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MEDICAL AND VETERINARY ENTOMOLOGY

Effects of Terbufos (Organophosphate) on Larval Behaviour of Two Forensically Important Diptera Species: Contributions for Entomotoxicology

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Abstract

Neurotoxicant compounds interfere with the behaviour and biology of insects, significantly altering their locomotion patterns. However, little is known about the effect of organophosphates, neurotoxicants for agricultural, domestic and industrial use, on the larval movement of necrophagous flies, although being responsible for frequent cases of poisoning and accidental or intentional deaths. Thus, we aimed to study the influence of Terbufos (organophosphate) on the activity and mobility patterns of *Lucilia eximia* (Wiedemann 1819) (Calliphoridae) and *Peckia* (*Peckia*) *chrysostoma* (Wiedemann 1830) (Sarcophagidae) immatures collected from rat carcasses intoxicated with 5, 10 or 20 mg/kg of Terbufos, to evaluate (i) peristaltic movements and body contractions, and (ii) distance and shape of the trajectory travelled by the larva. Behavioural parameters were analysed in loco and through videos. We observed that the presence of Terbufos altered poisoned larvae's activity and body mobility in both taxon and dose-dependent manner. *Lucilia eximia* larvae were more active, with greater frequency of body movements and lateral contractions when intoxicated with high and intermediate doses of Terbufos. On the other hand, *P.* (*P.*) *chrysostoma* immatures were less active, with fewer body and lateral contractions when intoxicated with the high dose of the compound. This work experimentally demonstrates that the presence of Terbufos can alters the mobility and movement of intoxicated necrophagous Diptera, essential components of the cadaveric fauna.

Keywords Forensic entomology · pesticide · larval dispersion · blowflies · flesh flies · carrion

Introduction

Physiological effects of insect exposure to insecticides with neurotoxic action (e.g. organophosphates) range from death to sublethal effects (Sidhu et al. 2019), including altered life cycle (Jales et al. 2020), reduced fecundity (Hunter et al. 1958; Bariola 1984), lower weight of larvae and pupae (Cavalho et al. 2001) or even reduced longevity (Hamilton and Schal 1990). Of these, the effects on larval behaviour are, comparatively, less studied, despite their applicability in sampling, monitoring and controlling insects of forensic, medical and agricultural importance (Lee et al. 2000; Desneux et al. 2007; De França et al. 2017).

Necrophagous insects can be indirectly exposed to organophosphates when feeding on cadavers' victim of intoxication. Self-poisoning or attempted suicide by organophosphate is the most severe global form of acute intoxication, affecting more than one million people each year and killing an estimated 100,000 (Eddleston 2019). For example,

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mortality from occupational poisoning related to pesticides, organophosphate and carbamate poisoning, prevailed among men from Brazil's Northeast region (Silva et al. 2016). Easy access to pesticides that are highly fatal to humans, even after ingesting small amounts, has resulted in their use for self-poisoning and suicide (Eddleston 2019).

The increasing frequency of deaths from organophosphate poisoning is reflected in a greater likelihood of scavenging insects being exposed to contaminated resources. The presence of drugs or toxic, illicit and psychoactive substances can be manifested in changes in the development, feeding and dispersion of necrophagous larvae, which, in turn, affects the reliability of the collection and use of entomological evidence for investigation purposes, a scientific area known as forensic entomology (Catts and Goff 1992; Goff and Lord 1994).

Calliphoridae and Sarcophagidae species are the primary colonizers of corpses. They use the resource for developing their larvae, which feed on dead animal tissues, thus representing the most used taxa for estimating the minimum post-mortem interval (minPMI). The minPMI is based on the collection of older larvae at the post-feeding dispersal stage, a period in which the immature ones leave the substrate and search for an appropriate place to pupate (Andrade et al. 2002).

Diptera larvae can also be used as indicators of toxic substances in the corpse because insecticides, barbiturates and other drugs can be detected for a more extended period in larval tissues than in human corpses, a key concept in Forensic Entomotoxicology (Introna et al. 2001; Chophi et al. 2019). The presence of toxic substances also affects the dynamics of cadaveric colonization and produces variable effects on the duration of larval development (Cophi et al. 2019). However, much remains to be known about the alteration of these behavioural parameters, especially for neurotoxic compounds.

The study of the influence of chemical substances on the larval dispersal process requires prior knowledge of the immature's migratory potential since the distance travelled by a larva in the dispersion stage can vary widely depending on the species (Singh and Bala 2010), interspecific competition (Andrade et al. 2002; Reigada and Godoy 2005) and biotic and abiotic conditions (Arnott and Turner 2008; Charabidze et al. 2008; Robinson et al. 2018). In addition to the dispersion per se, larval movement is also manifested in body contractions and resting time, which can make them more conspicuous to predators. On the other hand, these biological parameters can further aggravate the dynamics of toxic compounds in food chains and expand their spatial distribution, causing more significant soil contamination and increasing the vulnerability of non-target organisms.

Organophosphates are irreversible acetylcholinesterase inhibitors whose intoxication causes typical symptoms of cholinergic overexpression, including involuntary body contractions and changes in mobility in invertebrates (Haynes 1988; de França et al. 2017). Therefore, it is valid to question whether dipteran larvae that feed on corpses exposed to organophosphates will exhibit changes in movement speed, distance covered and pattern of body contraction and movement. In this aspect, organophosphates such as Pestanal (Malloy et al. 2019) and Malathion (Hoy and Dahlsten 1984) directly influence the larval movement behaviour by increasing the frequency and speed of body contractions, neuronal hyperexcitability and involuntary contractions in intoxicated insects. Unravelling how these changes manifest in different species' larvae and the implications for standardized specimens' collection at death sites is a tremendous challenge for forensic entomology.

Our research was motivated by the need to use empirical data on the effect of organophosphates on behavioural parameters intrinsic to the larval movement process of necrophagous flies. The information obtained advances in three areas of applied entomology: (a) to elucidate how sublethal effects of organophosphates can manifest in non-target organisms; (b) to introduce Entomotoxicology approaches to decomposition ecology and (c) to strengthen the methodological framework of Forensic Entomology, discussing the implications for the collection of entomological evidence at crime scenes. This work aimed to describe the movement behaviour of immature dispersants of the Calliphoridae and Sarcophagidae families collected from rat carcasses intoxicated with different doses of Terbufos (Organophosphate). Specifically, we evaluated the effect of Terbufos on the following behavioural parameters: (i) peristaltic movements and body contractions and (ii) distance and shape of the trajectory travelled by the larva.

As models, we chose the species *Lucilia eximia* (Wiedemann 1819) (Diptera: Calliphoridae) and *Peckia* (*Peckia*) *chrysostoma* (Wiedemann 1830) (Diptera: Sarcophagidae) due to their occurrence in carcasses and cadavers in the Neotropical Region (e.g. Meira et al. 2020; Jales et al. 2020). We tested the following hypotheses: (i) the larval movement will be affected by the organophosphate in a dose-dependent manner; (ii) higher doses will make the larvae more agitated, which can compromise movement and displacement control; (iii) the manifestation of the compound's effect on larval mobility does not differ in both species.

Materials and methods

Insect collection and identification

The experiments were performed using larvae of *L. eximia* and *P.* (*P.*) *chrysostoma* collected in the field on 150 g rat carcasses (licence CEUA 018/2019) placed in suspended

traps (Carmo et al. 2017) (Fig. 1). We exposed the traps for 96 h in a tropical rainforest fragment in the municipality of Natal, Northeastern Brazil (05°50′33.3″S; 35°12′05.6″W) in 2019.

The collection of immatures was performed as follows: after the entrance of flies into the trap (Fig. 1) and subsequent oviposition/larviposition and development of larvae in compartment "A", the dispersed larvae fell into compartment "B" through 40 openings of 2 cm each. The "B" compartments containing dispersing larvae were taken to the laboratory for preliminary identification and confirmation of the larval stage (Oliveira-Costa 2003).

Experimental design

To detect thresholds for behavioural responses, we designed a gradient of Terbufos exposure in four treatments: (i) non-intoxicated (control), (ii) carcasses exposed to low (5 mg/kg), (iii) intermediate (10 mg/kg) or (iv) high (20 mg/kg) concentration of Terbufos (Organophosphate, authorization n°160/2019). The Terbufos gradient of exposure was determined following its toxic tolerance range to rodents (EPA 2006) and insects (Siegfried and Scharf 2001; Jales et al. 2020). According to the U.S. Environmental Protection Agency (EPA 2013), Terbufos is a highly toxic substance, it is easily absorbed through the skin, mucous membranes, gastrointestinal and respiratory tracts, causing poisoning in rodents with doses ranging 1 and 9 mg/kg depending on the route of administration, sex of the animals and formulation (EPA 2006). In insects, this concentration varies according to the history of resistance linked to the species (Siegfried and Scharf 2001). Jales et al. (2020) showed that doses of 5 mg/kg and 10 mg/kg were sublethal in insects that fed on carcasses intoxicated with those doses, inducing changes in the diversity and life cycle of colonizers.

Female rats were orally intoxicated by the gavage method (200 µL final volume), following the protocol proposed by Jales et al. (2020) according to the toxic tolerance range established by the Environmental Protection Agency (EPA 2013) for rats. The administrations were carried out under controlled conditions in the Bioterium of the Biophysics and Pharmacology Department of the Federal University of Rio Grande do Norte, and 30 min following administration, the animals were euthanized by cervical dislocation. This period was required for the Terbufos to be metabolized and reach the bloodstream of the intoxicated animals (EPA 2013) and equally reach the animals' tissues. No other body lesion was caused to the animals after Terbufos administration once

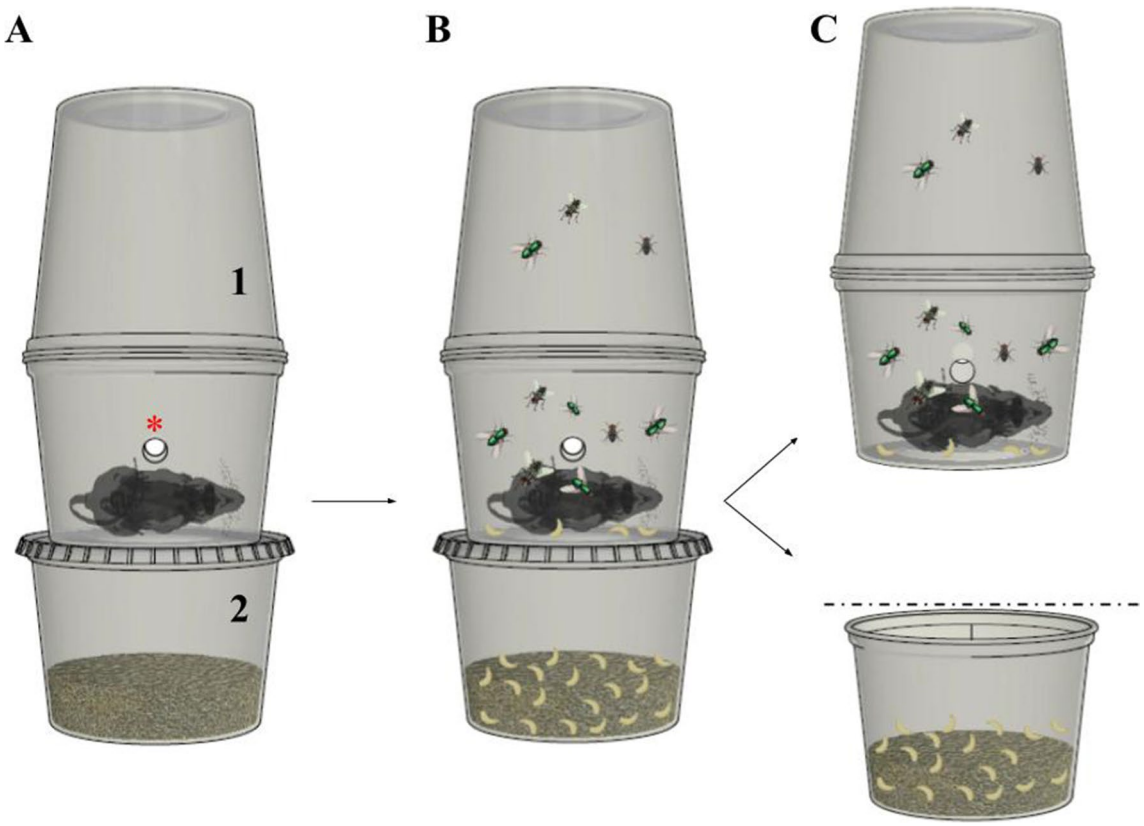

Fig. 1 Trap for collecting dispersing larvae (adapted from Carmo et al. (2017)). **A** Trap set before exposure in the field. **B** After exposure in the field. **C** Removal of dispersing larvae. The red asterisk represents the entry site of insects

tissue lesions could compromise the decomposition process. In addition to metabolization time, signs and symptoms of organophosphate intoxication (i.e. bristling fur, tremors, increased salivation, disorientation) were identified in all intoxicated animals. We used three replicates of each treatment

The field-collected larvae were kept in the laboratory under controlled conditions of temperature (27.0 ± 1.0°C) and relative humidity (50 ± 10%), without food, grouped by treatment for a maximum period of 6 h prior to the experiments to reduce intrinsic variations caused by age or stress. The instar (3rd) and larval length (Calliphoridae = 11.0–13.0 mm, Sarcophagidae = 13.0–16.5 mm) were standardized. After performing the behavioural tests, the larvae were kept in the laboratory under controlled conditions until the emergence of adults, of which more than 95% emerged and had their identification confirmed (Carvalho and Mello-Patiu 2008).

We built two experimental set-ups—Chamber for Observation of Larval Mobility (referred to as COLM onwards), one for each species, for visualization of movement and mobility behaviour. The COLM consisted of a glass arena (40 cm × 40 cm × 0.3 cm), adhered to a black background and inserted into a containment chamber constructed of odourless material (Fig. 2). At the top of the COLM, a camera with a 3968 × 2976 pixels resolution was attached, positioned in a 2-cm opening with a superior view of the arena to enable full capture of the displayed behaviour (Robinson et al. 2018). Pilot tests were performed to maximize image capture and register each type of behaviour. All tests were carried out in an environment without the circulation of people and under the same rearing conditions of temperature and relative humidity. We minimized the effect of external stimuli (e.g. smells, vibrations, sounds) and direct light entering the camera during observations.

We used 30 larvae of *L. eximia* and 30 of *P.* (*P.*) *chrysostoma* for each treatment (control, low, intermediate and high concentration), totalling 240 videos. Each larva was placed individually in the arena using soft forceps. Every recorded observation lasted 90 s, a sufficient time to characterize the movement within the spatial and temporal limits, after pilot tests. To minimize contamination, the arena was cleaned with water and neutral detergent between observations.

Behavioural parameters

The following behavioural parameters were evaluated from video analysis: (i) larval movement and (ii) larval mobility. "Movement" depicted the intensity of body activity and was characterized by the following variables (1): proportion of motionless and moving larvae, (2) movement frequency and (3) lateral contractions, also called body spasms. "Mobility" represented the individual's ability to move and change spatially and was characterized by the variables (1): effective body contraction, (2) total distance travelled and (3) spatial trajectory, following a model adapted from Berrigan and Pepin (1995) and Nichols et al. (2012). All variables are described in Table 1.

The movement trajectories were mapped and analysed using the open-source software Tracker© (version 5.1.4) with speeds of 6 and 10 fps, based on the protocol by Berrigan and Pepin (1995). The starting point of the analysis was determined from the first larval movement. The trajectory was marked using a reference point in the anterior region of the larvae, which was used by the software to analyse the movement of the cephalic piece since the larvae guide their movement from this region (Berrigan and Pepin 1995). The data were used to calculate the total distance covered with the aid of the equation of the distance between the two points below:

$$d = \sum_{i=1}^{n} \sqrt{\left(x_i - x_{i-1}\right)^2 + \left(y_i - y_{i-1}\right)^2} \quad (1)$$

where "d" is the total distance covered by the larva (in mm), "x" and "y" represent Cartesian coordinates and the index

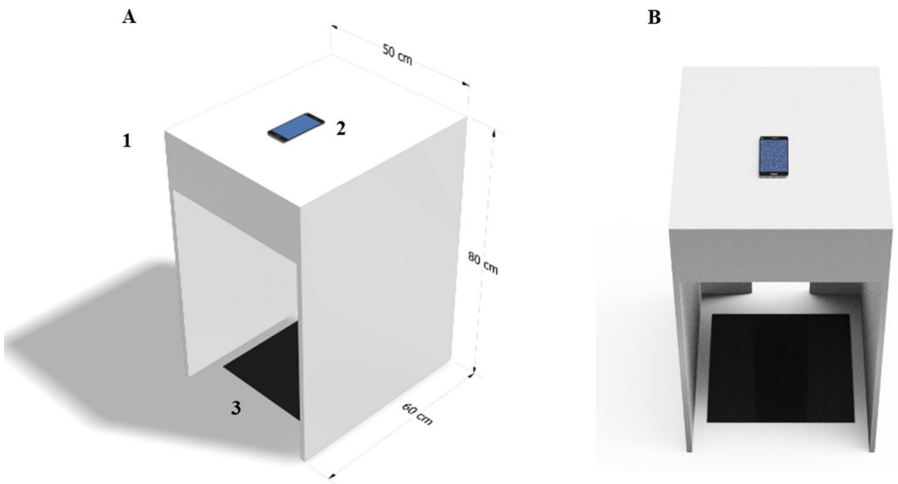

Fig. 2 Chamber for Observation of Larval Mobility (COMOL). **A** Side view—(1) containment chamber built in Styrofoam, (2) cell phone positioned over the containment chamber with a top view for filming; (3) glass arena. **B** Front view

Table 1 Parameters analysed in the chamber of observation of larval movement, main variables analysed and their characteristics

Parameter	Variables involved	Description
Larval movement	Moving larvae (%)	% of larvae that moved the body during the observation
	Speed contraction	Mean number of body contractions per minute of observation
	Lateral contractions (%)	% of larvae that presented lateral body contractions, characterized as de-synchronization of the lateral musculature
Larval mobility	Effective contraction (mm/mov)	Distance travelled per body movement, calculated as the ratio between the total distance covered by the larvae and the number of body movements per minute
	Total travel distance (mm)	Distance covered by the larva, in one minute of uninterrupted run
	Orientation of trajectory (%)	Running direction of the larva for at least 75% of the observation period: *Straight*; *Curve* or *Mixed*

"I" indicates the current position of the determined mass point. The orientation of the trajectory was considered when the larvae remained in a pathway shape for at least 75% of the analysed time.

Statistical analysis

Means (and standard deviations) of the following variables were calculated: speed contraction, total distance and effective contraction. The percentage of stationary and mobile individuals, those that presented lateral contractions, and the trajectories were calculated considering 100% the total of individuals that moved during the course. The speed contraction, total distance, effective contraction and trajectories were compared using the ANOVA test-one way with Tukey's posttest. All data were tested for normality using Shapiro-Wilk's. The percentage of moving individuals and lateral contractions were compared using the chi-square test. We used the BioStat 5.0 program, with a significance of 5%.

Results

Terbufos effect on larval movement

Terbufos altered the movement of both species. The effects varied according to species and concentration; more conspicuous effects were registered in larvae fed on carcasses exposed to the intermediate and high concentrations of Terbufos. Control larvae of both species remained active (moving) for most of the observation time (*L. eximia* = 86.7%; *P.* (*P.*) *chrysostoma* = 90.0%) (Table 2) (Video 1 and 3 of the supplementary material).

Lateral contractions varied according to species and treatment during the observation period. In control, this behaviour was observed in approximately 30% of *L. eximia* and 4% of *P.* (*P.*) *chrysostoma* larvae (Table 2). The muscle de-synchronization was more intense in intoxicated individuals (Fig. 3), being higher in the intermediate ($\chi^2_{\textit{L. eximia}} = 52.41$; $P < 0.01$ and $\chi^2_{\textit{P. (P.) chrysostoma}} = 19.53$; $P < 0.01$) and the high concentrations ($\chi^2_{\textit{L. eximia}} = 30.05$; $P < 0.01$ and $\chi^2_{\textit{P. (P.) chrysostoma}} = 34.62$; $P < 0.01$) (Fig. 3) when compared to the control and low concentration.

Terbufos increased larval movement in both species, visualized by lateral contractions and uncoordinated movements (Video 2 and 4 of the supplementary material). When fed on non-intoxicated rat carcasses, *L. eximia* larvae presented an average of 75 mov/min, while *P.* (*P.*) *chrysostoma* performed an average of 81 mov/min (Table 2). In the case of carcasses intoxicated with high concentrations of Terbufos, the body movements of *L. eximia* larvae were significantly more frequent than in control ($F_{3;112} = 4.632$; $P < 0.01$) (Table 2). However, for *P.* (*P.*) *chrysostoma*, we did not observe a pattern; there were differences in the number of movements of

Table 2 Body movement of *L. eximia* and *P.* (*P.*) *chrysostoma* larvae not intoxicated and intoxicated with different doses of Terbufos

	Control	Low	Intermediate	High
Lucilia eximia				
Moving larvae (%)	86.7	86.7	76.6	96.7
Speed contraction (mov/min)	75.0 ± 36.6[a]	78.0 ± 25.9[a]	90.7 ± 29.3[ab]	101.0 ± 23.1[b]
Lateral contractions (%)	26.9[a]	24.1[a]	65.1[b]	58.6[b]
Peckia (*Peckia*) *chrysostoma*				
Moving larvae (%)	90.0	45.9	93.4	68.3
Speed contraction (mov/min)	81.0 ± 19.8[a]	79.8 ± 18.2[b]	89.3 ± 29.5[a]	77.0 ± 18.2[bc]
Lateral contractions (%)	3.8[a]	18.1[a]	39.2[b]	30.7[b]

[a, b and c]Values followed by different letters represent statistical differences

the control group when compared to intermediate and high doses ($F_{3;112} = 20.444$; $P < 0.01$).

Terbufos effect on larval mobility

Terbufos altered the spatial mobility of immatures of both species. In the control group, *L. eximia* larvae moved 200 mm in 1 min of observation (Fig. 4), 2.5 mm per body movement (Table 3). *Peckia* (*P.*) *chrysostoma* travelled an average of 300 mm/min (Fig. 4), with 3.6 mm per movement (Table 3). When exposed to a high concentration of Terbufos (20 mg/kg), *L. eximia* larvae moved faster and longer when compared to the other groups ($F_{3;112 =}$ 15.842; $P < 0.01$). Larvae from this group travelled approximately 390 mm, with 3.6 mm per movement.

The distance covered by *L. eximia* exposed to control, low and intermediate doses did not differ from each other ($P > 0.05$). *Peckia* (*P.*) *chrysostoma* exposed to an intermediate dose of Terbufos were faster ($F_{3;112} = 7.173$; $P < 0.01$) than under other treatments, covering an average distance of 370 mm, with 3.9 mm per movement, differing from the other groups (Table 3). However, the distance covered by *P.* (*P.*) *chrysostoma* from carcasses intoxicated with high dose of Terbufos was lower than the non-intoxicated ($P < 0.05$),

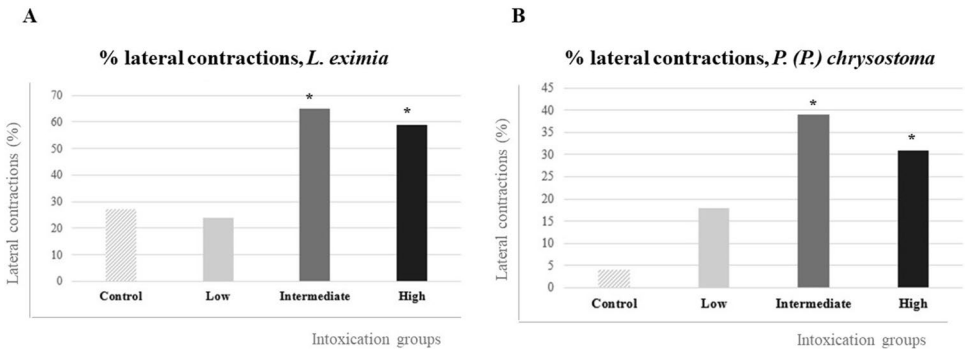

Fig. 3 Percentage of lateral contractions in non-intoxicated and intoxicated *Lucilia eximia* (**A**) and *Peckia* (*P.*) *chrysostoma* (**B**) larvae with different concentrations of Terbufos. *Significant difference when compared to the control

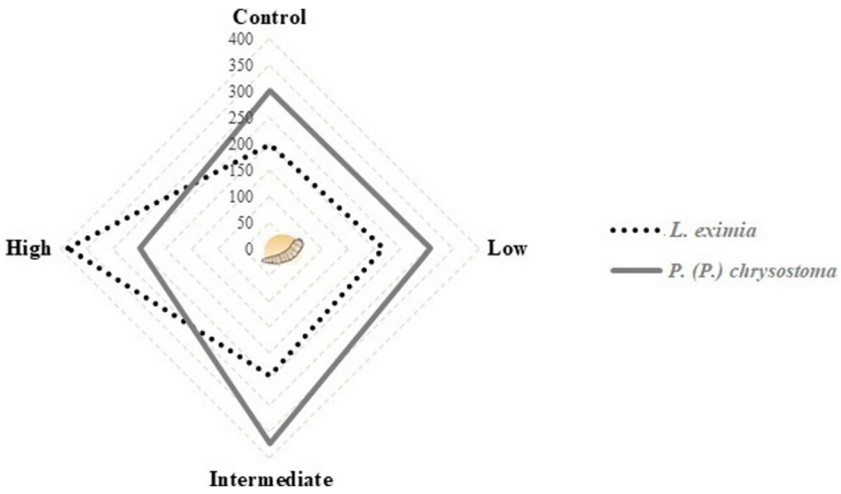

Fig. 4 Total distance travelled (mm) by *Lucilia eximia* and *Peckia* (*P.*) *chrysostoma* not intoxicated and intoxicated with different concentrations of Terbufos

Table 3 Body mobility of *L. eximia* and *P.* (*P.*) *chrysostoma* larvae not intoxicated and intoxicated with different doses of Terbufos

Larval mobility	*Lucilia eximia*				*Peckia* (*Peckia*) *chrysostoma*			
	Control	Low	Intermediate	High	Control	Low	Intermediate	High
Effective contraction (mm/mov)	2.5 ± 0.9^{a}	2.5 ± 1^{a}	2.7 ± 0.9^{a}	3.6 ± 1^{b}	3.6 ± 0.7^{a}	3.7 ± 1^{ab}	3.9 ± 0.9^{c}	3.7 ± 0.5^{b}
Total travel distance (mm)	199 ± 57^{a}	217 ± 44^{a}	242 ± 77^{a}	386 ± 124^{b}	302 ± 11^{a}	306 ± 122^{b}	372 ± 161^{c}	289 ± 76^{b}
Trajectory (%)								
Straight	52.0	38.4	60.9	51.7	33.3	45.4	36.6	7.7
Curved	20.0	23.2	21.7	31.0	59.2	45.4	48.0	84.6
Mixed	28.0	38.4	17.4	17.3	7.5	9.2	16.0	7.7

[a, b and c]Values followed by different letters represent statistical difference

even with lateral contractions. Finally, the shape of the trajectory did not differ between control and intoxicated larvae under the tested conditions. *Lucilia eximia* larvae showed a mostly linear trajectory in all studied groups, while *P.* (*P.*) *chrysostoma* maintained a mostly mixed configuration, regardless of the group.

Discussion

This study empirically demonstrates that feeding on Terbufos-contaminated rat carcasses alters the body movement and mobility of *L. eximia* and *P.* (*P.*) *chrysostoma* larvae. Parameters such as the frequency of body contractions and lateral contractions, effective contractions and distances travelled are affected by exposure to the neurotoxicant in a dose-dependent response.

Sublethal effects of insecticide exposure affect insects' behavioural parameters, such as foraging, selection of oviposition sites and locomotion patterns, especially in cases related to neurotoxicants such as organophosphates (De França et al. 2017). The different results found here for body activity of *L. eximia* and *P.* (*P.*) *chrysostoma* suggest that the effect of Terbufos may differ between species of the same trophic.

Two types of cholinergic receptors can be activated during immature movement, the muscarinic and nicotinic receptors. The increase in acetylcholine in the synaptic clefts by inhibition of acetylcholinesterase increases muscle contractions and movement of Drosophilidae larvae. However, activating only muscarinic receptors through the agonist muscarine causes a reduction in excitation and subsequent lethargy of them (Malloy et al. 2019). Thus, it is valid to hypothesize that the difference in excitability between *L. eximia* and *P.* (*P.*) *chrysostoma* may result from specific intrinsic biochemical factors involved in the permanence of acetylcholine in synaptic clefts. In this case, exposure to Terbufos may lead to greater activation of muscarinic receptors, reducing the activity of *P.* (*P.*) *chrysostoma* larvae. The increment in distance covered and frequency of body contractions observed here may be associated with the effect of Terbufos as an acetylcholinesterase inhibitor (EPA 2013), causing an increase of acetylcholine in the neuromuscular clefts and, consequently, alteration in the intoxicated larvae muscular contraction pattern.

Enhanced frequency of body contractions and distance covered by intoxicated *L. eximia* and *P.* (*P.*) *chrysostoma* larvae are supported by studies on insect exposure to sublethal doses of Malathion and Pestanal, which induced hyperactivity, increased body contractions and movement speed in *Encyrtus saliens* Prinsloo and Annecke 1978 (Hymenoptera: Encyrtidae) (Hoy and Dahlsten 1984) and *Drosophila* sp. (Diptera: Drosophilidae) (Malloy et al. 2019). Implications of the enhanced movement/mobility include changes are depicted in Fig. 5.

Gosselin et al. (2011) and Tracqui et al. (2004) report the limitations inherent to entomotoxicological studies, especially those related to the direct association between the concentration of the toxicant in the environment (either in an animate or inanimate substrate) and those found in living insects. We overcame these caveats by using contaminated carcasses in a feasible range of concentrations.

From a forensic entomology standpoint, the reliability and feasibility of insects or insect tissue as biological evidence of

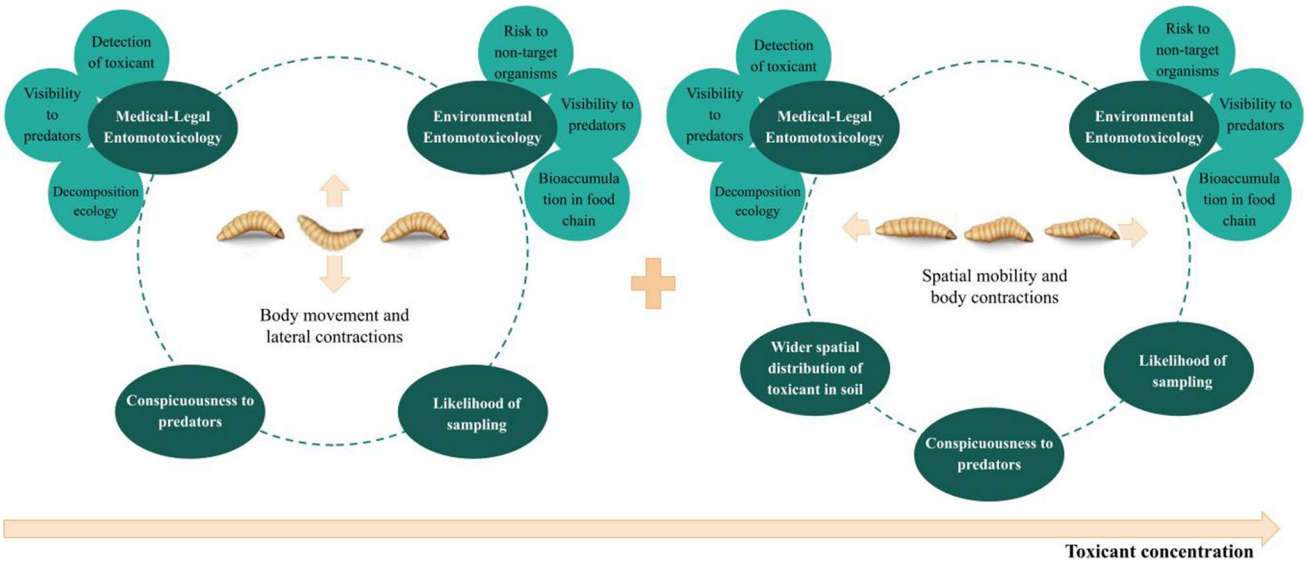

Fig. 5 Scheme showing the implications of changes in body movement and mobility of larvae exposed to carcasses intoxicated with Terbufos (Organophosphate)

toxicant contamination in carrion depend not only on insect taxa but also on the feeding substrate and toxicant administration (Gosselin et al. 2011; da Silva et al. 2017; Chophi et al. 2019). Intoxication of carcasses with organophosphate cause changes in the diversity of colonizing insects, the time of development and the survival of necrophagous larvae fed on Terbufos-intoxicated carcasses (Jales et al. 2020).

Sampling entomological pieces of evidence at crime scenes under suspected intoxication will be affected by the hyperactivity of larvae with involuntary body contractions. Non-intoxicated larvae of Calliphoridae at the post-feeding stage can disperse up to 3 m away from a cadaver/carcass and can bury themselves up to 5 cm in depth (Godoy et al. 1995). It is possible that more agitated/mobile larvae can disperse further and bury more profoundly into the soil, which will widen the sampling space, in a tridimension scale, to collect older larvae. That is because the estimation of minPMI is based on older dispersant insect larvae (Introna et al. 2001; Oliveira-Costa 2003).

Some fundamental aspects of larval dispersal have been taken into account in the design of mathematical models fitted to standard diffusion equations, which describe passive diffusion and larval velocity (Von Zuben et al. 1996). Our data can be fitted into such models to predict the spatial spread of entomological evidence and contribute directly to insect collections at crime scenes.

The differential effect of Terbufos on *L. eximia* and *P.* (*P.*) *chrysostoma* can directly impact complex interactions between necrophagous species, altering processes such as competition and predation. Less mobile larvae of *P.* (*P.*) *chrysostoma* become more vulnerable to predation by larvae of *Chrysomya albiceps* (Wiedmann 1819) (Barbosa et al. 2021). On the other hand, more significant agitation and mobility of *L. eximia* may make them more conspicuous for vertebrate predators, such as lizards and birds (Schricker and Stephen 1970). The result of this balance—between the risks and benefits of greater mobility—deserves attention in future ecological studies.

Jales et al. (2020 and 2021) show that the presence of Terbufos altered both the visitation and colonization profile and the development time and mortality of scavenger dipterans associated with intoxicated carcasses. Thus, by altering the resource decomposition, the presence of Terbufos can hinder efficient nutrient cycling, compromising the role of flies as natural decomposers. As described in the present study, we showed that Terbufos alters larval movement and mobility and may favour the development of some taxa in the face of critical biological events such as predation. Therefore, our study complexes the indirect effect of toxic agents on the ecological interactions of necrophagous flies.

Organophosphates are lipophilic compounds with long half-lives so that they can accumulate in the environment, and their metabolites can be transferred from one organism to another (Jayaraj et al. 2016). By acting as recyclers of organic matter (Cicková et al. 2015), necrophagous larvae can alter the trophic chain locally—the toxicant can be transferred from the carcass to the larvae and from the larvae to the soil, water or predators. The toxicant's wider spatial distribution expands the environmental contamination cycle, and this process can be detected in necrophagous larvae. Its indiscriminate use has significantly increased environmental contamination, affecting air, soil and water quality (Kaushal et al. 2021).

In conclusion, sublethal Terbufos intoxication induces non-linear effects in necrophagous larvae, whose responses depend on the dose and the species. This information can help us understand how physiological-behavioural changes can influence critical ecological processes and interactions in the necrobiome. In opposition to worldwide environmental policies, the Brazilian government has approved a record number of high-persistence pesticides forbidden in Europe and the United States since 2019 (Alpino et al. 2020). The consequences of indiscriminate use—and the fate of—these toxicants are yet to be fully understood.

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