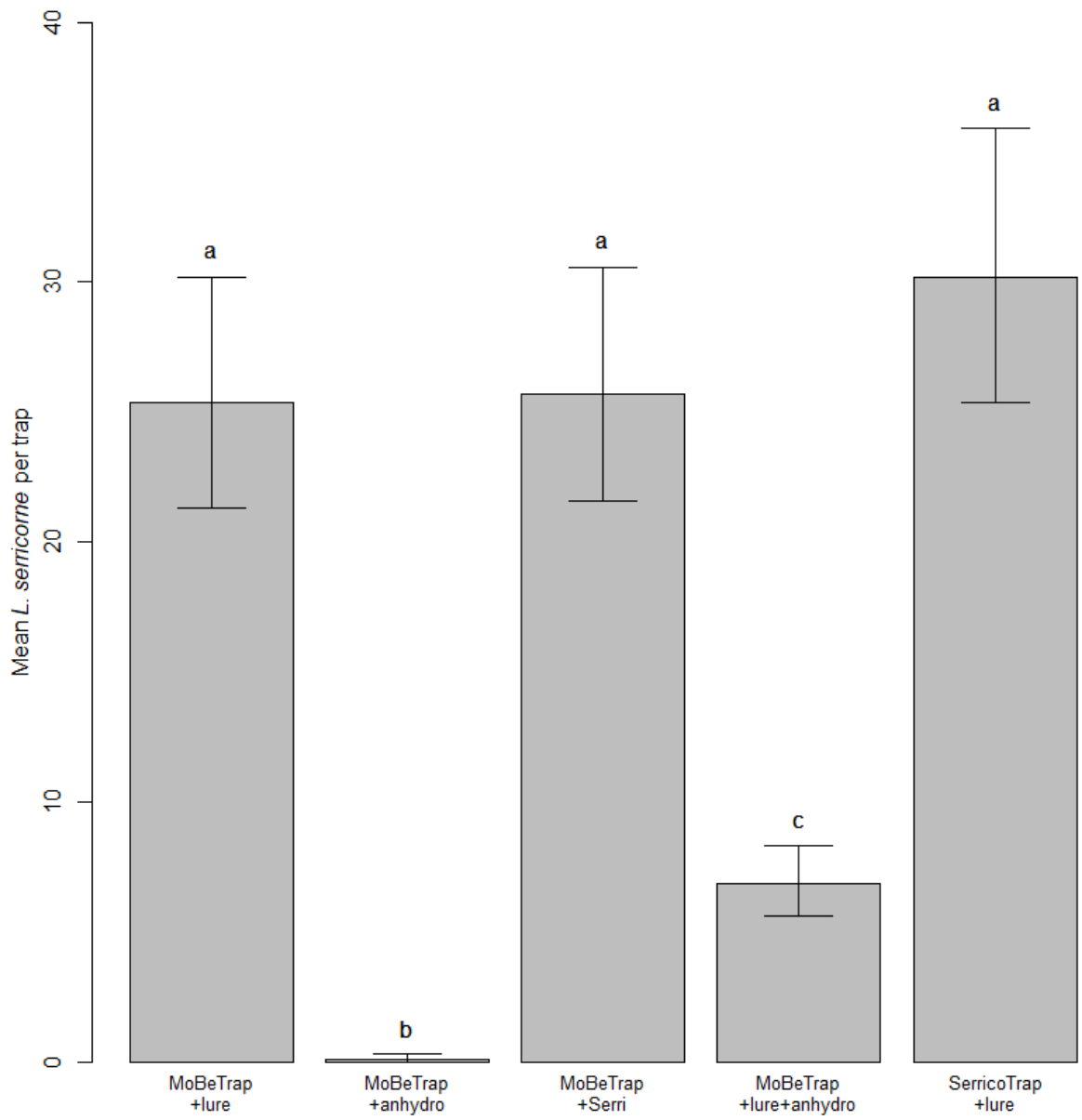

Figure 1

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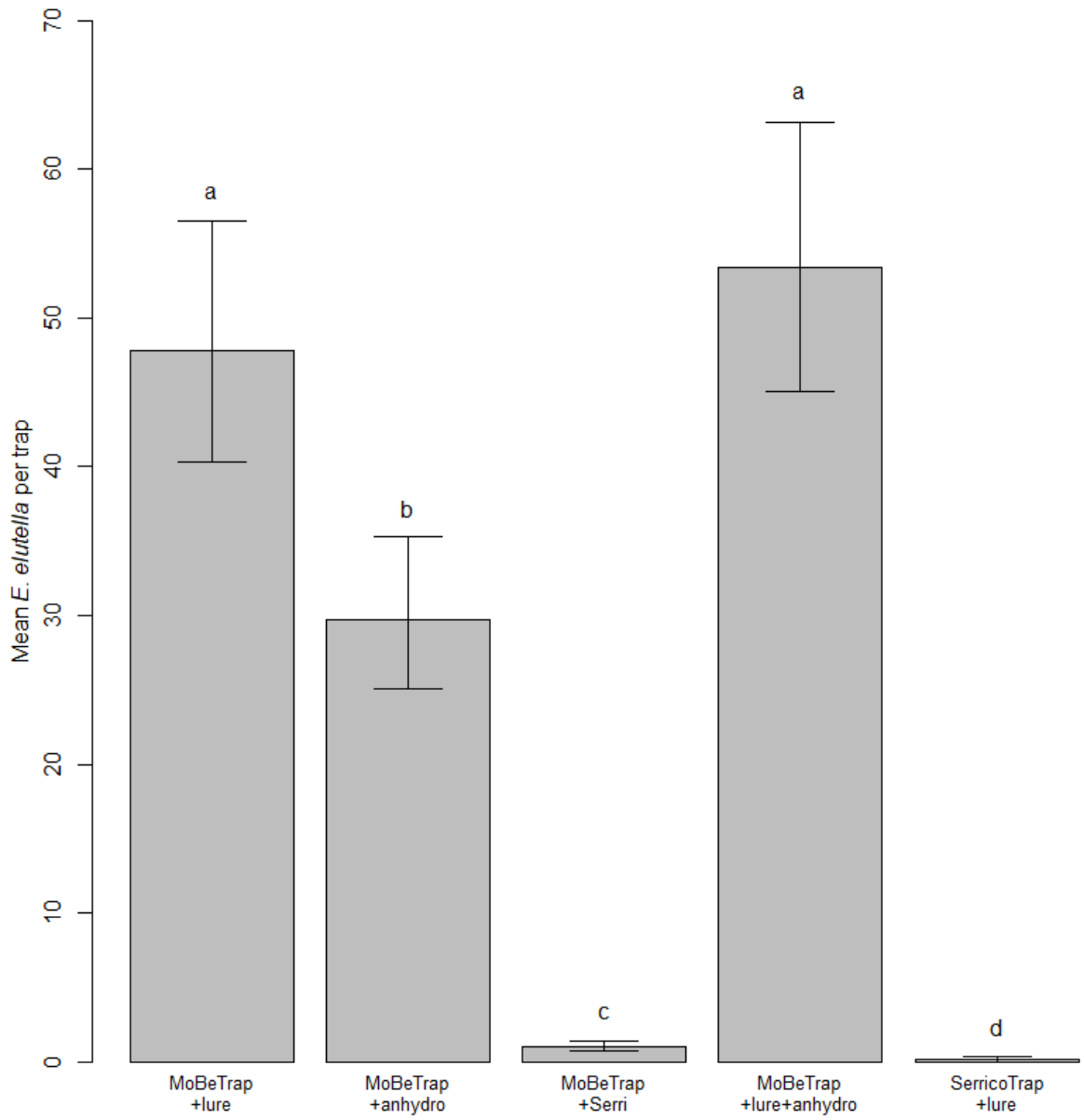

Figure 2

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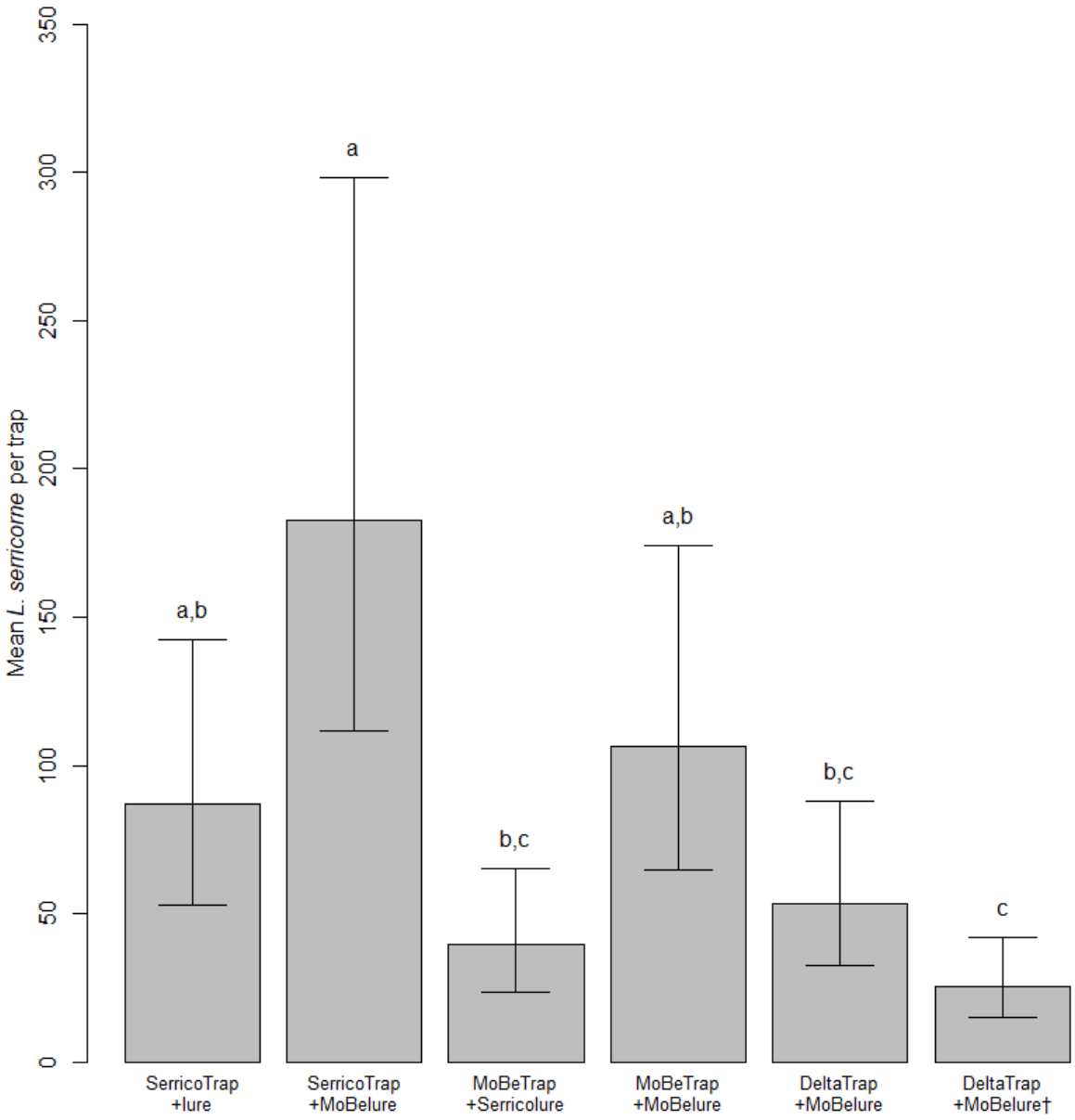

Figure 3

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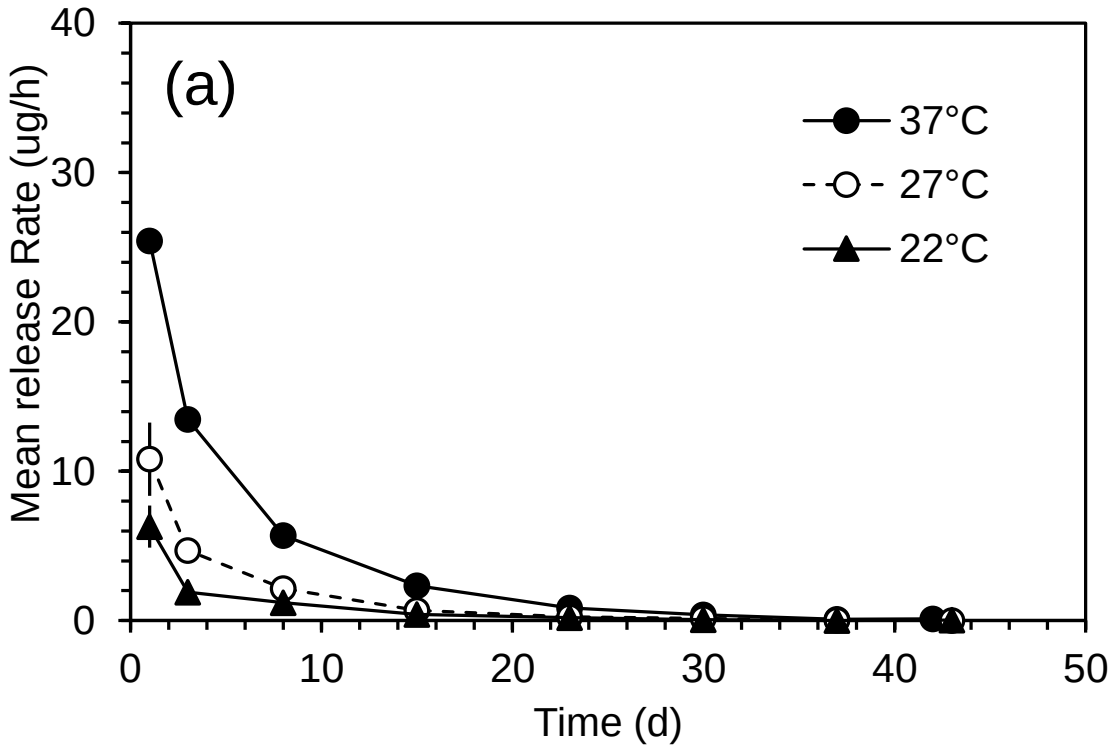

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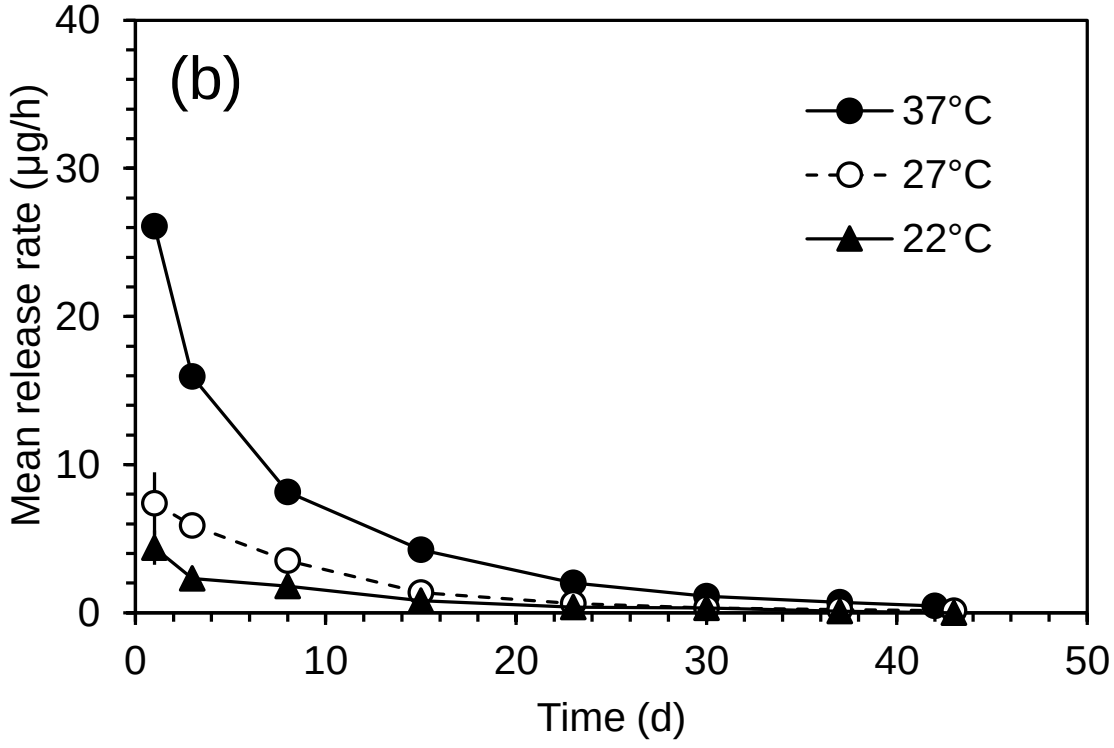

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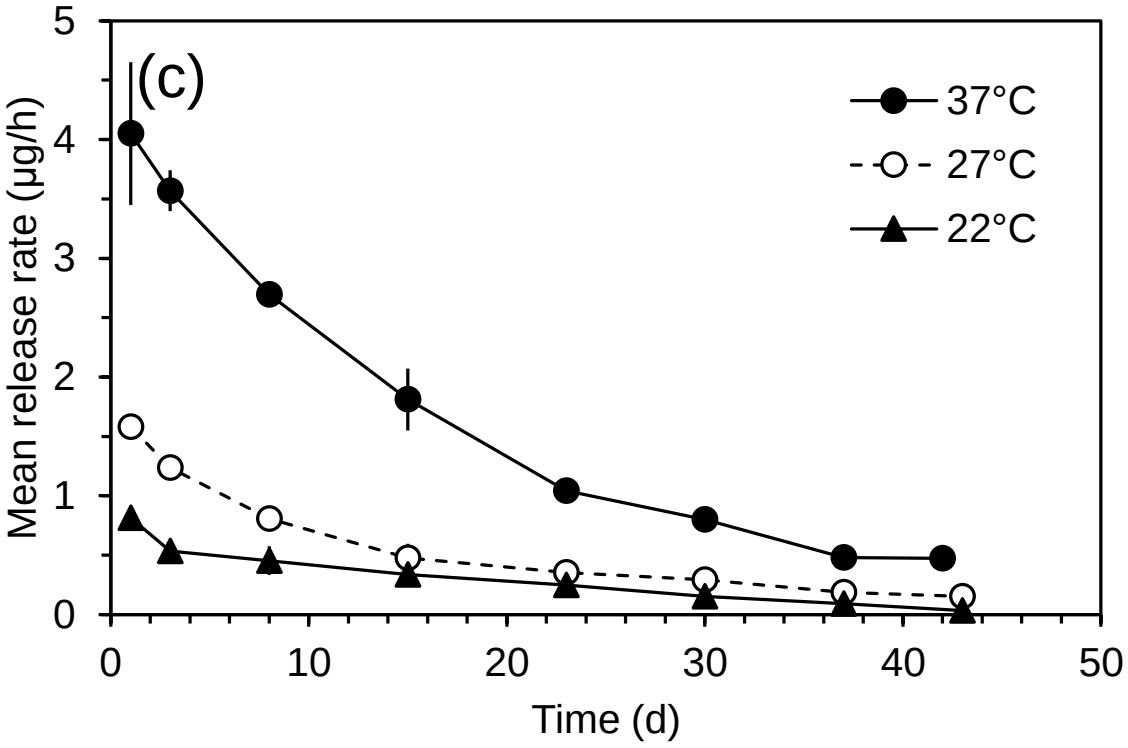

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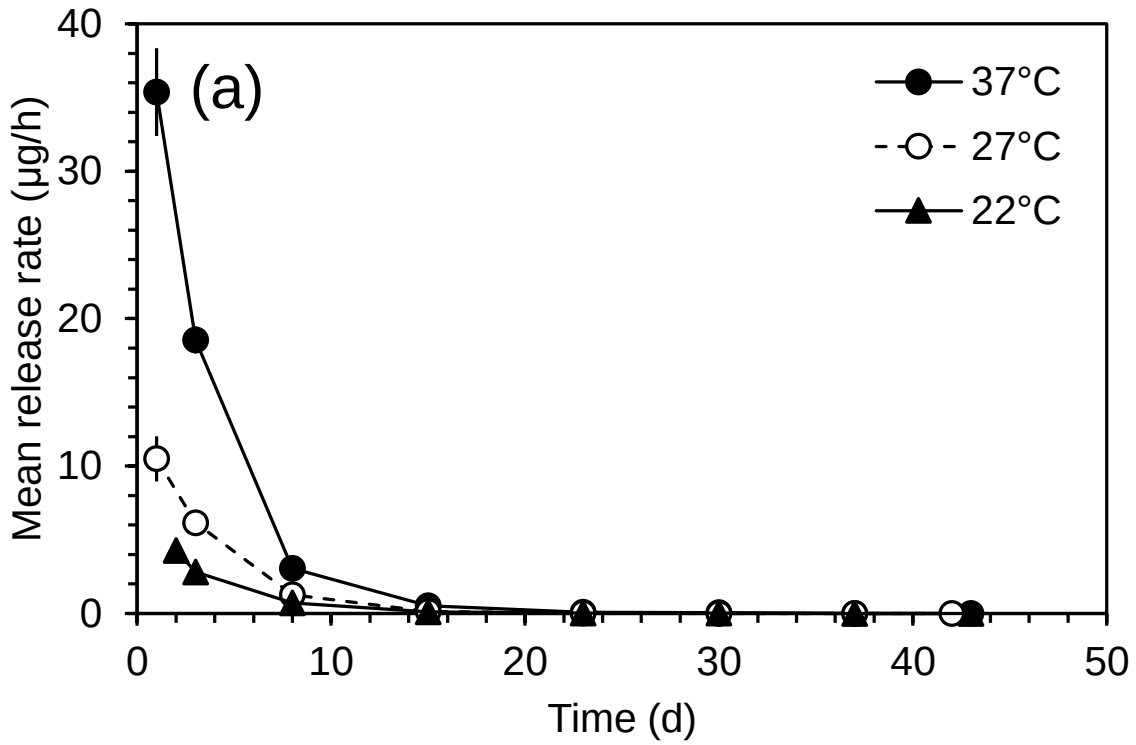
Figure 4



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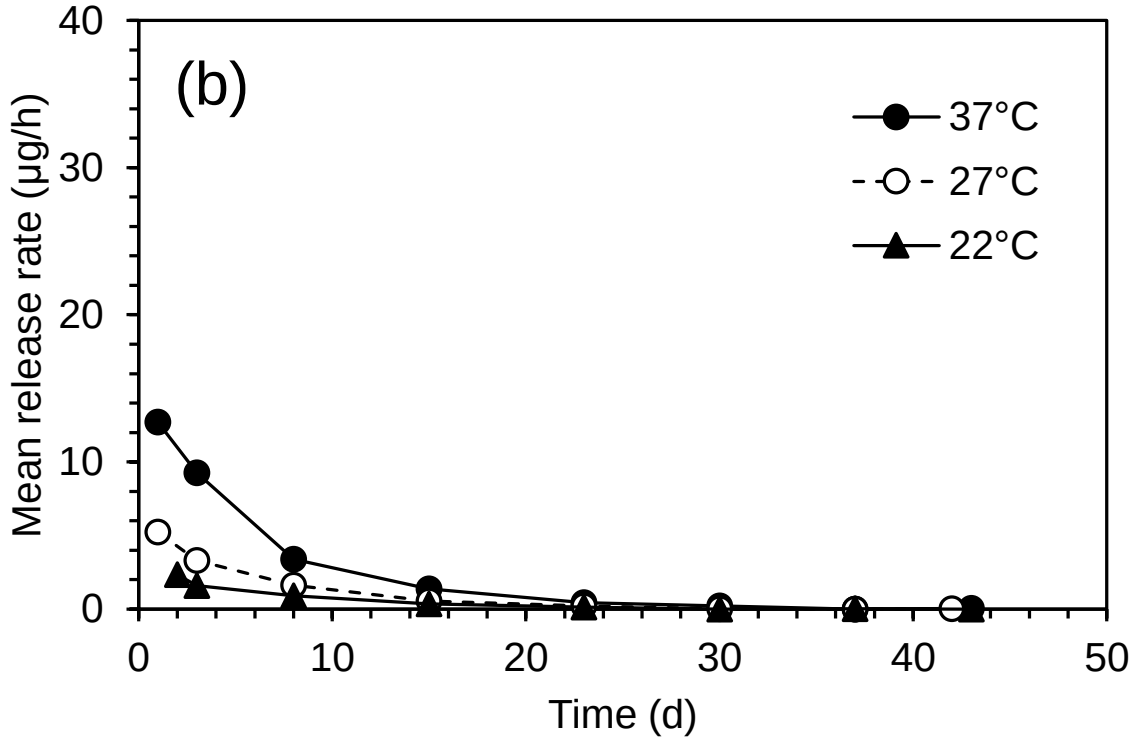

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Figure 5