Sublethal exposure to deltamethrin impairs maternal egg care in the European earwig *Forficula auricularia*

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HIGHLIGHTS

- This is the first test of pesticide effects on maternal care in an insect
- We tested the impact of 4 sublethal doses of deltamethrin on earwig's behavior
- Deltamethrin impaired 3 of the 6 measured forms of maternal egg care
- Deltamethrin increased maternal self-grooming at the expense of egg-grooming
- Deltamethrin did not affect female exploration, mobility and reproductive success

ABSTRACT

The application of pesticides typically leads to lethal and sublethal exposure of non-target insects. Whereas our current understanding of these sublethal effects typically focuses on reproductive and physiological parameters, recent works emphasize that sublethal effects on behaviors such as maternal care could be of major importance in non-target species. However, it remained unknown whether these sublethal effects occur in insects. Here, we tested if exposure to sublethal doses of deltamethrin - a pyrethroid insecticide commonly used in crops - alters the expression of maternal egg care in females of the European earwig *Forficula auricularia*, a predator insect and pest control. Our results first reveal that deltamethrin exposure impaired the expression of three forms of maternal egg care: It decreased the likelihood of mothers to gather their otherwise scattered clutch of eggs, increased the time during which the female abandoned the clutch after a predator attack and reduced egg grooming duration. These sublethal effects did not reflect a lower activity of deltamethrin-exposed females, as these females increased their expression of self-grooming, and deltamethrin exposure did not affect females' exploration and mobility. Finally, we found that the negative effects of deltamethrin on egg care did not modify egg development, hatching rate and juvenile weight, possibly due to the transient effects of deltamethrin on maternal behaviors. Overall, our results reveal that sublethal exposure to a pesticide may diminish maternal egg care in a natural pest control and call for the integration of this measurement in assays on pesticides application.

Keywords: Behaviour; Dermaptera; Pyrethroid; Insect; Parental care; Pesticide.

1. INTRODUCTION

The widespread and increased applications of pesticides inherently lead to the exposure of a great number of non-target insects (Aktar et al., 2009; Guedes et al., 2016). Among these insects, some can be of key agricultural, economic and ecological importance, as they often offer long-term sustainable pest control solution by consuming herbivore invertebrates and plant pathogen vectors, provide pollination services facilitating propagation and fruit production for many plants, and represent keystone species maintaining ecosystem balances. Understanding how pesticides affect the survival, physiology and behavior of these beneficial insects is therefore critical to safely and effectively use these compounds.

Pyrethroids are one of the most common class of pesticide used in agrosystems (Davies et al., 2012; Liao et al., 2018) and includes allethrin, bifenthrin, cyhalothrin, cypermethrin, permethrin and deltamethrin. Deltamethrin in particular is a type II synthetic pyrethroid which is well known to affect the sodium channel and trigger a broad release of neurotransmitters at the synapse (Enan and Matsumura, 1992). Due to its low toxicity for vertebrates, high efficiency against insects, and low persistence in soil (Dietz et al., 2009; Li et al., 2019), it is applied in many crops worldwide (e.g. apples, pears, peaches, sorghum, pineapple, coffee, and eucalyptus), as well as used in the public health field to control mosquitoes, flies, cockroaches, and ticks (Davies et al., 2012; Liao et al., 2018). In addition to its well-known lethal effects (e.g. Gutiérrez *et al.* 2017; Shaw *et al.* 2019; Vander Pan *et al.* 2019), sublethal concentrations of deltamethrin can entail major physiological damages in a multitude of target and non-target insects (Cutler, 2013; Müller, 2018). For instance, exposure to sublethal doses of deltamethrin reduces females' fecundity in honeybees, parasitoid wasps and cockroaches (Dai et al., 2010; Lee et al., 1998; Teder and Knapp, 2019), impairs larval development in honeybees and the wasp *Trichogramma achaeae* (Oliveira et al., 2018; Yang et al., 2020) and inhibits molting processes in the stable fly *Stomoxys calcitrans* (Reissert-Oppermann et al., 2019).

Exposure to sublethal concentrations of deltamethrin may also indirectly and directly alter crucial behaviors in insects. Indirect alterations often take the form of an avoidance of deltamethrin contaminated areas by non-exposed individuals. This is the case, for instance, in the maize weevil *Sitophilus zeamais* where individuals modify their walking activity in presence of grains exposed to deltamethrin (Vélez et al., 2019), and in the mosquito *Anopheles sinensis*, where adults show longer flying time and more frequent takeoffs when maintained close to deltamethrin-treated bed nets (He et al., 2019). On the other hand, direct behavioral alterations after deltamethrin exposure have been reported in few insects, such as in Trichogramma species where it disrupted the discrimination of sex pheromones (Delpuech et al., 2012), in the cotton leafworm *Spodoptera littoralis*, where it had an effect on peripheral olfactory system and sexual behavior (Lalouette et al., 2016), and in the backswimmer *Buenoa tarsalis*, where it disrupted swimming activity (Gutiérrez et al., 2017). In the honeybee *Apis mellifera*, it also impaired olfaction and learning capabilities (Decourtye et al., 2004), altered homing flight (Vandame et al., 1995), disturbed orientation and dancing communication (Thompson, 2003; Zhang et al., 2020), and modified foraging activity and memory (Ramirez-Romero et al., 2005).

Whereas a growing number of studies shed light on the broad diversity of sublethal effects associated with pesticide exposure on insect behaviors (Haynes, 1988; Mazzi and Dorn, 2012; Müller, 2018; Parkinson et al., 2020), their impacts – and the impact of deltamethrin - on maternal care remain unexplored in insects. This is surprising, as maternal care is a common phenomenon in insects and forms the backbone of key insect societies such as in many bees (Klug and Bonsall, 2014; Kramer and Meunier, 2019; Machado and Trumbo, 2018; Wong et al., 2013). Moreover, sublethal effects of pesticides on maternal care have been recently suggested to be of central importance because the resulting alterations in offspring could shape the long-term efficiency of pesticide use, and the maintenance and population dynamics of non-target organisms (Cummings et al., 2010; Fong-Mcmaster et al., 2020). To date, however, these

sublethal effects on maternal care were investigated in only a few studies conducted in rats and mice (de Castro et al., 2007; Keller et al., 2019; Punzo, 2003; Stürtz et al., 2008; Udo et al., 2014; Venerosi et al., 2009). These studies showed, for example, that mothers fed with lindane (an organochlorine pesticide) exhibit a lack of retrieval behavior and a consequential litter loss (Matsuura et al., 2005), that mothers exposed to a glyphosate-based herbicide (an organophosphorus pesticide) increased maternal licking behaviors (Dechartres et al., 2019) or that mothers fed with methoxychlor (an organochloride pesticide) reduced the amount of time spent in nursing (Palanza et al., 2002). Whether and how sublethal effects of pesticide exposure (such as deltamethrin) alter maternal care in non-mammalian species remains unknown.

The European Earwig *Forficula auricularia* L. is a common Dermapteran in many agro-ecosystems worldwide and is well-known to exhibit multiple forms of maternal care (Albouy and Caussanel, 1990; Lamb, 1976; Orpet et al., 2019). It is considered an effective generalist predators of several pests such as aphids, leafrollers, and psyllids in pip-fruit orchards (Dib et al., 2010, 2011; Lordan et al., 2015; Moerkens et al., 2011; Orpet et al., 2019; Sauphanor and Sureau, 1993) and data are available on the lethal and physiological effects (or absence of effects) of a number of pesticides commonly applied in crops and vineyards (Colvin and Cranshaw, 2010; ffrench-Constant and Vickerman, 1985; Le Navenant et al., 2019; Malagnoux et al., 2014; Orpet et al., 2019). Individuals of this non-target species (it is sometimes considered as a pest in North America; Colvin and Cranshaw, 2010) are nocturnal and may therefore encounter pesticides only in the form of residuals through direct contacts with (dried) treated surfaces (leaf and soil; Orpet et al., 2019). Although many pesticides used in orchards are toxic to this species (Fountain and Harris, 2015; Malagnoux et al., 2015b), direct exposures to normal application rates of deltamethrin have no impact on their survival and predator activities (Malagnoux et al., 2015a). The effects of deltamethrin on earwig maternal egg care remain unknown. Earwig females produce a first and second (if any) clutch of eggs in late autumn and early spring, respectively, with which they remain in a nest for several weeks to

provide extensive egg care (Meunier et al., 2012; Van Meyel et al., 2019). This egg care is considered essential for egg development and survival, as it allows the removal of external pathogens and mold from the egg shell, the deposition of chemical compounds (hydrocarbons) on the eggs to limit the risk of desiccation, and the expression of fierce maternal behaviors to deter predators (Boos et al., 2014; Buxton and Madge, 1974; Diehl and Meunier, 2018).

In this study, we exposed females to different concentrations of deltamethrin and subsequently measured the expression of six forms of egg care, three forms of non-egg care behaviors, and measured the resulting effects on egg hatching date, hatching rate and juveniles' fresh weight at egg hatching. We also investigated the sublethal effects of deltamethrin on females' allocation between self- and egg-grooming, two key behaviors that can trade-off and typically allow insects to respectively clean their cuticle and egg shell from pathogens and dirt of different natures (Boos et al., 2014; Weiß et al., 2014). To confirm that the reported sublethal effects of deltamethrin do not reflect population-specific expression of egg care (Ratz et al., 2016; Tourneur and Meunier, 2020), we tested females from two distant populations.

2. MATERIAL AND METHODS

2.1.Earwig sampling and maintenance

Our study involved a total of 120 *Forficula auricularia* females that were field-sampled in pip fruit orchards under Integrated Pest Management (IPM) during the first week of July 2019 in Pont-de-Ruan, France (n = 71) and Joué-les-tours, France (n = 49). These females were then maintained under standard laboratory conditions until egg-laying. In brief, earwig adults were caught with wood traps previously placed on tree branches for one week. On the day of field-sampling, males and females were transferred in large plastic containers to allow uncontrolled mating (Sandrin et al., 2015). All the containers were then maintained at 18°C under a 12:12h Light:Dark cycle. Four months later, each female was isolated in a Petri dish (diameter 9 cm) to mimic natural dispersion and encourage egg production (Körner et al., 2018). The Petri dish

was grounded with humid sand and maintained at 10°C under constant darkness to trigger females' nest construction (Koch and Meunier, 2014). Each female was provided with an *ad libitum* amount of an artificial diet mainly consisting of pollen, cat food, carrots and agar (see details in Kramer et al., 2015). These last rearing conditions were maintained until egg hatching. Each Petri dish was checked once a week to record the week of egg laying. Because females cease feeding between egg-laying and egg hatching (Kölliker, 2007), we removed the food present in each Petri dish at egg-laying.

2.2.Deltamethrin exposure

Sixteen days after egg laying (i.e. half egg development time; Ratz *et al.* 2016), each mother was randomly exposed to a deltamethrin or a control (absolute ethanol) solution using a protocol adapted from Malagnoux *et al.* (2015a) and mimicking commercial sprayers (Sauphanor et al., 1992). We used four (sublethal) concentrations of deltamethrin (68.750, 13.750, 6.875 or 3.438 ng/cm^2) obtained by diluting the active deltamethrin molecule (SIGMA #45423) in pure analytical grade (>99.5% absolute) ethanol. These concentrations were respectively 4 times larger, and 1.25, 2.5 and 5 times smaller than the normal application rate (NAR) allowed in French apple orchards that is 0.75 g/hl active ingredient equivalent to 17 ng/cm^2 (Malagnoux et al., 2015a). Maternal exposures were conducted by directly applying 88 µL of one of the four deltamethrin solutions or an absolute ethanol solution (control) on the surface of a Petri dish (diameter 3.5 cm). The Petri dish was then immediately and gently rotated to fully cover its ground and walls with the pesticide or control solution. These solutions were allowed to evaporate for 30 min under an extractor hood. Each mother was subsequently removed from its clutch of eggs, transferred to this Petri dish and allowed to walk freely on its ground contaminated with deltamethrin (or evaporated ethanol for the control). Four hours later, each mother was returned to her clutch of eggs in the original Petri dish and we subsequently conducted egg care measurements. Overall, this process allowed us to obtain 120 exposed

mothers, of which 24 mothers exposed to deltamethrin at a level of 68.750 ng/cm^2 (14 and 10 from Pont-de-Ruan and Joué-les-tours, respectively), 24 mothers exposed at a level of 13.750 ng/cm^2 (14 and 10, respectively), 24 mothers exposed at a level of 6.875 ng/cm^2 (15 and 9, respectively), 24 mothers exposed at a level of 3.438 ng/cm^2 (14 and 10, respectively) and 24 mothers exposed to (evaporated) ethanol only (14 and 10, respectively). During exposure, mothers were maintained under a constant artificial (white) light to stimulate their walking activity (the European earwig is a lucifugous species) and thus maximise contacts with molecules deposited on the Petri dish. The tested mothers were randomly distributed among the five treatments, so that there was no association between the number of tended eggs and deltamethrin concentration (Linear model, $F_{4,155} = 0.07$, $P = 0.992$).

Sublethal doses of pesticides are typically defined as doses inducing no apparent mortality in the tested population, but potentially causing physiological and/or behavioral effects on individuals that survive the pesticides exposure (Desneux et al., 2007; Müller, 2018). In our study, we therefore defined the four doses of deltamethrin as sublethal on the basis of previous works showing that deltamethrin doses corresponding to normal application rates (NAR) in French apple orchards do not induce mortality in *F. auricularia* adults (Malagnoux et al., 2015a), and on our current results showing that there was no excess of mortality with the four tested doses (see results).

2.3.Behavioural and reproductive measurements

The 120 mothers exposed to deltamethrin or evaporated ethanol (control) were then used to measure the expression of six forms of egg care (egg gathering, egg defence against a predator, delay of maternal return after clutch abandonment, egg grooming, egg displacement and egg antennation), three female behaviours that are not associated with egg care (see below), females' allocation between self- and egg-grooming, as well as to record females' reproductive outcome in terms of date of egg hatching, egg hatching rate and larval weight at egg hatching.

For egg gathering, we carefully scattered the clutch of each female in its original Petri dish during the 4h maternal exposure (see above), then transfered each mother to its scattered clutch and, 17h later, recorded whether each mother gathered (or not) all the eggs, i.e. whether all eggs were within one egg distance from each other. The time spent by mothers on egg grooming, egg displacement and egg antennation was measured just after egg gathering measurements and involved all the tested mothers, i.e. includes the few mothers that did not gather their eggs after 17h (see results). We first isolated each mother for 15 min to increase their future motivation to interact with the eggs (Van Meyel et al., 2019). We then put back mothers in their Petri dish at a distance of 5 cm from the eggs and finally video recorded their behaviours for the next 20 min (SONY© Handycam HDR-CX700 camera). The total durations of egg grooming, egg displacement and egg antennation were respectively defined as the total amount of time each female spent on cleaning eggs with their mandibles, displacing eggs within or away from the clutch, and antennating at least one egg without mandibular contacts (Boos et al., 2014). These three measurements are classical forms of maternal care and reflect the amount of time earwig mothers allocate to the cleaning or application of chemicals (hydrocarbons) on the eggs, the moving of eggs to safer area and/or to the re-organisation of clutch structure and to the touching of the eggs before triggering other care behaviours, respectively (Van Meyel et al., 2019). Movies were analysed using the software BORIS v4.0.3 (Friard and Gamba, 2016). The measurements of egg defence (Thesing et al., 2015), which reflects females' willingness to protect their eggs from a predator attack, were conducted just after the above-detailed movies recordings. We standardly poked females on the pronotum with a glass capillary (1 poke per second) and recorded the number of pokes required until the female moved more than 1 body length away from its initial position. Finally, the delay of maternal return after clutch abandonment (Van Meyel et al., 2019) was measured by recording the time the female took to return to its clutch just after the end of the egg defence measurement. At the end of these behavioral measurements, all females were returned with their clutch of eggs and then

maintained under standard laboratory conditions. All the behavioural measurements (here and below) and video analyses were done under red light (earwigs are nocturnal) and blind regarding the type of female exposure.

The effects of deltamethrin exposure on three non-care behaviours (self-grooming, exploration and inactivity) and females' allocation between self- and egg-grooming were measured on the 20 min movies used to measure egg care behaviors (see above). The total duration of females' self-grooming, which is a behavior during which individuals typically clean off dirt, remove external parasites and apply chemical protections on their cuticle (Weiß et al., 2014), was defined as the total amount of time each female spent on cleaning itself with its mandibles. Females' exploration, which is a behavior reflecting the general mobility of an individual, was defined as the total amount of time each female spent on being mobile neither interacting with the eggs nor expressing self-grooming. Females' inactivity was defined as the total amount of time each mother did not show any movement or behaviour directed at themselves or the eggs during the 20 min video recordings. These three behaviours were analysed following the same protocol than for maternal egg care. Finally, females' allocation between self- and egg-grooming, which may reflect a selfish versus cooperative maternal strategy, was calculated for each female by dividing the total amount of time spend in egg-grooming by the total amount of time spend in both egg-grooming and self-grooming.

All females and eggs used in the behavioural measurements detailed above were then maintained under standard conditions during the 40 days following female exposure. They were checked daily to record the date of death (if any) and thus confirm our use of sublethal doses of deltamethrin, as well as to record the date of egg hatching and, one day later, to count and weight the resulting larval. For the weighing, a group of 10 juveniles (called nymphs) was randomly sampled per clutch (or all of them if less than 10 were available), weighed to the nearest 0.01 mg using a microbalance (OHAUS© Discovery DV215CD) and these measurements then used to calculate mean nymph weight.

2.4.Statistical analyses

We used three generalised linear models (GLM) fitted with binomial error distribution to analyse egg gathering (1 or 0), hatching rate and females' allocation between self- and egg-grooming, while we used a series of nine GLM fitted with Poisson error distribution corrected for over-dispersion to analyse egg defence, delay of maternal return, egg grooming, egg displacement, egg antennation, self-grooming, exploration, inactivity and mean nymph weight. In each of these models, females' exposure (68.750, 13.750, 6.875, 3.438 ng/cm^2 or evaporated ethanol), females' population of origin (Pont-de-Ruan or Joué-les-tours) and the interaction between these two factors were entered as categorical factors, while the number of eggs produced by a female was used as a covariable to control for its possible impact on the level of egg care. Egg hatching rate and females' allocation between self- and egg-grooming were entered in the models using the *cbind* function in R (number of juveniles at hatching, number of unhatched eggs; and time spend in egg grooming, time spend in self grooming, respectively). We also analyzed whether mothers exposed to each tested dose of deltamethrin allocated the same amount of time between egg- and self-grooming using one sample t-tests, in which we compared females' allocation between self- and egg-grooming in each treatment to the value 0.5. For egg gathering, pairwise comparisons between deltamethrin treatments were conducted using a series of fisher exact tests and the p-values were corrected for multiple testing using the mean false discovery rate method (Benjamini and Hochberg, 1995; Kramer et al., 2015). For the delay of maternal return, egg grooming and self-grooming, pairwise comparisons between deltamethrin treatments were conducted using the estimated marginal means and the p-values were corrected for multiple testing using Tukey methods. All models were checked for homoscedasticity and normal distribution of model residuals. All statistical analyses were performed with the software R v3.6.3 (http://www.r- project.org/) loaded with the packages *car* and *emmeans*.

3. RESULTS

Out of the 120 females involved in this study, only 11 (9.2%) died within the 40 days following experimental exposures. These deaths were independent of deltamethrin exposure (Fisher's exact test, $p = 0.949$), as 3 (12.5%) females died after exposure to the 68.750 ng/cm^2 solution of deltamethrin, 3 (12.5%) after exposure to the 13.750 ng/cm^2 solution, 2 (8.3%) after exposure to the 6.875 ng/cm^2 solution, 2 (8.3%) females after exposure to the 3.438 ng/cm^2 solution and 1 (4.2%) female after exposure to evaporated ethanol (control) solution.

There were contrasting dose-dependent effects of deltamethrin on maternal care in terms of likelihood of egg gathering, total time spent egg grooming and delay of maternal return after clutch abandonment (Figure 1; Table 1). First, the likelihood of females to gather their eggs within the 17h post-exposure period was significantly lower when they were exposed to the highest quantity of deltamethrin (45.9% of the females at 68.750ng/cm^2) compared to the control (100% of the control females) or any others doses of deltamethrin (Tables 1 & 2 , Figure 1A). This likelihood was not different among the three lowest doses of deltamethrin and the control (Table 2). Second, the total duration of egg grooming was about 3.5 times shorter when females were exposed to the highest quantity of deltamethrin (51.4 sec of the females at 68.750ng/cm^2) compared to the control (177.9 sec) or any other doses of deltamethrin (Tables 1 & 2, Figure 1B). This duration was also not different among the three lowest doses of deltamethrin and the control (Table 2). Finally, the delay of maternal return after clutch abandonment was about 9 times longer when females were exposed to the highest dose of deltamethrin (194.5 sec of the females at 68.750ng/cm^2) compared to the control (21.5 sec), a delay also present for all the other tested quantities of deltamethrin (Tables 1 & 2, Figure 1B). The delay of maternal return was comparable among the four tested doses of deltamethrin (Table 2). Contrary to these effects, there was no impact of deltamethrin on the total amount of

time spend in egg displacement and egg antennation, nor on the level of egg defense against a simulated predator attack (Figures 1D, 1E and 1F; Table 1).

Dose-dependent effects of deltamethrin were present in self grooming behavior, but not on the time spent in exploration and inactivity (Tables 1 & 2, Figure 3). In particular, the total duration of self-grooming was about 2.4 times longer when females were exposed to the highest quantity of deltamethrin (270.2 sec of the females at 68.750ng/cm^2) compared to the control (111.6 sec) and the two lowest doses of deltamethrin (Tables 1 & 2, Figure 2A). Females exposed to the second highest quantity of deltamethrin (13.750 ng/cm^2) spent 197.4 sec in self-grooming on average, and intermediate value that was not different from the other tested doses of deltamethrin and the control (Tables 1 & 2; Figure 2A).

Dose-dependent effects of deltamethrin also shaped the presence and outcome of females' allocation between self- and egg-grooming (Figure 3, Table 1). Of the total amount of time spend in grooming behaviors (i.e. egg- plus self-grooming), control females allocated on average 64.2% of their grooming time to their eggs, whereas females exposed to the two highest doses of deltamethrin (68.750 and 13.750 ng/cm^2) allocated a 4 and 1.7 times smaller proportion of time (Tables 1 & 2, Figure 3). Females exposed to the two smallest doses of deltamethrin exhibited a proportion in egg grooming that was intermediate between the control and highest doses of deltamethrin (Table 2; Figure 3). Interestingly, females exposed to control allocated a larger proportion of their total grooming time to their eggs (one sample t-test, $t = 2.94$, df = 23, $P = 0.007$; Figure 3), females exposed to the smallest (8.750 ng/cm^2) and second smallest (3.438 ng/cm^2) quantities of deltamethrin shared their total grooming time between the eggs and themselves ($t = 1.14$, df = 23, $P = 0.266$ and $t = 1.26$, df = 23, $P = 0.220$, respectively), while females exposed to the highest (68.750 ng/cm^2) and second highest (13.750 ng/cm^2) doses of deltamethrin allocated a larger proportion of their total grooming time to themselves ($t = -10.59$, df = 21, $P < 0.0001$ and $t = -2.15$, df = 23, $P = 0.043$, respectively).

There were no effects of deltamethrin exposure on egg hatching date, egg hatching rate and on the mean weight of the resulting juveniles (Figure 4; Table 1). Interestingly, the eggs from Joué-les-tours overall hatched 8.30 ± 0.03 (mean ± SE) days after exposure, a value that was shorter than the 10.05 ± 0.04 days of the eggs from Pont-de-Ruan (Table 1). Females from Joué-les-tours also spent 419.9 ± 41.6 (mean ± SE) sec in inactivity, which was longer than the 306.1 ± 32.0 sec of females from Pont-de-Ruan (Table 1). Nevertheless, the population had no other effect (neither a main effect nor in interaction with deltamethrin exposure) on the six measured forms of egg care, on females' allocation between self- and egg-grooming, on the two other non-care behaviors, and on the two other measurements of reproductive outcome (Table 1). Similarly, egg number was negatively associated with the likelihood of egg clustering 17h post-exposure (model estimate ± SE = -0.37 ± 0.019; Table 1) and with the mean juvenile's weight at egg hatching (model estimate ± SE = -0.0052 ± 0.0019; Table 1), while it was positively associated with maternal allocation in egg- compared to self-grooming (model estimate ± SE = 0.03 ± 0.01; Table 1). Egg number had no effect on the ten other measured traits (Table 1).

4. DISCUSSION

Whereas exposure to sublethal doses of pesticides is well known to shape a broad diversity of insect behaviors (Haynes, 1988; Mazzi and Dorn, 2012; Müller, 2018; Parkinson et al., 2020), their effects on maternal egg care remained surprisingly unknown. In this study, we addressed this gap in knowledge by testing whether exposures to different concentrations of deltamethrin (a commonly used pesticide in agriculture and public health fields (Davies et al., 2012; Li et al., 2019; Liao et al., 2018) altered the expression of maternal egg care in the European earwig. Our results first revealed that sublethal exposure to deltamethrin indeed impaired earwig maternal egg care by reducing their propensity to gather eggs, shortening the time spent in egg

grooming and prolonging the time spent away from the clutch after a predator attack. These effects were unlikely to result from a general lower activity of deltamethrin exposed mothers, as we also found that this exposure increased the time spend by mothers in self-grooming, while it had no effects on egg displacement, egg antennation, females exploration and inactivity, as well as on the number of pokes received before escaping from a simulated predator attack. Interestingly, deltamethrin exposure modified females allocation between self- and egg-grooming: mothers exposed to the control solution exhibited a higher allocation of time in egg- compared to self-grooming, whereas exposure to the two smallest concentrations induced mothers to equally share their time between egg- and self-grooming, and exposure to the two highest concentrations of deltamethrin induced mothers to favor self- over egg-grooming. Finally, our results confirm that the low concentrations of deltamethrin used in our study did not trigger a higher mortality in the exposed mothers (and are thus sublethal doses), as well as show that it did not impair their reproductive outcomes in terms of egg hatching date, egg hatching rate and juveniles' weight at hatching. All these findings were independent of the population of origin of the tested females.

It has been recently proposed that studying sublethal effects of pesticides on maternal care could be of central importance to improve our general use and regulation of pesticides (Cummings et al., 2010; Fong-Mcmaster et al., 2020; Keller et al., 2019). To date, however, only few studies in rodents did investigate the occurrence and nature of these sublethal effects (Cummings et al., 2010; Fong-Mcmaster et al., 2020; Keller et al., 2019). Our results in earwigs thus confirm that sublethal effects of pesticides can impair the expression of maternal care in a broader number of animal species and, importantly, reveal for the first time that these effects can apply both before juveniles' emergence (i.e. toward eggs) and in non-mammalian species. Given that maternal egg care is taxonomically widespread in insects (Klug and Bonsall, 2014; Machado and Trumbo, 2018; Wong et al., 2013), our results emphasize that this currently neglected sublethal effects could have a broad impact on the target and non-target insects

present in crops, and therefore call for a better integration of this measurement in future assays on sublethal effects.

The effects of sublethal exposures to pesticides on insect behaviors can take multiple forms ranging from, for instance, increased locomotor activity in the predatory beetle *Platynus assimilis* (Tooming et al., 2014), over altered calling behaviors in tortricid moth pests (Navarro-Roldán et al., 2017), to impaired mating behaviors in the predatory stink bug *Podisus nigrispinus* (Oliveira et al., 2012). The sublethal effects of deltamethrin on insect behaviors have been well studied in honeybees, where deltamethrin exposure typically impairs orientation and memory, increases stimulus sensitivity and general excitement, and triggers disordered movements (Decourtye et al., 2004; Ramirez-Romero et al., 2005; Thompson, 2003; Zhang et al., 2020). The sublethal effects reported on earwig behaviors only partly support these modes of action. On one hand, disordered movements, overexcitement and impaired orientation may explain why earwig females exposed to deltamethrin were less likely to gather their eggs and then spent less time in egg grooming. On the other hand, these modes of action fail to explain why deltamethrin exposure increased the expression of self-grooming and did not affect the level of reaction to a stimulus (poking number in egg defense) and the time spent in exploration and inactivity. This apparent discrepancy may suggest specificity in the mode of action of deltamethrin on maternal care and/or earwig behaviors. In *Spodoptera littoralis,* it has been shown that deltamethrin can disrupt peripheral olfactory system and sexual behaviors (Lalouette et al., 2016). It might thus be interesting to study whether deltamethrin affects the receptivity of earwig mothers to recognition signals emitted by the eggs, even if we do not observe any hormetic effect on their maternal behaviors (contrary to the study on *S. podoptera littoralis*).

Studying sublethal effects of pesticides on maternal care may provide novel tools to unravel the neurobiological, physiological and/or molecular pathways regulating maternal behaviors in animals (Keller et al., 2019). This could be particularly decisive in insects, where

these pathways remain poorly understood (Bridges, 2020; Körner et al., 2020; Trumbo, 2019; Wu et al., 2020). The principal molecular mode of action of deltamethrin in insects is the prolonged opening of the sodium channel, which results in membrane depolarization of neurons, repetitive discharges and synaptic disturbances leading to hyperexcitation of the nervous system (Narahashi et al., 1992). How these effects can modulate specific behaviors is still unclear. A recent study suggests that the impaired honeybee waggle dance observed after exposure to deltamethrin is associated with a deregulation of the signaling pathway of neurotransmitter dopamine, which in turn impairs learning and memory (Zhang et al., 2020). Further studies should test whether deltamethrin can alter maternal care behaviors by disrupting neurotransmitter systems and more generally, decipher which molecular mode of action could lead to this behavioral effect.

Although maternal egg care is considered essential to ensure egg development and hatching in the European earwig (Boos et al., 2014; Buxton and Madge, 1974; Van Meyel et al., 2019), our results surprisingly showed that the sublethal effects of deltamethrin on egg care did not translate into a delayed hatching date, a lower hatching rate nor into the production of lighter larvae at hatching. These findings first suggest that earwig mothers did not transfer the deltamethrin molecules present on their body to their eggs during egg care and/or that the quantities of transferred molecules are not toxic after the eggs have reached half of their development time. The first hypothesis is unlikely to explain our results, as frequent and tight contacts between individuals often facilitate the horizontal transfer of pesticides from exposed to non-exposed insects, a process reported in cockroaches, termites and ants (Buczkowski and Schal, 2009; Choe and Rust, 2008; Rust and Saran, 2006). On the other hand, earwig mothers regularly clean their eggs during the period of egg care (Boos et al., 2014), a behavior that might help them removing the few molecules possibly transferred on the egg shell. Determining whether this horizontal transfer is possible with deltamethrin and occurs during egg care will require future works and measurements of deltamethrin on the eggs.

The absence of effects of maternal exposure to deltamethrin on the egg fate may also suggest that the observed sublethal effects on maternal care are transient and return to a normal level a few days after maternal exposure (a behavior that was not recorded in the present experiment) and/or that impaired maternal care occurring after the eggs have reached half of their development time is not detrimental in absence of natural constraints such as pathogens and predators. In insects, the duration of sublethal effects of pesticides on insect behaviors are difficult to predict, as they strongly depend on the type and dose of pesticide, mode of pesticide exposure, species, and measured behavior (Haynes, 1988; Mazzi and Dorn, 2012; Müller, 2018; Parkinson et al., 2020). Our findings therefore pave the way for follow-up studies disentangling among the formulated hypotheses and investigating the long-lasting effects of deltamethrin on the physiology and behaviors of earwig mothers, as well as the short- and long-term effects of deltamethrin exposure on the impact of impaired egg care under natural conditions. These studies should also investigate the effects of (more natural) repeated maternal exposures to deltamethrin, as earwig females are likely to repeatedly encounter deltamethrin when exploring treated areas (deltamethrin residues can be found on plants and soil several weeks after application; Dietz et al., 2009; Mukherjee et al., 2015).

On a more general level, our results highlight the benefits and novel opportunities provided by the use of non-model organisms (such as the European earwig) to study the impacts of pesticides and reveal novel markers of fitness reduction. Developing a comprehensive understanding of the environmental impacts of pesticides typically requires us to consider species' idiosyncrasies, the diversity of fitness-relevant parameters, and the multiple routes of contamination. These three factors, however, cannot be necessarily taken into account when focusing on a small number of model organisms. For instance, the effects of pyrethroids have been recently investigated in insect aquatic models (Gutiérrez et al., 2017), because this ecosystem is the receptacle of numerous routes of contaminations. Beyond tests of toxicity, assays on sublethal effects on specific behaviors such as swimming and position within the

water body column have been developed, as they are relevant with regard to the consequences of these behaviors in terms of food seeking and predator avoidance (Gutiérrez et al., 2017). Concerning terrestrial insect species, pollinating bees are the model organisms that have attracted most attention in the literature, particularly regarding the sublethal effects of deltamethrin on insect behaviors (Decourtye et al., 2005; Desneux et al., 2007; Lalouette et al., 2016; Ramirez-Romero et al., 2005; Thompson, 2003). However, the sublethal effects of deltamethrin on maternal egg care remained unexplored in these studies (possibly due to difficulties in its measurements), overall fostering the neglect of this key behavior (Klug and Bonsall, 2014; Machado and Trumbo, 2018; Wong et al., 2013) in the insect literature.

To conclude, our results demonstrate that sublethal exposure to deltamethrin, a pesticide commonly applied in a broad diversity of crops worldwide, alters the expression of maternal egg care in the European earwig, a pest control insect present on almost every continent (Quarrell et al., 2018; Tourneur and Meunier, 2020; Wirth et al., 1998). These findings provide important insights in our general understanding of the diversity of sublethal effects possibly occurring among insects and highlight the benefits and novel opportunities provided by the use of non-model organisms to study these effects. They also shed light on novel potential markers of fitness reduction associated with pesticide exposure, and thus open new research avenues to allow safe and effective use of these compounds in crops and orchards.

Credit Author Statement

JM, MR and CL: Conceptualization, Methodology and Validation. JM, JD and SvM: Data acquisition. JM: Formal analysis. JM and CL: Manuscript writing – original draft. JM, JD, SvM, MR and CL: Manuscript reviewing and editing.

Declaration of competing interests

The authors declare that there are no conflicts of interests.

Data availability

The complete data set is archived in the open data repository Zenodo (https://doi.org/10.5281/zenodo.3892264).

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Table 1 – Effect of exposure to different concentrations of deltamethrin, females' population and egg number on the expression of six forms of egg care, three female behaviors not associated with egg care, the trade-off between self- and egg-grooming, and on three proxies of females' reproductive outcome. Statistical values from generalized and general linear models conducted on each measurement (one model per line; see methods). Significant p-values are in bold. LRχ^2 stands for Likelihood Ratio χ^2.

	Exposure			Population			Egg number			Exposure : Population		
	F / LRχ^2	df	P	F / LRχ^2	df	P	F / LRχ^2	df	P	F / LRχ^2	df	P
Egg care behaviors												
Egg gathering	28.48	4	**<0.0001**	1.03	1	0.310	6.45	1	**0.011**	9.18	4	0.057
Egg grooming	27.64	4	**<0.0001**	0.21	1	0.643	3.18	1	0.075	2.49	4	0.647
Maternal return	29.62	4	**<0.0001**	2.06	1	0.151	0.08	1	0.774	3.43	4	0.489
Egg displacement	1.08	4	0.897	2.37	1	0.124	4.06	1	0.044	1.68	4	0.794
Egg antennation	5.17	4	0.270	0.54	1	0.462	0.62	1	0.432	2.99	4	0.560
Egg defense	7.32	4	0.120	1.44	1	0.230	0.73	1	0.392	1.37	4	0.849
Non-care behaviours												
Self-grooming	15.81	4	**0.003**	2.35	1	0.125	1.96	1	0.162	0.79	4	0.939
Exploration	2.50	4	0.644	0.34	1	0.561	0.25	1	0.619	2.13	4	0.712
Inactivity	3.17	4	0.530	4.5	1	**0.034**	0.02	1	0.886	5.71	4	0.222
Allocation self/egg grooming	71.87	4	**<0.0001**	0.28	1	0.594	9.3	1	**0.002**	2.47	4	0.649
Reproductive outcome												
Hatching date	0.73	4	0.571	27.22	1	**<0.0001**	2.92	1	0.091	0.87	4	0.488
Hatching rate	4.61	4	0.329	0.22	1	0.638	0.19	1	0.663	2.34	4	0.674
Nymph weight	2.38	4	0.057	1.50	1	0.223	7.24	1	**0.008**	1.65	4	0.168

Table 2 – Pairwise comparisons among deltamethrin treatments (C1 = 68.750 ng/cm^2, C2 = 13.750 ng/cm^2, C3 = 6.875 ng/cm^2, C4 = 3.438 ng/cm^2) and control (evaporated ethanol). The values represent the estimated marginal means of the statistical models on egg gathering, egg grooming, delay of maternal return, self-grooming and allocation self/egg grooming (see methods). Significant p-values are in bold.

			Egg gathering	Egg grooming			Delay of maternal return			Self-grooming			Allocation self/egg grooming		
			P	Est.	SE	P	Est.	SE	P	Est.	SE	P	Est.	SE	P
C1	-	C2	**0.036**	-1.01	0.33	**0.019**	0.58	0.34	0.431	0.31	0.24	0.196	-1.28	0.32	**0.001**
C1	-	C3	**0.001**	-1.23	0.32	**0.001**	0.57	0.31	0.357	0.52	0.25	**0.036**	-1.82	0.32	**<0.001**
C1	-	C4	**0.015**	-1.33	0.32	**<0.001**	0.65	0.32	0.263	0.64	0.25	**0.012**	-1.98	0.32	**<0.001**
C1	-	Control	**<0.001**	-1.27	0.32	**0.001**	2.14	0.51	**<0.001**	0.82	0.27	**0.002**	-2.10	0.32	**<0.001**
C2	-	C3	0.416	-0.22	0.22	0.859	-0.01	0.33	1.000	0.21	0.26	0.427	-0.54	0.26	0.251
C2	-	C4	1.000	-0.32	0.22	0.587	0.07	0.34	1.000	0.33	0.27	0.220	-0.70	0.26	0.062
C2	-	Control	0.050	-0.26	0.22	0.751	1.56	0.52	**0.023**	0.52	0.29	0.072	-0.82	0.27	**0.021**
C3	-	C4	0.666	-0.10	0.21	0.990	0.08	0.31	0.999	0.12	0.28	0.657	-0.16	0.26	0.970
C3	-	Control	0.489	-0.04	0.21	1.000	1.57	0.50	**0.015**	0.31	0.29	0.299	-0.28	0.27	0.829
C4	-	Control	0.109	0.05	0.20	0.999	1.49	0.51	**0.029**	0.18	0.30	0.547	-0.12	0.27	0.992

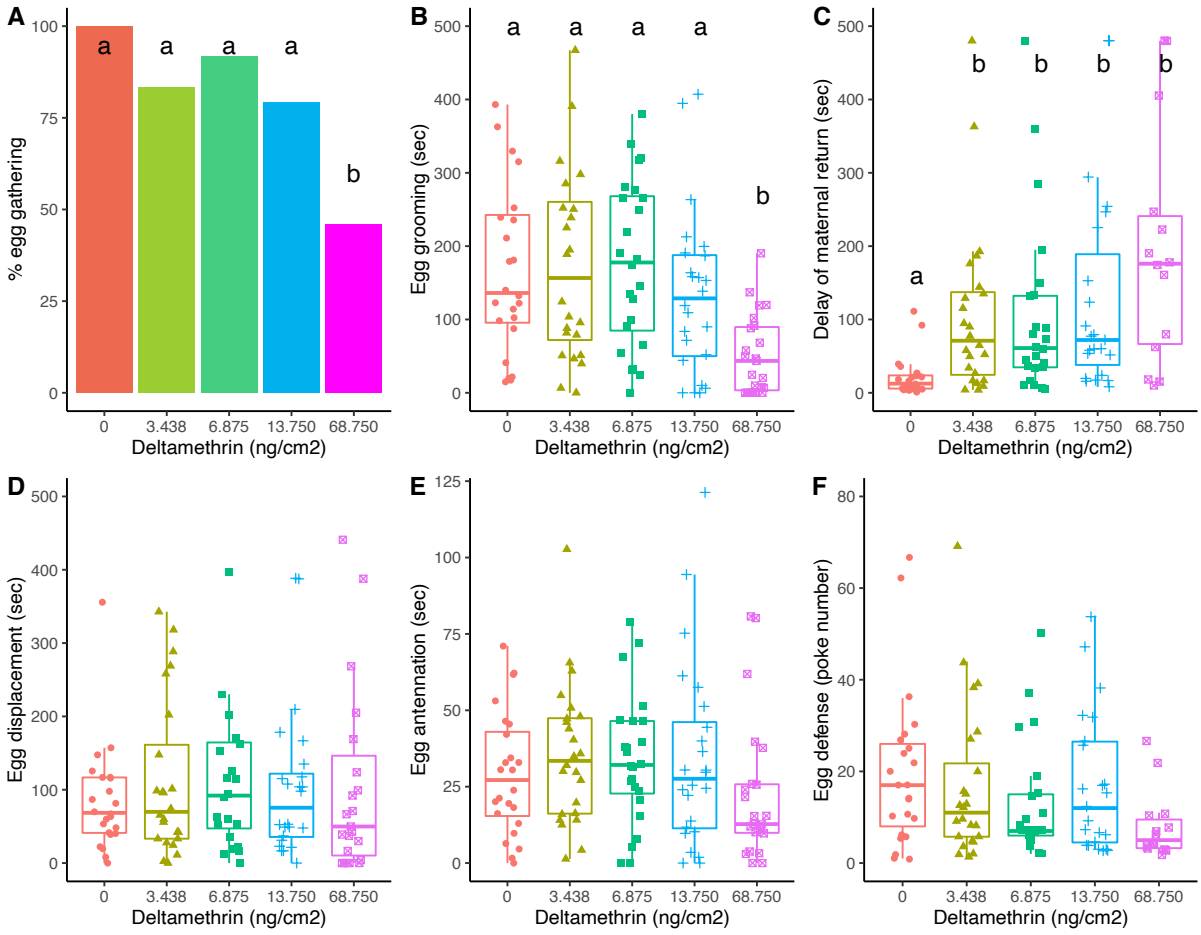


Figure 1 – Effects of deltamethrin on maternal egg care in the forms of A) egg gathering, B) egg grooming, C) delay of maternal return after clutch abandonment, D) egg displacement, E) egg antennation and F) egg defence against a predator. Boxplots depict median (bold bar) and interquartile range (light bar), with whiskers extending to 1.5 times the interquartile range and dots representing experimental values. For pairwise comparisons, different letters correspond to P <0.05. The absence of letter on a figure indicates an absence of significant difference between all the tested doses. Colour figures can be viewed in the online version.

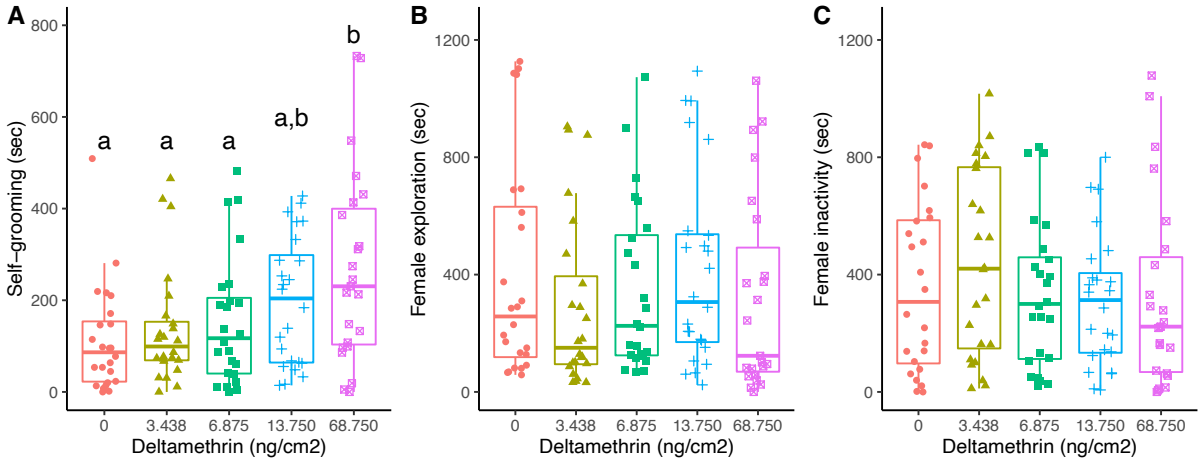


Figure 2 – Effects of deltamethrin on the total durations of females' A) self-grooming, B) exploration and C) inactivity. Boxplots depict median (bold bar) and interquartile range (light bar), with whiskers extending to 1.5 times the interquartile range and dots representing experimental values. For pairwise comparisons, different letters correspond to $P < 0.05$. The absence of letter on a figure indicates an absence of significant differences between all the tested doses. Colour figures can be viewed in the online version.

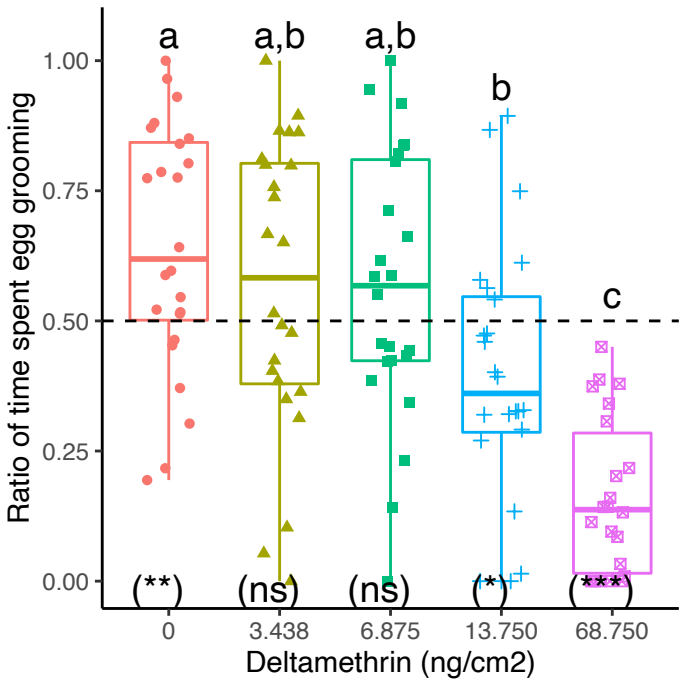


Figure 3 – Effects of deltamethrin on female's allocation between egg- and self-grooming. A ratio of time spent on egg grooming of 0.5 indicates an absence of the trade-off, while ratios between 0.5 and 1 indicates a trade-off in favour of egg grooming and between 0 and 0.5 a trade-off in favour of self-grooming. Values between brackets (bottom) compare each column to 0.5 (using one sample t-tests), i.e. tests whether females from each category did express the same amount of egg- and self-grooming. *** stands for $P < 0.001$; ** for $P < 0.01$, * for $P < 0.05$ and *ns* for $P > 0.05$. For pairwise comparisons, different letters correspond to $P < 0.05$. Colour figures can be viewed in the online version.

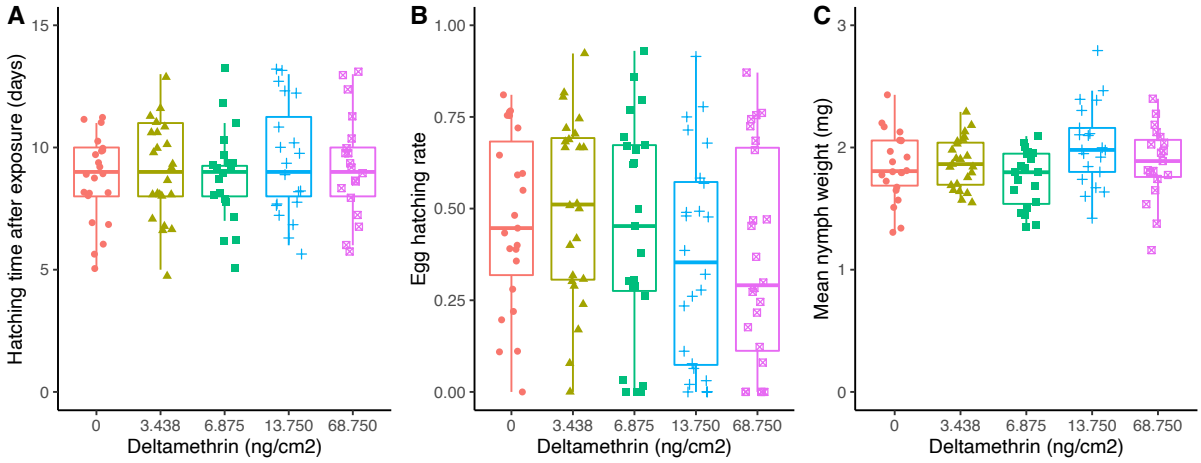


Figure 4 – Effects of deltamethrin on reproductive outcomes in term of A) hatching time after exposure, B) egg hatching rate and C) mean nymph weight at egg hatching. Boxplots depict median (bold bar) and interquartile range (light bar), with whiskers extending to 1.5 times the interquartile range and dots representing experimental values. For the three measurements, there was no significant differences between the tested doses. Colour figures can be viewed in the online version.