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Control of *Leiodes cinnamomeus* (Coleoptera: Leiodidae) in Cultivated Black Truffle Orchards by Kairomone-Based Mass Trapping

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Abstract

The monoculture situation of truffle cultivation is favoring the appearance of pests that would not be economically important in naturally balanced forest ecosystems. The most prominent of them is the European truffle beetle *Leiodes cinnamomeus* (Panzer) (Coleoptera: Leiodidae), for which there are no effective control methods capable of reducing its populations. The potential of the mass trapping technique against this beetle, based on adapted pitfall traps and the semiochemical methyl disulfide as an attractant, is explored in the present work. Two trap densities (40 and 80 traps/ha) were tested in 2-yr field trials carried out in the region of Teruel (Spain) with black truffle cultivation tradition. Kairomone dispensers were placed in the field immediately before adult outbreak and remained active there throughout the season. The efficacy of each treatment was measured according to the reduction in beetle populations and the damaged truffles in the center of the treated areas. The results showed that both trapping densities reduced adult populations (mean 57% catch reduction), but 80 traps/ha were needed to significantly lower damage parameters (>40% reduction), percentage of attacked truffles and number of galleries/g truffle. The cost effectiveness of these treatments and possible improvements are discussed.

Key words: truffle beetle, *Tuber melanosporum*, kairomone, IPM

Black truffle is the fruiting of *Tuber melanosporum* Vittad. (Pezizales: Tuberaceae), a hypogeal fungus that establishes symbiotic relationships with different phanerogam species, mainly of the *Quercus* genus. Worldwide, there are ~180 truffle species (Bonito et al. 2010), although only 13 are of commercial interest (Bonito et al. 2009). Some truffle species have been cultivated, such as *Tuber aestivum* (Wulfen) Spreng, *Tuber borchii* Vittad., and the *Tuber indicum* complex (Chevalier and Frochot 1997, Zambonelli et al. 2000, Hu et al. 2005, Wang et al. 2006). Nevertheless, efforts to date have focused on *T. melanosporum* cultivation given its successful domestication in nurseries and plantations, and its high profitability.

Thanks to the improvement in farming techniques, the average European truffle production has increased in the last decades, which has gone from 15.9 tons during 2003–2012, to 47 tons from 2013 to 2017 (GETT 2016). It is estimated that 40,000 ha of plantations in Spain, France, and Italy annually generate approximately €50 million (Oliach et al. 2020).

All this leads to a clear monoculture situation that is favoring the appearance of pests which did not exist in naturally balanced forest ecosystems. The mycophagous beetle *Leiodes cinnamomeus* (Panzer) (Coleoptera: Leiodidae) is prominent among them, also known as the 'European truffle beetle' (Arzone 1971, Pacioni 1989, Bratek et al. 1992, Callot 1999, Barriuso et al. 2012, Martín-Santafé et al. 2014). It bores multiple galleries in *T. melanosporum* fruiting bodies, which can be produced by both larvae (to feed) and adults (to take shelter). According to Barriuso et al (2012), the main consequences of infestation are depreciation of the product, economic losses in weight and quality, rot acceleration and organoleptic damage. Only distributed in Europe, *L. cinnamomeus* is univoltine and its populations comprise several adult cohorts that successively emerge after the summer diapause. In the region of Teruel (Spain), adults are observed from mid-September to mid-May, eggs from October to January and mycophagous larvae (L1 to L3) from November to March. Hence, long periods elapse during which all insect stages simultaneously feed on truffles and cause severe damage.

In the present-day, no effective control methods are capable of reducing this insect's populations to acceptable levels, and the truffle sector remains in a defenseless situation. Good cultural practices recommend performing frequent recollections to minimize the number of uncollected truffles that act as shelters for beetles, or collecting truffles with the surrounding soil when grown in wells to remove any accompanying eggs, larvae or adults (Martín-Santafé et al. 2014). Traps are also employed to monitor *L. cinnamomeus* populations using truffle kairomones as attractants (Pérez-Andueza 2015). They are based mainly on dimethyl sulfide, a compound detected in almost all hypogeous genera of fungi, which clearly attracts mycetophilus insects (Pacioni et al. 1990, Pacioni et al. 1991). No known pheromones have been described in the Leiodidae family, but studies conducted on *L. cinnamomeus* behavior suggest using food attractants to manage it (Pacioni et al. 1991, EFSA 2020). In particular, Hochberg et al. (2003) demonstrated that *L. cinnamomeus* was not attracted to mature truffles, but by those infested in soil. More recent studies have found that other compounds released by truffles, such as 1-octen-3-ol and 4-methyl-3-octanol, elicited consistently a marked electroantennographic response in *L. cinnamomeus* (Ortiz et al. 2014). However, none has been demonstrated to be effective attractants alone or combined with dimethyl sulfide in the field.

Although efficacy in adult captures using adapted pitfall traps baited with dimethyl sulfide has already been demonstrated (Pacioni et al. 1991, Ruiz-Babarin et al. 2010, Pérez-Andueza 2015), the potential of the mass trapping technique has not yet been reported. The objective of this work was to assess the efficacy and efficiency of mass trapping to control *L. cinnamomeus* at two densities, 40 and 80 traps/ha. For this purpose, we conducted mass trapping experiments over two seasons in commercial black truffle orchards in Teruel (Spain).

Materials and Methods

Traps and Kairomone

The employed traps were white 600-ml polypropylene pots (Kartell, Fisher Scientific SL, Madrid, Spain) with screw caps (Fig. 1A) (Ruiz-Babarin et al. 2010). Twelve 10-mm-diameter holes were made in the upper pot part to allow beetles to enter traps. A small piece of polystyrene foam placed at the bottom of the pot held a kairomone dispenser, which consisted of a 20-ml polypropylene screw-cap vial (Fig. 1B) (Fisher Scientific SL, Madrid, Spain) filled with 10 ml of >99% dimethyl sulfide (DMS) (Merck, Darmstadt, Germany) (Fig. 1B). Traps were buried up to the level of the top of the trap, with holes 1–2 cm below ground to facilitate the entrance of digging beetles.

Trial Fields and Mating Disruption Treatments

Four trial replicates were conducted in three holm oak (*Quercus ilex* L, *Quercus faginea* Lam or *Quercus coccifera* L. (Fagales: Fagaceae)) orchards, located in the municipality of Sarrión (Teruel, Spain) (Table 1). They were all irrigated by micro-aspersion to help truffle growing. Each trial contained a randomized block design with three 1-ha plots: two of them received the mass trapping treatment with different trap densities, while the third was left untreated (Table 1 and Fig. 2). The tested densities were 40 and 80 traps/ha, which were achieved by placing traps in a grid pattern of 16 × 16 and 11 × 11 m, respectively.

In the first year of field trials (season 2016–2017), traps were placed on 18 October 2016 and remained in the field until April 2017. In the second year, traps were placed on 24 September 2017 and were left in the field until 30 April 2018.

Efficacy Assessments

The efficacy of the mass trapping treatments compared with the untreated areas, was evaluated according to two parameters: number of captured *L. cinnamomeus* adults and truffle damage. Both adult population and truffle damage were measured in a 18 × 24 m^2 area (assessment area) delimited in the geographical center of each plot to avoid edge effects.

Each assessment area contained four monitoring traps (identical to the mass trapping ones) to delimit the 18 x 24-m rectangle in order to follow the adult population. Traps were revised weekly and captures were identified by a binocular microscope to record male and female *L. cinnamomeus* captures separately. The kairomone vials did not need replacing throughout each season because the release rate studies showed that the emission of DMS was constant during the entire trial. The DMS release rate was studied by the gravimetric method by periodically weighing 10 vials from randomly selected monitoring traps. Weight differences over each period were referred to as the amount of emitted DMS. To obtain the mean emission level, the recorded weights were fitted to linear regression models, $y = a$

Fig. 1. (A) Trap and (B) kairomone dispenser employed in the field trials.

Table 1. Characteristics of the trial fields

Orchard	Coordinates	Plantation age (years)	Trial	Area (ha)	Plot	Trap density (traps/ha)	Planting pattern (m)
1	40°06′04.8″ N 0°49′40.6″ W	25	1	3.1	1	40	8 × 4
					2	80	
					3	0	
			2	3.2	4	40	
					5	80	
					6	0	
2	40°06′05.6″ N 0°49′49.6″ W	15	3	7.46	7	80	6 × 6
					8	40	
					9	0	
3	40°06′00.8″ N 0°50′04.8″ W	17	4	4.72	10	40	6 × 6
					11	80	
					12	0	

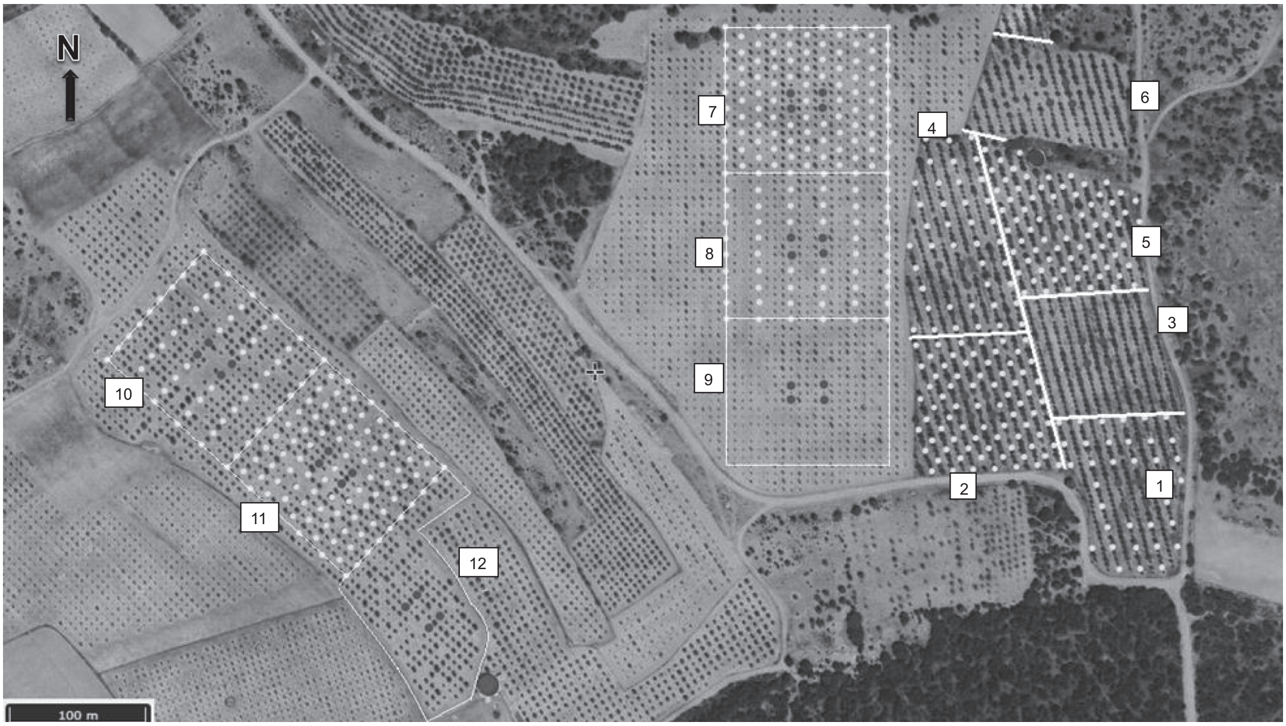

Fig. 2. Distribution of plots in orchards 1, 2, and 3. White points indicate a trap for mass trapping and black points indicate the monitoring traps in the center of each plot. The numbers indicate the plot number according to Table 1.

+ *bx*, where *y* is the dispenser weight and *x* is the corresponding aging days.

Damage was assessed by visually inspecting all the truffles harvested only inside the assessment area, which were located with the help of a trained dog. When the dog pointed the location, truffles were gently removed with a small shovel. All the collected truffles in each assessment were closed in a cloth bag and transported to the laboratory, at less than 5 km from the orchards for visual inspection. Truffles were weighed and the number of galleries counted in less than 6 h after harvesting to prevent their loss of quality. Harvesting and inspection were conducted in less than 12 h to allow producers to sell the truffles as soon as possible. Recollections were performed approximately on a weekly basis from November to April, except some weeks in January and February when soil was frozen and it was impossible to look for truffles. The percentage of attacked truffles and the number of galleries/g truffle were the infestation features recorded for each plot. A truffle was considered damaged when at least one gallery was present (Fig. 3).

Statistical Analysis

The GLM Repeated Measures procedure was employed to compare the number of individuals trapped in the center of the different plots. The number of individuals captured per trap and day (males, females, and total beetles separately) were employed as the dependent variable, time (study period week) was considered as within-subjects factor and density of traps (treatment) and location (trial) as between-subjects factors. Interactions between factors have been included in the model and Tukey HSD post hoc tests were applied to evaluate the differences among specific means. When the sphericity assumption was not met, the Greenhouse–Geisser correction results were used. Trapping data were log-transformed prior to apply the statistical analyses to fulfill the homoscedasticity and normality requirements. These analyses were carried out using SPSS statistical software (SPSS for Windows, Version 16.0, SPSS Inc., Chicago, IL).

Fig. 3. Heavy infested truffle showing multiple galleries bored by *Leiodes cinnamomeus*.

Truffle infestation data were grouped into three periods (November-December, January-February and March-April) and the differences observed between treatments were assessed by generalized linear mixed model (GLMM) techniques, using the *glmer* function from the *lme4* package in R version 4.0.3 (R Core Team 2020). The *binomial* error distribution was assumed for the data of the attacked truffles. In the case of the number of galleries found per truffle, the *poisson* distribution was applied and an offset vector was introduced to relate these numbers with the weight of each inspected truffle. In all cases, the fixed factor treatment and the random factor location were included in the models. The significance of the treatment effects was assessed by removing them from the model and comparing models with likelihood ratio tests. Then, the *glht* function in the *multcomp* package was used to perform Tukey HSD tests for post hoc pairwise comparisons.

Results

Release Profile of the Kairomone Dispensers

Loss of weight of polypropylene vials fit the linear model depicted in Fig. 4, which means that DMS emission remained practically constant during the study period. Hence, the mean release rate was given by the slope of the fitted model (R^2 = 0.978) and equaled 58.7 mg/d.

Season 2016–2017

The capture dynamics of the male and female adults were similar (Fig. 5), with no delay between male and female populations. The most abundant emergence peak took place in the first week of November and a second smaller peak was detected at the end of December, which was more prominent in the control plots. The statistical analyses indicated that the considered categorical factors, namely treatment, time (study period week) and location, had significant effects on male, female and total captures (Table 2). The factor time was significant, as was the factor location, which highlighted the different pest pressure among orchards (Table 2). The

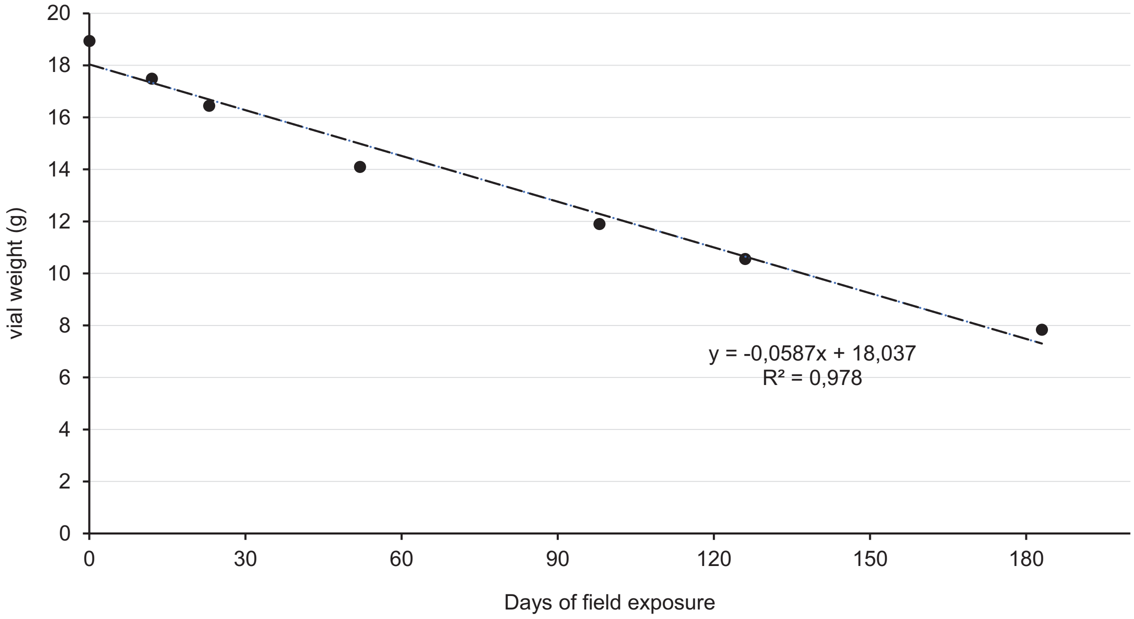

Fig. 4. Release profile of the polypropylene kairomone vials studied by the gravimetric method. The slope of the fitted linear model means that dimethyl sulfide was emitted at a mean constant rate of 58.7 mg/day.

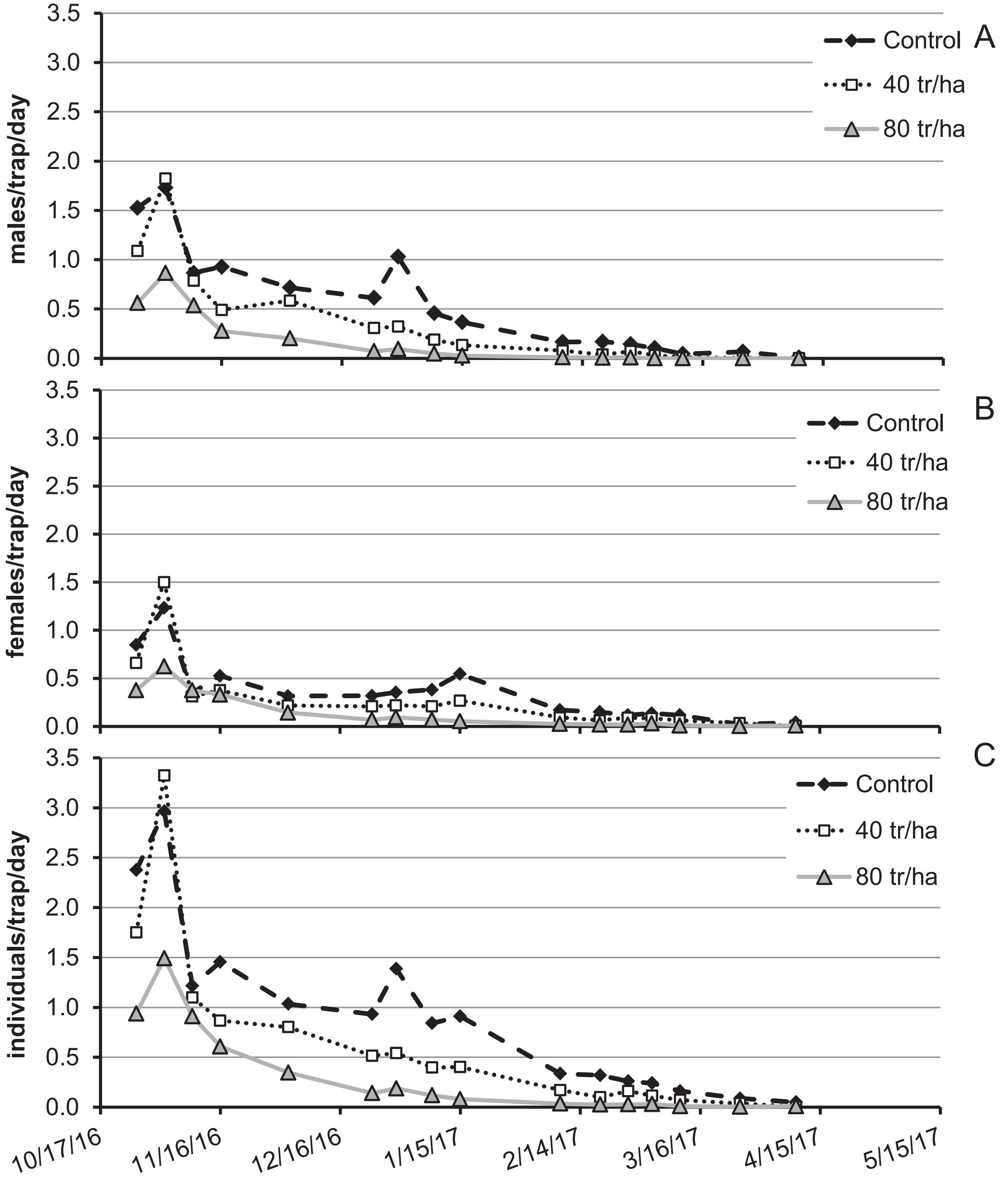

Fig. 5. Mean male (A), female (B), and total (C) *Leiodes cinnamomeus* captures throughout the season 2016–2017 in the assessment areas of the untreated (control) and the mass trapping plots that received 40 or 80 traps/ha.

effect of the treatment applied to each plot was also significant, which indicates that the installed trap density had a significant effect on the *L. cinnamomeus* male (F = 37.92; df = 2,36; $P < 0.001$), female (F = 19.50; df = 2,36; $P < 0.001$), and total (F = 137.40; df = 2,36; $P < 0.001$) captures. The Tukey post hoc tests indicated that the male, female and total captures in the untreated control plots were significantly higher than in the plots treated with 40 traps/ha ($P < 0.001$ in all cases, except for female captures in the 'control vs 40 traps/ha' with $P = 0.047$) which, in turn, were significantly higher than those recorded in the plots treated with 80 traps/ha ($P < 0.001$ in all comparisons except for female captures in the '40 traps/ha vs 80 traps/ha' with $P = 0.002$). When considering the

Table 2. Mean annual captures per trap and day (±SE) of *Leiodes cinnamomeus* in the two seasons and statistical results

		Captures (mean ± SE)[a]			Statistics[b]	
Season	Sex	Control	40 traps/ha	80 traps/ha	Treatment	Trial
2016–2017	M	0.56 ± 0.14 **a**	0.37 ± 0.13 **b**	0.17 ± 0.07 **c**	$F_{2,36} = 37.92$; $P < 0.001$	$F_{3,36} = 21.38$; $P < 0.001$
	F	0.35 ± 0.08 **a**	0.27 ± 0.09 **b***	0.14 ± 0.05 **c**	$F_{2,36} = 19.51$; $P < 0.001$	$F_{3,36} = 22.86$; $P < 0.001$
	T	0.91 ± 0.22 **a**	0.65 ± 0.22 **b**	0.31 ± 0.11 **c**	$F_{2,36} = 37.40$; $P < 0.001$	$F_{3,36} = 26.85$; $P < 0.001$
2017–2018	M	1.28 ± 0.30 **a**	0.47 ± 0.13 **b**	0.18 ± 0.06 **c**	$F_{2,36} = 115.71$; $P < 0.001$	$F_{3,36} = 20.26$; $P < 0.001$
	F	1.16 ± 0.21 **a**	0.46 ± 0.10 **b**	0.19 ± 0.06 **c**	$\mathrm{F}_{2,36} = 113.84$; $P < 0.001$	$\mathrm{F}_{3,36} = 19.56$; $P < 0.001$
	T	2.44 ± 0.49 **a**	0.93 ± 0.23 **b**	0.36 ± 0.11 **c**	$F_{2,36} = 136.82$; $P < 0.001$	$F_{3,36} = 24.49$; $P < 0.001$

[a] Mean (±SE) captures of males (M), females (F) and total (T) obtained in the plots treated with the different treatments. For each sex and season, means noted with different letters are significantly different in the *post-hoc* tests at $P \le 0.001$, except those noted with (*). The mean female captures in the control and 40 traps/ha plots (season 2016–2017) were significantly different at $P = 0.047$.

[b] Statistical significance of the between-subjects factors considered for the GLM Repeated Measures procedure: *F*-test, followed by post-hoc pairwise comparisons (Tukey HSD tests).

total mean captures during the season, the captures from the center of the plots treated with 40 and 80 traps/ha were 16 and 64% lower, respectively, than the control plots.

The truffle damage assessments revealed that the mass trapping strategy with 40 traps/ha was unable to generally reduce infestation in relation to the control plots throughout the season when considering both the percentage of damaged truffles and number of galleries/g truffle (Fig. 6A and Table 3). Conversely, the percentage of damaged truffles was significantly lower, especially 2 mo after treatments began (January-February 2017), in the plots that received 80 traps/ha compared with the control plots ($P < 0.001$), and damage reductions went from 20 to 70%. Moreover, those truffles collected in the plots treated with 80 traps/ha also had significantly fewer galleries/g truffle in all the assessments (Fig. 6A and Table 3). In global, the reduction percentages of attacked truffles in the center of the plots treated with 40 and 80 traps/ha were 3 and 40.4%, respectively, compared with the control plots. Likewise, these attacked truffles had 15.4 and 59.6% fewer galleries/g truffle from the center of the plots treated with 40 and 80 traps/ha, respectively.

Season 2017–2018

The dynamics of male and female adults were also synchronized, with no delay between male and female populations (Fig. 7). In general, the truffle beetle population was larger during this second season of field trials but, similarly to what occurred during the previous season, adult peak emergence took place at the end of October and the beginning of December. The effect of the treatment applied to plots was also significant on male ($F = 115.71$; df = 2,36; $P < 0.001$), female ($F = 113.84$; df = 2,36; $P < 0.001$), and total ($F = 136.82$; df = 2,36; $P < 0.001$) beetle captures (Table 2). Both mass trapping densities reduced adult populations from the center of their plots compared with the untreated ones ($P < 0.001$ in the post hoc tests). However, the 80 traps/ha treatment obtained better results than the 40 traps/ha density as the total adult captures lowered by 85 and 62%, respectively. The GLM analyses also indicated the significant effect of the factor time (Table 2). Likewise, orchard location also affected captures because the different locations had distinct pest pressure levels (Table 2).

Damage level was generally higher during this season, and coincided with higher beetle population levels and lower truffle yields (Fig. 6B). The damage assessment results for this second year highlighted that the mass trapping strategy with 40 traps/ha was generally unable to reduce infestation versus the control (Fig. 6B and Table 3), except for a significant reduction at the end of the season (March-April assessment) in both the percentage of attacked truffles ($P < 0.001$) and the number of galleries/g truffle ($P = 0.034$) (Table 3). The treatment with 80 traps/ha significantly lowered the percentage of attacked truffles from the beginning of treatments until the end of the study (38–91% attack reduction compared with the control). Moreover, those damaged truffles that were collected in the plots treated with 80 traps/ha also displayed a lower degree of attack, with significantly fewer galleries/g truffle throughout the season (Table 3 and Fig. 6B). In global, the reduction percentages of attacked truffles in the center of the plots treated with 40 and 80 traps/ha were 15.5 and 47.7%, respectively, *versus* the control plots. The attacked truffles collected from the center of the plots treated with 80 traps/ha had 47.1% fewer galleries/g truffle; whereas the treatment with 40 traps/ha had no statistically significant global effect on this parameter.

Discussion

Mass trapping of *L. cinnamomeus* using DMS as an attractant proved effective in reducing adult populations and the damage inflicted to truffles from the center of treated areas. However, with this attractant and the described trap, more than 40 traps/ha were needed to obtain significant damage reduction percentages. In fact the strategy with 80 traps/ha lowered the percentage of damaged truffles to 40 and 47% for the first and second season, respectively, and damage level (galleries/g truffle) was also clearly lower, by between 47 and 60%, respectively, during both seasons compared with the untreated areas. When considering the current mean prices of the first-quality black truffle in Spain (around 400 €/kg) and an average yield of around 50 kg truffle/ha (Lefevre 2012), the damage reduction achieved by installing 80 traps/ha leads to a reduction in economic losses of about 900 €/ha, without considering the superior quality of the collected truffles as a result of less infestation. Although this trap and the attractant are quite cheap, which can be manufactured ca. at 5 €/device (trap + attractant), the cost of preparing and burying traps can increase the final cost to over 5.5 €/device. According to a basic calculation, the amount earned by damage reduction (€900) certainly offsets the cost of the devices and their installation (440 €/ha), and this profit will increase with the higher truffle quality obtained in the mass trapping-treated area. However, when using 40 traps/ha, although the cost of traps, attractants and their installation is cut almost by half (220 €/ha), an average damage reduction of 9% (3% during the first and 15% during the second season) will only

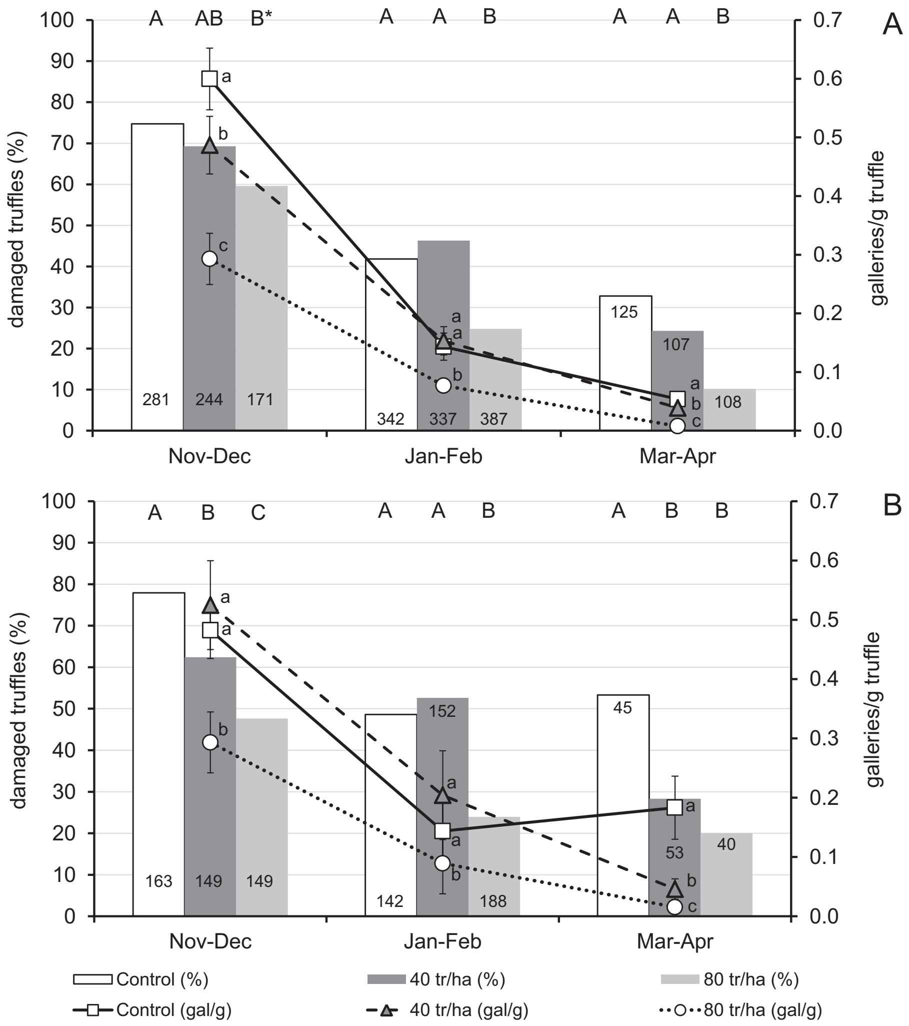

Fig. 6. Results of the truffle damage inspections performed during (A) 2016–2017 and (B) 2017–2018 seasons in the assessment areas. Damage parameters recorded: (%) percentage of attacked truffles (truffles with one or more galleries) in bars and (gal/g) number of galleries per gram of truffle (mean ± SE) as data points. The values showed inside the bars (*n*) are the total number of inspected truffles in the assessment area of each treatment in each period. Capital letters on the top of the graph show the statistical significance of the differences observed in the percentages of attacked truffles (bars) (results according to Table 3). Low case letters next to data points show the statistical significance of the differences observed in the number of galleries per gram of truffle (results according to Table 3).

produce a damage reduction valued at 180 €/ha. In the plantations in full production, where truffle yields can reach 200 €/ha/week (Letizi et al. 2001), the mass trapping cost can be easily supported. However, we must bear in mind that these economic calculations are based on the data obtained in plots with medium pest pressure levels and cannot be extrapolated to plots with very high or very low pest levels.

Moreover, to calculate the final cost, the cumulative effect of mass trapping on pest populations must be considered. Mass trapping applied during successive years can have a cumulative effect as demonstrated for many pests (El-Sayed et al. 2006, Navarro-Llopis and Vacas 2014). Regarding Coleoptera, this effect has been observed for bark beetles, such as *Ips duplicatus* (Sahlberg), which indicated a continuous decrease in dead trees after 3 yr of mass trapping applications (Schlyter et al. 2001). Nevertheless, a similar study was carried out with other bark beetles, such as *Ips typographus* (L.) and *Trypodendron lineatum* (Olivier) over a 5-yr period and demonstrated that population density was not substantially influenced by massive and continuous trapping with pheromone-baited traps (Dimitri et al. 1992). In the present 2-yr field trial, more damage reduction was achieved during the second year compared with the first year, from 40 to 47% with 80 traps/ha and from 3 to 15% with only 40 traps/ha. Therefore, we suspect that this accumulative effect exists, but the present study did not allow us to conclude reduced pest populations for successive years.

Another point that should be taken into account is the effect of the treated area size. A species' invasive capacity depends on its mobility and, therefore, the minimum treated area required to achieve effective treatments should be studied depending on the insect's biology. However, information about the dispersal capacity of the studied insect is too scarce to establish a minimum treated area size. In the present study, the basic plot size was established as 1 ha. Therefore, if *L. cinnamomeus* is able to move easily beyond 40 m, the efficacy of this control method might be compromised. Although this coleopteran has an undeniable flight capacity as observed during the field trials, no data about dispersal capacity are available in the literature. However, other species from the Leiodidae family, such as *Drimeotus viehmanni* (Ienistea), have been reported to move

Table 3. Statistical results of the truffle damage assessments performed in the two seasons

Parameter[a]	Period	Post hoc results[b]	Statistics[c]
Galleries/g truffle 2016–2017	Nov.–Dec.	0–40:**; 0–80: ***; 40–80: ***	$\chi^2 = 193.7$; df = 2; $P < 0.001$
	Jan.–Feb.	0–40: ns; 0–80: ***; 40–80: ***	$\chi^2 = 230.4$; df = 2; $P < 0.001$
	Mar.–Apr.	0–40: **; 0–80: ***; 40–80: *	$\chi^2 = 35.8$; df = 2; $P < 0.001$
% attacked truffles 2016–2017	Nov.–Dec.	0–40: ns; 0–80: (0.08); 40–80: ns	$\chi^2 = 4.7$; df = 2; $P < 0.001$
	Jan.–Feb.	0–40: ns; 0–80: ***; 40–80: ***	$\chi^2 = 45.5$; df = 2; $P < 0.001$
	Mar.–Apr.	0–40: ns; 0–80: ***; 40–80: *	$\chi^2 = 17.1$; df = 2; $P < 0.001$
Galleries/g truffle 2017–2018	Nov.–Dec.	0–40: ns; 0–80: ***; 40–80: ***	$\chi^2 = 207.0$; df = 2; $P < 0.001$
	Jan.–Feb.	0–40: ns; 0–80: ***; 40–80: ***	$\chi^2 = 69.1$; df = 2; $P < 0.001$
	Mar.–Apr.	0–40: ***; 0–80: ***; 40–80: *	$\chi^2 = 58.3$; df = 2; $P < 0.001$
% attacked truffles 2017–2018	Nov.–Dec.	0–40: *; 0–80: ***; 40–80: *	$\chi^2 = 31.5$; df = 2; $P < 0.001$
	Jan.–Feb.	0–40: ns; 0–80: ***; 40–80: ***	$\chi^2 = 37.1$; df = 2; $P < 0.001$
	Mar.–Apr.	0–40: *; 0–80: **; 40–80: ns	$\chi^2 = 11.4$; df = 2; $P = 0.003$

[a] Parameters recorded to evaluate the damage inflicted to truffles: percentage of attacked truffles (truffles with one or more galleries) and number of galleries per gram of truffle.

[b] Significance of the post hoc tests (Tukey HSD) performed for the fixed factor treatment: ***$P \le 0.001$, **$P \le 0.01$, *$P \le 0.05$, (ns) not significant at $P > 0.05$, or specific value.

[c] Statistical significance of the fixed factor treatment by likelihood test ratio.

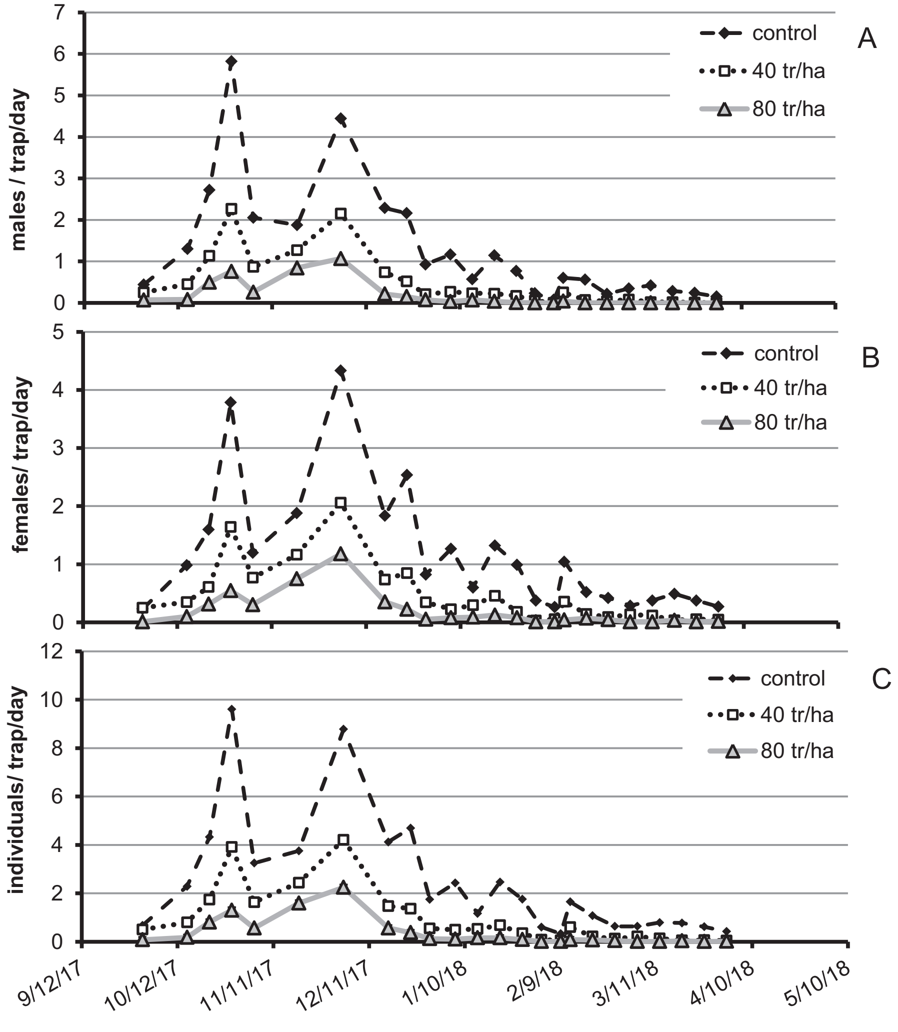

Fig. 7. Mean male (A), female (B) and total (C) *Leiodes cinnamomeus* captures throughout the season 2017–2018 in the assessment areas of the untreated (control) and the mass trapping plots that received 40 or 80 traps/ha.

distances longer than 200 m (Fejér and Moldovan 2013). For other *Leiodes* species, dispersal ability is presumed to lead to the rapid recolonization of younger forests as food sources become available (Chandler and Peck 1992). Moreover, Leiodids have been described as active fliers that search for their spatially and temporally limited food sources of slime molds, fungi, and carrion (Lawrence and Newton 1980). In spite of these reported spread capacities and the relatively small plot sizes of our field trials, our results indicate a significant truffle damage reduction, so applying mass trapping to larger areas could lead to better treatment efficacies. Given that the cultivation of truffle holm oaks is concentrated in specific zones and these zones cover thousands of hectares, the combined treatment of these productive areas could provide better results.

Truffle attack took place mainly during the first period of the season (November to December). This could be due to the fact that *L. cinnamomeus* is more attracted by the volatiles emitted by immature truffles (Pacioni et al. 1991) and they can easily find truffles to infest. Furthermore, it could also happen because adults emerge mainly during this period. The population dynamics observed in our study area showed that adult population varies from October to April, but maximum population occurred between November and December. Then, most eggs and larvae occur from October to December (Pérez-Andueza 2015), and therefore, in these months the highest damage should be expected. In any case, the data on the most damaging period herein obtained should be considered to improve the trapping strategy, for example, by advancing placement of traps at the end of summer in an attempt to minimize damage by affecting the most early adult emergence.

The number of traps required to achieve effective truffle damage reduction depends on trap efficacy and the semiochemical's attractiveness for *L. cinnamomeus*. Distance between traps could be determined by the effective attraction radius (EAR), which depends on the joint trap + attractant efficacy effect. The EAR has been proposed as an attractive strength index for traps that release semiochemicals (Byers et al. 1989), defined as the radius that a spherical passive trap would need to catch, merely by interception, as many dispersing insects that were actually caught in the baited trap. Therefore, the EAR positively correlates with attractant strength and trap efficacy, and independently of insect density, locality or test duration. For these reasons, improvements made to the employed attractant (improved composition or aggregation pheromone availability) or trap design could reduce the number of required traps and increase the efficiency of this method, which would mean an economically viable alternative for controlling *L. cinnamomeus*.

In conclusion, mass trapping using the current pitfall traps and DMS dispensers has shown potential to control *L. cinnamomeus*, with densities greater than 40 traps/ha when these were installed in orchards of at least 1 ha.

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