RESEARCH ARTICLE



An exploratory study of energy reserves and biometry as potential tools for assessing the effects of pest management strategies on the earwig, Forficula auricularia L

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Received: 8 February 2018 / Accepted: 22 May 2018 / Published online: 31 May 2018 © Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

Apple orchards are heavily treated crops and some sprayed insecticides are recognized to have toxic effects on non-target arthropods. Earwigs are important natural enemies in pip-fruit orchards and contribute to the biological control of aphids. In addition, due to their ease of capture and identification, they are an interesting potential bioindicator of the possible detrimental effects of different orchard management strategies. In this study, we measured the energy reserves and some morphological traits of *Forficula auricularia* L. sampled in apple orchards under management strategies (organic versus integrated pest management (IPM)). We observed a significant decrease in mass (22 to 27%), inter-eye width (3%), and prothorax width (2 to 5%) in earwigs from IPM compared to organic orchards. Energy body reserves also confirmed these results with a significant decrease of 48% in glycogen and 25 to 42% in lipid content in earwigs from IPM compared to organic orchards. However, the protein content was approximately 70% higher in earwigs from IPM than in organic orchards. Earwigs sampled in IPM orchards may adapt to minimize the adverse toxic effects of pesticide treatments using a large number of strategies, which are reflected in changes to their energy reserves. These strategies could, in turn, influence the population dynamics of natural enemies and impair their role in the biological control of pests in apple orchards.

Keywords Earwig · Energy reserves · Glycogen · Lipid · Protein · Biometry · Pest management strategy

Introduction

The United Nations Food and Agricultural Organization reported that the world's population will reach to nearly

10 billion by 2050. This will lead to an increase in world food production in order to keep pace with the demand of this growth (Gill and Garg 2014). Agricultural chemicals can enhance crop productivity. However, pesticides can also have

Highlights

- Females and males from organic orchards have higher weight
- Inter-eye and prothorax widths are lower in IPM orchards
- Lipid and glycogen contents decrease in earwigs coming from IPM orchards
- · Protein increase in females and males from IPM versus organic orchards

Responsible editor: Philippe Garrigues

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11356-018-2371-x) contains supplementary material, which is available to authorized users.

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negative effects on the environment, ecosystem, and biodiversity. Pesticides can have an impact on non-target and beneficial species through direct or indirect routes, such as water contamination and runoff, pesticide residues, and by consuming food that has been sprayed (Martinou et al. 2014). The destruction of these beneficial arthropods can exacerbate pest problems as they play an important role in regulating pest population levels. In France, apple orchards are heavily treated with, on average, more than 30 pesticide applications per year (Sauphanor et al. 2009; Mazzia et al. 2015). Two pest management strategies are generally identified in commercial apple orchards: integrated pest management (IPM) and organic farming. The European earwig, Forficula auricularia L. (Dermaptera: Forficulidae) is a common, sub-social, and omnivorous insect in agroecosystems worldwide. These arthropods are commonly found in apple orchards. Adults and nymphs are nocturnal and hide during the day under shelters such as bark or in holes in the ground. They can be found in high abundance in artificial shelters and traps (Sauphanor 1992; Fountain and Harris 2015). In apple orchards, earwigs are a key biological control agent against numerous insect pests such as aphids, psyllids, and spider mites (Dib et al. 2010; Debras et al. 2007; Romeu-Dalmau et al. 2012). Their ease of capture and identification make them an interesting potential bioindicator of the intensity of pesticide use in orchards (Dib et al. 2010).

Earwigs are sensitive to different management strategies in IPM apple orchards where several pesticides can be applied, e.g., organophosphates (Ffrench-Constant and Vickerman 1985; Malagnoux et al. 2015a), pyrethroids (Badji et al. 2004), or neonicotinoids (Shaw and Wallis 2010; Malagnoux et al. 2015b). Other agricultural practices such as tillage and the presence and quality of hedges can also influence earwig abundance (Sharley et al. 2008; Moerkens et al. 2012) and the presence and quality of hedges (Debras et al. 2007). Management strategies in apple orchards also influence diversity in the earwig community, with F. auricularia being less sensitive to insecticides than Forficula pubescens (Malagnoux et al. 2015b). Studies concerning F. auricularia focused on its ecological role in orchards (Kölliker and Vancassel 2007; Malagnoux et al. 2015a) or the direct impact of commonly used pesticides on its populations (Ffrench-Constant and Vickerman 1985; Campos et al. 2011; Malagnoux et al. 2015a; Fountain and Harris 2015). However, there is, to our knowledge, little available data on the negative physiological effects of pesticides on energy reserves in these earwigs.

Glycogen and the lipid, triacylglycerol, are the predominant energy reserves stored in the fat bodies distributed throughout the body of the insect (Arrese and Soulage 2010). This compartment plays an essential role in energy storage and has important functions throughout the insect's life cycle including growth, timing of metamorphosis, and

egg development. In addition, the fat body also synthesizes most of the hemolymph proteins and circulating metabolites. Glycogen can be rapidly hydrolyzed to glucose in the hemolymph of insects to be used as a glycolytic fuel (Steele 1982). The glycogen content may vary depending on physiological state (Anand and Lorenz 2008) or environmental conditions (Chowanski et al. 2015).

Triacylglycerol is formed by fatty acids that can be used for energy production through β -oxidation. Lipids are always the main component of the fat body which, in insect, represents more than 50% of their dry weight (Ziegler 1991). Lipids provide energy during diapause (Hahn and Denlinger 2007) for embryo development (Ziegler and Van Antwerpen 2006) and sustained flight activity (Van der Horst 2003).

Proteins can also supply energy through catabolism and are essential in tissue building and repair. For this reason, it is also relevant to study the variability of insect protein content under different conditions.

Living organisms use reserves to maintain their bodies, growth, development, and reproduction (Arrese and Soulage 2010). Under stressful conditions, there is an increased expenditure associated with detoxification which can have a negative impact on life history traits in the life cycle. After exposure to pesticides, it has been shown that energy reserves decreased in several insects (Ribeiro et al. 2001; Nath 2003; Da Silva et al. 2004; Rharrabe et al. 2008).

A low level of energy reserves could potentially influence the population dynamics of earwigs and decrease their role in pest management in apple orchards. Markow (1995) and Nestel et al. (2016) showed that ecological stressors can affect developmental instability, which leads to reduced fitness. Morphological parameters, e.g., mass or insect size can be used to detect the effects of environmental stress on an organism.

The aim of our study was to assess the effects of two orchard management strategies, organic and IPM practices, on earwig (*F. auricularia*) weight; energy reserves (glycogen, lipid, and protein contents); and two morphometric parameters (inter-eye and prothorax widths).

Materials and methods

Chemical compounds

Sodium chloride, Tris-HCl, trichloroacetic acid, ethanol, oyster glycogen, glucose, sodium acetate, amyloglucosidase from *Aspergillus niger*, phosphovanillin, sulfuric acid, and bovine serum albumin were purchased from Sigma-Aldrich® (Saint Quentin-Fallavier, France) while glucose RTUTM was purchased from bioMérieux SA® (Geneva, Suisse).



Earwigs

Male and female adults of F. auricularia L. earwigs were caught in July 2015 using cardboard traps placed on apple tree branches in five orchards located in Noves, near Avignon (south-eastern France). The landscape around these orchards is similar and characterized by the presence of a dense network of windbreak hedgerows, which protects orchards against the prevailing northern winds. Since this species is univoltine, these adults were of roughly the same age. The five orchards had two management strategies: three were under IPM management and thus applied synthetic plant protection products and two were under organic farming and used a restricted range of products (such as Carpovirusine, spinosad, copper, sulfur ...). Twenty male and 20 female earwigs were collected from each management system (i.e., 40 and 60 earwigs per sex for organic and IPM strategies, respectively), frozen and stored at -20 °C until biochemical and morphometry measurements were carried out.

Morphometric measurements

Frozen earwigs were slightly thawed to avoid change to morphometric measurements. All morphometric measurements of earwigs were carried out using an eyepiece micrometer on a Nikon® binocular microscope. Measurements were first recorded as units of the ocular micrometer scale to the nearest 1 μm and were then converted into millimeters ($\pm\,0.1$ mm). Each earwig was positioned carefully to avoid bias in measuring and to achieve the same plane of view. A single person carried out all the measurements to avoid user bias. We measured the maximum prothorax and inter-eye (i.e., distance between the eyes) widths. The inter-eye width was measured in a straight line between the two eyes and the prothorax width between two reference points (Fig. 1).

Scanning electron microscopy

We used scanning electron microscope photography (Philips XL 30 ESEM with detector SE) to obtain images (e.g., Fig. 1) of dried specimens coated with a gold layer of 10^{-12} m. The equipment was set to operate at 20 kV acceleration voltage, at $\times 35$ for the magnification and at 28.9 mm for the working distance.

Crude extract preparation

After morphometric measurements, earwigs (male and female) were weighed individually using a Precisa® XT120A electronic precision balance with a readability of 0.1 mg. The whole body of the earwig was homogenized on ice in 10% (w/v) low-salt buffer containing 10 mM Tris-HCl (pH 7.3) and 10 mM NaCl and centrifuged for 10 min at 3000g. This

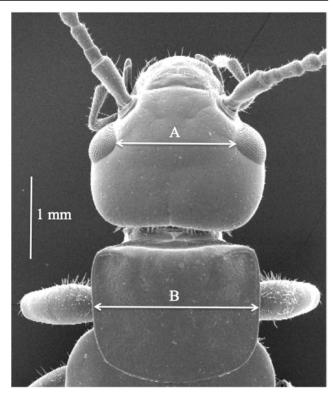


Fig. 1 Scanning electron microscopy dorsal view of head and prothorax of a male earwig showing the measurement method applied to selected body parts: the inter-eye (a) and the prothorax (b)

supernatant was the crude extract on which further glycogen, lipid, or protein measurements were carried out.

Biochemical measurements

Determination of glycogen content

The method, based on enzymatic hydrolysis of glycogen by amyloglucosidase (EC 3.2.1.3), was used according to Parrou and François (1997). Solution trichloroacetic 4% acid was added to 250 µL of crude extract (v/v) for deproteinization and the solution was centrifuged at 3000g for 1 min at 4 °C. After centrifugation, two volumes of 95% ethanol were added to precipitate the glycogen, which was present in the supernatant. Glycogen was finally pelleted by centrifugation at 5000g for 5 min at 4 °C. Ethanol was removed and the pellet was dried at room temperature. The dried pellet was incubated for 2 h at 60 °C in 500 µL of 0.2 M sodium acetate, pH 5.2, containing 7 UI of amyloglucosidase. After incubation, the solution was cooled on ice for 5 min and the amount of glucose generated from glycogen was determined using the Glucose RTUTM method adapting to 96-well microplate format. The reaction mixture (275-µL final volume), containing 250-µL Glucose RTUTM and 25 µL glucose produced above, was left to stand for 20 min at room temperature before glucose



detection by measuring the absorbance at 505 nm. The amount of glucose was calculated from a standard curve (A 505 = f [glucose]) containing pure glucose as a standard treated within the same conditions. Because the final values were included, the amount of glycogen was corrected for the glucose content in samples that were not incubated with amyloglucosidase.

Determination of lipid content

Total lipids were determined using a sulfo-phospho-vanillin reaction according to the method of Frings et al. (1972) adapted to a 96-well microplate format. Two hundred ninety-four-microliter concentrated sulfuric acid was added either to 6 μ L of earwig crude extract or to 6 μ L of a standard solution (olive oil). The solutions were homogenized and placed in boiling water for 10 min and cooled on ice for 5 min. A 700 μ L of phospho-vanillin reagent was added to each extract or lipid standard, homogenized and incubated at 37 °C in a water bath for 15 min. After the samples were allowed to cool for 5 min, the absorbance was recorded at 540 nm.

Determination of protein content

Total protein content was determined by the Lowry method modified according to Markwell et al. (1978), with bovine serum albumin as the standard. The energy reserve values were derived directly from a standard curve prepared with known concentrations of bovine serum albumin, glycogen, and lipid. All assays were run in triplicate. All the above components were expressed as milligrams per gram of earwig.

Statistical analysis

For all the parameters (energy reserves and morphometric measurements), the effects of orchard management (organic versus IPM) and earwig sex were included in a mixed model with management and sex as fixed factors and apple orchard as a random factor. The assumptions of normality and homogeneity of variance were first tested, and log transformation was applied when required (weight and morphometric measurements). We compared all the means for both factors using the "Ismeans" package. The effects of the factor were thus assessed with the general model but sometimes also on simplified models (one factor only) depending on the significance of the interaction between the factors. All computations were carried out using R (version 2.15.3).

Results

Weight

Both earwig sex and orchard management had a significant effect on earwig weight (p < 0.001) and no significant interaction was observed between the two factors (p = 0.37). Regardless of orchard management strategy, male earwigs were significantly heavier than females. The weight of both sexes of earwigs sampled in organic orchards was also higher than those sampled in IPM orchards (Fig. 2).

Morphometric parameters

Prothorax and inter-eye widths

Similar observations to above were obtained with the morphometric analysis of the earwigs. For both prothorax and intereye widths measurements, sex did not have a significant effect, and the interaction between sex and management strategy was also not significant. Only management strategy had a significant effect with significantly smaller prothorax (p=0.032) and inter-eye widths (p=0.026) for earwigs sampled in IPM orchards compared to those sampled in organic orchards (Fig. 3). The decrease in inter-eye width was about 3% and between 2 to 5% for the prothorax width.

Biochemical analysis

Glycogen

The interaction between sex and management strategy was highly significant (p < 0.001) for the glycogen content. For both management practices (p < 0.001), sex had a significant effect with females having higher glycogen content than males. Management practices had no overall effect but when only females were considered, higher glycogen contents were

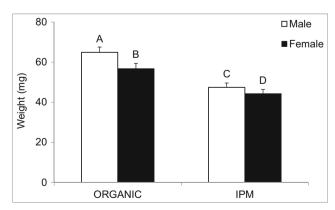


Fig. 2 Mean weight (+SE) of the female and male adults of earwigs depending on the management strategy in apple orchards. (n = 40 for males and n = 40 for females for each strategy)



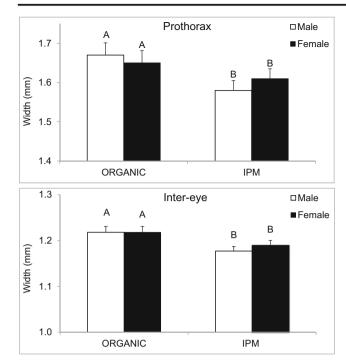


Fig. 3 Measurements of morphometric parameters: prothorax and intereye widths (means + SE) according to the management strategy in apple orchards. For each morphometric parameter, bars with the same letters are not significantly different from each other (n = 40 for males and females for each strategy)

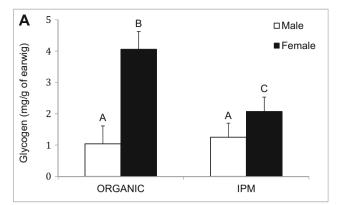
observed in earwigs from organic compared to IPM orchards. No significant difference was observed for males explaining the significant interaction.

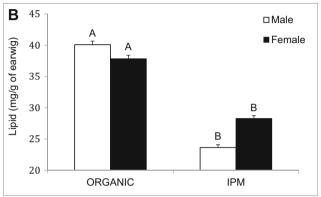
Lipids

Sex and the interaction between sex and management strategy had no significant effect on the earwig lipid contents. However, management strategy had a significant effect (p = 0.008) with lower lipid content in earwigs sampled in IPM orchards than those sampled in organic orchards (23.49 \pm 10.57 mg versus 32.10 \pm 10.39 mg and 24.23 \pm 11.12 mg vs 29.49 \pm 10.57 mg, respectively, for females and males (Fig. 4b)). On average, earwigs sampled in IPM orchards had 25 to 42% less triacylglycerol than those under organic management.

Proteins

Sex and the interaction between sex and management strategy had no significant effect on earwig protein contents. However, management strategy had a significant effect (p=0.016) with higher body protein content in earwigs from IPM than organic orchards (Fig. 4). Earwigs in IPM orchards had 70% more protein than those under organic management for both female and male.





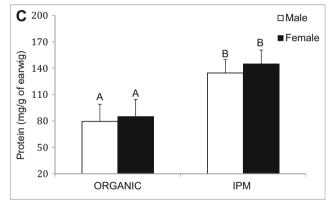


Fig. 4 Energy reserves content of the female and male of earwigs for each management strategy in apple orchards. **a** Glycogen. **b** Lipid. **c** Protein. Data represent the means (+SE). Bars with the same letters are not significantly different form each other (n = 40 for males and females for each strategy)

Discussion and conclusion

It is well recognized that agricultural management practices including the use of pesticides affects non-target species and leads to a decline in biodiversity in agroecosystems (Mclaughlin and Mineau 1995; Kromp 1999; Nash et al. 2008). The hazard of pesticide usage depends on the application rate, intrinsic toxicity, and the level of uptake, persistence, volatilization, metabolization, and bioavailability of the active ingredient (Sánchez-Bayo 2009). The exposure time is also a critical variable in risk assessments of agrochemicals. Organisms may develop a number of strategies to minimize



the negative toxic effects induced by chemical stress. All responses to resist toxicants are costly for the organism in terms of metabolic resources and energy demand (Calow 1991). Because the energy resources of organisms are limited and used for growth, reproduction, and basal metabolism, the additional metabolic costs due to pesticide management results in a reallocation of energy resources and can increase energy consumption leading to reduced energy reserves (Congdon et al. 2001; Yasmin and Souza 2010). Resource allocation and life history strategies are inevitably interconnected for all organisms because of the competition and the limitation of available energy reserves either in the environment or within the individual itself.

Our results confirm that pest management strategy can result in changes to energy reserves in earwigs. Our measurements of energy reserves indicated a lower lipid and glycogen contents in earwig bodies collected from IPM compared to organic orchards. The major differences between the organic and IPM strategies were lower total treatment frequency index (TFI: total number of pesticide treatment weighted by the ratio of the dose used to the recommended dose (Jørgensen 1999), and agricultural practices such as tillage, total weed removal, or the presence and quality of hedges in the organic orchard. In organic strategy, pest control is based on a greater use of mineral fungicides (copper and sulfur) and natural insecticides (granulovirus, mineral oils, and spinosad). In contrast, nonorganic orchards mainly used synthetic pesticides which are known to be more efficient against pests. Beside the fact that the total TFI was slightly but significantly lower in organic orchards (22.2) than in IPM orchards (26.6), it has been previously shown that the earwig community of F. auricularia was lower in IPM orchards compared to organic orchards and was correlated to a higher insecticide use (4.9 in organic versus 9.2 in IPM) (Malagnoux et al. 2015b). By their toxicity, insecticides increase lethality and/or affect the behavior of non-target organisms. For example, high sensitivity of F. auricularia to many insecticides was observed after exposure to organophosphates (Ffrench-Constant and Vickerman 1985; Malagnoux et al. 2015a), pyrethrinoids (Badji et al. 2004), and neonicotinoids (Shaw and Wallis 2010; Malagnoux et al. 2015b). Moreover, insecticide exposure induced disturbed behavior in predation activity of earwigs (Malagnoux et al. 2015a), cast production of earthworms (Jouni et al. 2018), feeding rate of the white shrimp (García-de la Parra et al. 2006), or locomotion of zebrafish (Tilton et al. 2011). Because the process of food detection involves complex nervous activity that can be disrupted by neurotoxic pesticides (Desneux et al. 2007), impairments on behavior modify the feeding ability of the organisms and leads to a decrease in their energy reserves. Then, despite the possible loss in prey availability induced by higher TFI in IPM orchards (Sauphanor and Stäubli 1994; Altieri 1999; Pekar 1999; Dib et al. 2016), we assumed that the main factor responsible for depletion in energy reserves lies to a direct effect of insecticides on the organisms rather than on environmental modification (i.e., food availability). Our assumption was supported by several studies that have been conducted in laboratory experiments without food limitation. These studies have shown that exposure to organophosphorus insecticides, either severely disturbed glycogen metabolism in the Lepidoptera *Bombyx mori* (Nath 2003), or induced a significant depression of glycogen, lipid, and protein contents in the terrestrial isopod *Porcellio dilatatus* (Ribeiro et al. 2001).

Glycogen is a major glucose reserve and can be rapidly mobilized whereas lipids and proteins are considered as long-term reserves (Arrese and Soulage 2010). In our study, the protein values were higher in male and female earwigs sampled from the orchard under IPM. Our results are in agreement with those of Jeon et al. (2013) who observed an increase in protein of *Daphnia magna* after carbaryl exposure, associated to a decrease in lipid and carbohydrate contents, or with the results of Staempfi et al. (2007) in the soil arthropod, Folsomia candida after dinoseb exposure. Reallocation of energy reserves to maintain homeostasis under pesticide exposure mainly focuses on protein induction to improve defense mechanisms and they often involve overproduction of detoxification enzymes (Calow 1991). According to those results and ours, it is unlikely that the increase in protein levels in IPM practices may offset the reduction in glycogen and lipid but, on the contrary, the consumption of glycogen and lipid content allow protein synthesis, which is highly cost effective. According to the thermodynamics of each nutrient (17.5 kJ/g glycogen, 24 kJ/g protein, and 39.5 kJ/g lipids) (Gnaiger 1983), total energy reserves are higher in term of global kilojoules in IPM orchards by 19 and 28% for male and female earwigs, respectively. However, this amount of energy is not available fast enough. When the organisms become unable to cope with stress or pollutants, the energy budget results of imbalance between the rate of synthesis and rate of degradation. Then, after the use of glycogen and lipids, proteins constitute the last energy reserve to change or be used upon stress exposure (Ferreira et al. 2015). In this context, studies have reported that a decrease of protein content is associated to an increase in mortality. It was the case observed by Staempfi et al. (2007) where a biphasic concentration-response linked to the phenomenon of hormesis was observed, showing that after having produced an effort to increase growth and reproduction, lethality increases. Rharrabe et al. (2008) demonstrated that for Lepidoptera *Plodia interpunctella* Hübner, the insecticide azadirachtin caused a severe reduction in protein levels and led larval weight loss, developmental delay followed by high lethality. These authors postulated that azadirachtin exerts its effect on insects by modifying the protein synthesis capacity of the fat body as also observed for Spodoptera litura (Huang et al. 2004). Moreover, Ribeiro et al. (2001) observed a decrease in



protein content in parathion-intoxicated isopods along with an increase in mortality, indicating that physiological adaptability to compensate pesticide stress requires high energy but associated with ineffective demand.

In addition to agricultural practices, other factors can have an impact on rates of energy consumption, e.g., age and environmental conditions including temperature or dehydrating state. Anand and Lorenz (2008) reported that the cricket, Gryllus bimaculatus, fat body energy reserves were age-dependent. Climatic conditions also change abundance of energy reserves (Chowanski et al. 2015). F. auricularia is univoltine, and in south of France, earwigs reach the adult stage in early summer. It is noteworthy that because all the organisms analyzed were caught in July, they were of roughly the same age. Moreover this study was carried out on a same town and thus a reduced geographic area (less than 10-km radius), climatic conditions, and landscape type are very similar. In this region, all orchards are surrounded by hedges due to the prevailing wind. Moreover, earwigs are nocturnal, inactive, and hidden during the day inside the cardboard traps. When they are captured, we can assumed they are all in the same diet stage with an almost empty gut that will limit differences in energy reserves. Thus, in the studied apple orchards, the main factor governing energy budget in F. auricularia was assumed to be management strategy and suggested that insecticide use, quantitatively and qualitatively, is able to decrease F. auricularia energy reserves. Other pollutant molecules can also lead to changes in energy reserves under natural conditions. Indeed, studies of chronically polluted with metals effects on the beetle Pterostichus oblongopunctatus, showed a significant decrease in energy reserves (Bednarska et al. 2013). Givaudan et al. (2016) showed higher lipid and glycogen contents in earthworms collected in abandoned and organic orchards compared to IPM orchards. Thus, the depletion of such biochemical constituents can be due to the major mobilization of these nutrients in response to the toxic effect of the pesticides.

Biochemical parameters appear to be good biomarkers for monitoring environmental management strategies but they need to be complemented with morphological parameters to confirm the impact of orchard management practices. Weight and morphometric traits in our earwigs were lower in IPM than in organic orchards. Earwigs from IPM orchards have to make tradeoffs between maintenance, growth, storage, and resistance to agrochemicals so as to maximize their fitness. The observed reduction in their prothorax and inter-eye widths is likely to be the consequence of these tradeoffs induced by pesticides. A lot of studies in other insects showed that pesticides can lead to morphometric variations. Marcus and Fiumera (2016) showed that exposure to atrazine, the one of the most widely used herbicides significantly affected male and female adult body size of Drosophila melanogaster. In Pterostichus melasitalicus, a carabidae, morphometric analyses also showed that dimethoate application caused a reduction in body or elytra lengths in females (Giglio et al. 2011). Giglio et al. (2017) showed in another carabidae, *Calathus fuscipes*, that morphological parameters, e.g., body and pronotum width decreased after an exposure to the larvicide lambda-cyhalothrin. Moreover, studies of the effects of an inorganic insecticide (boric acid) against *Blattella germanica* showed a reduction in the size of basal oocytes, which affected its reproduction (Habes et al. 2013). All these studies suggest that agrochemical molecules can have an impact on the morphometric parameters of exposed organisms.

Our results underline the possible modifications in biochemical and morphometric parameters in earwigs due to orchard management strategies. Body energy reserves and prothorax and inter-eye widths in earwigs may be interesting biomarkers to characterize changes induced by agricultural farming both with pesticides and for other practices in orchards. This study shows more widely that F. auricularia, along with some other characteristics (ease of capture, identification, univoltine species) is an interesting potential bioindicator of the orchard management practices, especially in pip-fruit orchards, where it is considered an active natural enemy of aphids. In order to characterize the specific effects of pest management strategy on earwigs (direct and indirect effects), future studies should also be performed in abandoned orchards to assess the consequence of a pesticide-free environment. Other morphometric parameters could additionally be investigated to further determine if agricultural strategies influence other earwig life history traits and further weakens these non-target species. In conclusion, this present study highlights the need to develop an integrated approach to assess both the physiological and biochemical modifications induced by pest management strategies on natural enemies.

Acknowledgments We thank Alain Tonetto (Pratim, Aix Marseille University) for assistance in taking the electronic microscopy photos.

Funding Information We are grateful to the Rovaltain Fundation for its financial support of the I-ResPect project.

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