

# Use of earthworms as a pesticide exposure indicator in soils under conventional and organic management

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## ABSTRACT

The intensive use of agricultural soils reveals the massive application of agrochemicals. There is no follow-up of the presence of pesticide residues in soil or their toxic effects on organisms that are beneficial for agrosystems, such as earthworms (*Lumbricus terrestris* L.) The objective of this study was to evaluate the effect of pesticides used in horticultural orchards on earthworms, and the use of earthworms as an indicator through carboxylesterase (CbE) activity, which is an enzyme involved in the detoxification metabolism of organophosphorus, carbamates, and pyrethroids. Eight individuals were placed in each polyethylene container and the container was buried under the soil surface in two apple orchards, one under organic management and the other under conventional management. The experiment was carried out in triplicate. A control treatment was conducted in the laboratory. The experiment was repeated in autumn, winter, spring, and summer of the 2014–2015 study period. Three internal gut tissues of the earthworm were measured for CbE activity in the laboratory. Results showed higher CbE activity in the crop-gizzard of the control treatment with  $14.22 \pm 1.00 \mu\text{mol min}^{-1} \text{mg}^{-1}$  in winter; the lowest activity was recorded in soil under conventional management in summer with  $6.15 \pm 2.77 \mu\text{mol min}^{-1} \text{mg}^{-1}$  ( $p \leq 0.05$ ). There was a seasonal difference in enzymatic activity that was higher in winter and autumn with 14.22 and  $13.93 \mu\text{mol min}^{-1} \text{mg}^{-1}$ , respectively, and lower in summer and spring with  $6.15$  and  $6.31 \mu\text{mol min}^{-1} \text{mg}^{-1}$  ( $p \leq 0.05$ ), respectively; enzymatic activity was associated with higher pesticide application. It can be concluded that CbE activity is sensitive to the inhibitory action of pesticides and can therefore be used as a biological indicator of agrochemicals.

**Key words:** Carboxylesterase, conventional management, earthworm, *Lumbricus terrestris*, organic management, pesticide.

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## INTRODUCTION

Agricultural soils in Chile are under intensive use and this is revealed by an elevated application of agrochemicals (OECD, 2008). There are scarce studies about the presence of pesticide residues in soil and their toxic effects in soil organisms, such as earthworms, which are beneficial because of their physical, chemical, and biological soil functions (Blouin et al., 2013; Bottinelli et al., 2015). Earthworm interaction with soil is continuous exposure to contaminants present in the integument and its gastrointestinal tract, both of which are the main routes of entry of contaminants inside the earthworm.

This has led to the use of the earthworm in toxicity assays to evaluate the toxic potential of new agrochemicals or determine toxicity in contaminated soils (ISO, 1993; ISO, 1998). At the same time, earthworms can accumulate and respond to contaminants at different levels of complexity ranging from molecular, cellular, to ecological changes (Dimitrova et al., 2010; Sforzini et al., 2012; Hayashi et al., 2013).

Numerous enzymatic mechanisms have been studied to evaluate the ecotoxicological effects in soil that play an important role in the metabolism and detoxification of pesticides belonging to the organophosphorus (OP), carbamate (CB), and pyrethroid (PT) groups (Sanchez-Hernandez and Wheelock 2009; Nigam et al., 2014), such as carboxylesterase (CbE) which is an enzyme of increasing interest (Wheelock and Nakagawa, 2010). The higher sensitivity of CbE to the inhibitory action of pesticides is the reason why these enzymes are attracting attention in the environmental monitoring of the exposure of organisms to this group of pesticides, which are considered to be good exposure biomarkers (Sanchez-Hernandez et al., 2015). Few studies have examined the earthworm's response under natural conditions to evaluate the intensity of pesticide usage and consider it as an indicator of orchard management because it can modify the physical and chemical activity of organisms found in the soil. We hypothesize that CbE activity is affected by pesticide use in conventional agriculture.

Therefore, the objective of this study was to evaluate the effect of pesticides in the CbE activity in conventionally and organically managed horticultural soils in four different seasons using the earthworm as an indicator.

## MATERIALS AND METHODS

### Study sites characterization

Two apple orchards were selected as the two study sites, one with conventional management (historical use of agrochemicals)

and the other with organic management (free of pesticide application). These sites are located 30 km one from the other in an agricultural area with intensive and heterogeneous crops in the Biobío Region, Chile. Both sites have soils belonging to the Andisol order and are classified as medial, thermic Humic Haploxerands (Stolpe, 2006). The climate is Mediterranean with marked seasons, high precipitation and low temperatures in winter, and little rainfall and high temperatures in summer. Seasonal mean precipitation and temperatures in the 2014-2015 study period were 150.8 mm and 13.1 °C in autumn, 205.8 mm and 7.9 °C in winter, 51.2 mm and 13.7 °C in spring, and 19.2 mm and 18.2 °C in summer (Agromet, 2015). Some physical and chemical properties were determined for both soils in each season, such as moisture content by gravimetry (Sandoval et al., 2012), organic matter content by the oxidation method with dichromate in an acidic medium and determined by colorimetry, electrical conductivity by conductivity, and pH in water with a soil:water ratio of 1:2.5 determined by potentiometry (Sadzawka et al., 2006). Organic matter was high and its values were normal for volcanic soil; it was slightly higher in the organically managed orchard because greater residue cover remained on the soil. The same situation occurred for water pH where values were moderately acidic and slightly higher in the orchard under organic management. Electrical conductivity was low and had similar values for both orchards (Table 1).

### Acquisition of earthworms and experiment in orchards under conventional and organic management

The *Lumbricus terrestris* L. species was obtained from a supplier in Concepción, Chile; specimens were taken to the laboratory where they were reproduced in polyethylene containers at 20 °C in clean soils free of agrochemicals (pH  $7.58 \pm 0.05$ , organic matter  $2.03 \pm 0.23\%$ , and electrical conductivity  $1.32 \pm 0.03$  dS m<sup>-1</sup>) and fed contaminant-free horse manure. Earthworms were reproduced and adapted under controlled conditions to obtain a sufficient number of individuals grown under the same conditions for use in the assays. Before being taken to the experimental sites, earthworms underwent gut emptying, were placed in a clean container, and not fed for 24 h.

The assays in the apple orchards under organic and conventional management were repeated in four different seasons of the 2014-2015 study period. In each season,

juvenile individuals with an undeveloped clitellum (maturity indicator) and weighing between 2.8 and 3.73 g after gut emptying were selected. They were taken to each site and placed in experimental polyethylene containers that were circular, 20 cm long × 15 cm diameter, and completely perforated (0.2 mm) for good soil water diffusion. Each container was filled with 3 kg moist soil from the same site; soil was taken at 0-20 cm depth and sieved with a 2 mm. The top of the containers was covered with a fine polyethylene mesh to prevent the earthworms from escaping, and containers were left at surface level. Three containers per site were randomly placed on an equidistant row between two trees and maintained for 30 d. This procedure was repeated in each season in the orchard under organic and conventional management; a control for each stage of the assay was maintained in the laboratory.

The 13 pesticide applications in the conventionally managed orchard were recorded during the study period. An average of two applications per month was carried out in spring and summer while only one application per month in winter. The organophosphorus chemical group was the most frequently used and reached a total of seven applications. Only one application was at the soil (herbicide) and the rest were targeted to the foliage, that is, seven insecticide and five fungicide applications (Table 2). The second site was the organically managed apple orchard where no environmentally damaging pesticides were applied; cutting management was used to eliminate weeds while biological control of pests involved monitoring with pheromone traps. Refined sulfur and copper sulfate were applied once or twice to control diseases.

### Collection of earthworm tissues and determination of carboxylesterase activity

After each 30-d period, containers were taken to the laboratory to retrieve the earthworms. Soil in the containers was spread onto a surface to collect the individuals; these were then washed with distilled water and frozen at -80 °C in an ultrafreezer (BXC-86 HL-340, Biobase, Jinan, China) for later analysis. Earthworms were thawed for the analyses and each one was dissected by a longitudinal incision in the dorsal midline of the clitellum toward the mouth; crop-gizzard, foregut, and midgut were removed without scraping the epithelial tissue. Tissues were carefully washed to eliminate soil particles. Tissue samples were placed in 2.5 mL Eppendorf tubes and 2.0 mL 10 mM

**Table 1. Soil physical and chemical variables for apple orchard experimental sites under conventional and organic management in the four seasons.**

|                           | Conventional orchard<br>36°35.876' S 72°49.10' W |              |              |              | Organic orchard<br>36°33.813' S 71°49.560' W |              |              |              |
|---------------------------|--|--------------|--------------|--------------|--|--------------|--------------|--------------|
|                           | Autumn   | Winter       | Spring       | Summer       | Autumn                                       | Winter       | Spring       | Summer       |
| Dry soil base moisture, % | 30.38 ± 1.16                                     | 32.28 ± 1.26 | 36.61 ± 1.45 | 24.07 ± 1.24 | 32.10 ± 1.38                                 | 39.86 ± 1.56 | 32.63 ± 1.34 | 25.79 ± 1.28 |
| Water pH (1:2.5)          | 6.05 ± 0.02                                      | 5.98 ± 0.03  | 5.88 ± 0.01  | 6.01 ± 0.02  | 6.42 ± 0.02                                  | 6.44 ± 0.03  | 6.98 ± 0.02  | 6.55 ± 0.07  |
| Organic matter, %         | 10.7 ± 0.10                                      | 10.4 ± 0.07  | 10.9 ± 0.06  | 10.7 ± 0.10  | 12.7 ± 0.10                                  | 12.9 ± 0.01  | 12.6 ± 0.10  | 12.0 ± 0.02  |
| EC, dS m <sup>-1</sup>    | 1.33 ± 0.01                                      | 1.32 ± 0.01  | 1.33 ± 0.01  | 1.34 ± 0.02  | 1.26 ± 0.02                                  | 1.27 ± 0.01  | 1.30 ± 0.01  | 1.29 ± 0.02  |

EC: Electrical conductivity; ±: standard deviation.

**Table 2. Phytosanitary management of the conventionally managed apple orchard during the assay period.**

| Month of application | Commercial name       | Chemical group   | Active ingredient            | IUPAC name  | Pesticide   |
|----------------------|-----------------------|------------------|------------------------------|---|-------------|
| January              | Gusathion M 35% WP    | Organophosphate  | Azinphos-methyl              | S-3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3-ylmethyl <i>O,O</i> -dimethyl phosphorodithioate   | Insecticide |
| January              | Lorsban 50 WP         | Organophosphate  | Chlorpyrifos                 | <i>O,O</i> -Diethyl <i>O</i> -3,5,6-trichloro-2-pyridyl phosphorothioate  | Insecticide |
| February             | Calypso 480 SC        | Neonicotinoid    | Thiacloprid                  | ( <i>Z</i> )-3-(6-Chloro-3-pyridylmethyl)-1,3-thiazolidin-2-ylidenecyanamide  | Insecticide |
| February             | Glyphosate            | Aminophosphonate | Glyphosate                   | <i>N</i> -(Phosphonomethyl)glycine  | Herbicide   |
| April                | Cuprodul              | Copper compound  | Cuprous oxide                | Cuprous oxide   | Fungicide   |
| May                  | Nordox                | Copper compound  | Cuprous oxide                | Cuprous oxide   | Fungicide   |
| July                 | Mineral-phosphate oil | Oleo phosphate   | Fenitrothion                 | <i>O,O</i> -Dimethyl <i>O</i> -4-nitro- <i>m</i> -tolyl phosphorothioate  | Acaricide   |
| September            | Mancozeb 80%          | Dithiocarbamate  | Alkylenbis                   | Manganese ethylenebis(dithiocarbamate) (polymeric) complex with zinc salt   | Fungicide   |
| September            | Mystic 520            | Oximino acetate  | Trifloxystrobin              | Methyl ( <i>E</i> )-methoxyimino-{( <i>E</i> )- $\alpha$ -[1-( $\alpha,\alpha,\alpha$ -trifluoro- <i>m</i> -tolyl)ethylideneaminoxy]- <i>o</i> -tolyl}acetate       | Fungicide   |
| October              | Indar                 | Triazole         | Fenbuconazole                | ( <i>RS</i> )-4-(4-Chlorophenyl)-2-phenyl-2-(1 <i>H</i> -1,2,4-triazol-1-ylmethyl)butyronitrile   | Fungicide   |
| November             | Imidan                | Organophosphorus | Phosmet                      | <i>O,O</i> -Dimethyl <i>S</i> -phthalimidomethyl phosphorodithioate   | Insecticide |
| November             | Lorsban               | Organophosphorus | Chlorpyrifos                 | <i>O,O</i> -Diethyl <i>O</i> -3,5,6-trichloro-2-pyridyl phosphorothioate  | Insecticide |
| December             | Lorsban/Gusathion     | Organophosphorus | Azinphos-methyl/Chlorpyrifos | S-3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3-ylmethyl <i>O,O</i> -dimethyl phosphorodithioate/ <i>O,O</i> -Diethyl <i>O</i> -3,5,6-trichloro-2-pyridyl phosphorothioate | Insecticide |

Tris-HCl (pH 7.4) buffer solution were added; they were crushed in a glass micromortar and macerated to ensure membrane esterase extraction (Huang and Hammock, 1997). Tissue was centrifuged at 10 000 rpm at 4 °C for 30 min (Mikro 220 R, Hettich, Tuttlingen, Germany). A supernatant fraction of post-mitochondrial tissue was obtained and 1:50 dilutions were performed in the tissues that were stored in the ultrafreezer at -80 °C until further analysis.

Carboxylesterase activity was performed by determining the absorbance of the Red ITR-naphthol complex at 530 nm in a reaction medium with 200  $\mu$ L final volume (Thompson, 1999, adapted by Narváez et al., 2016); enzymatic activity was then calculated based on the individual protein content of each sample. The procedure involved placing 10  $\mu$ L sample on the flat-bottomed microplate of 96 dishes (TCL, Trueline, Santiago, Chile), adding 170  $\mu$ L solution of 25 mM Tris-HCl with 1 mM CaCl<sub>2</sub> (pH 7.6), and adding 20  $\mu$ L  $\alpha$ -naphthyl acetate substrate after a 5-min incubation period at 25 °C. Naphthyl formation stopped after 15 min when 50  $\mu$ L the mixture 2.5% Triton X-100 (25 mL), 25% SDS (25 mL), and 20 mg Fast Red 0.1% was added. The solution was left to stand in the dark for 30 min at room temperature (20 °C). A spectrophotometric analysis was then performed in a microplate reader (Synergy HT, BioTek, Winooski, Vermont, USA) and specific enzymatic activity was expressed in  $\mu$ mol per minute per milligram of protein ( $\mu$ mol min<sup>-1</sup> mg<sup>-1</sup>).

The protein content of each earthworm tissue sample was quantified by the Biuret method (Gornall et al., 1949) using the bovine serum albumin standard. This value was used to calculate the enzymatic activity based on milligrams of proteins.

## Statistical analyses

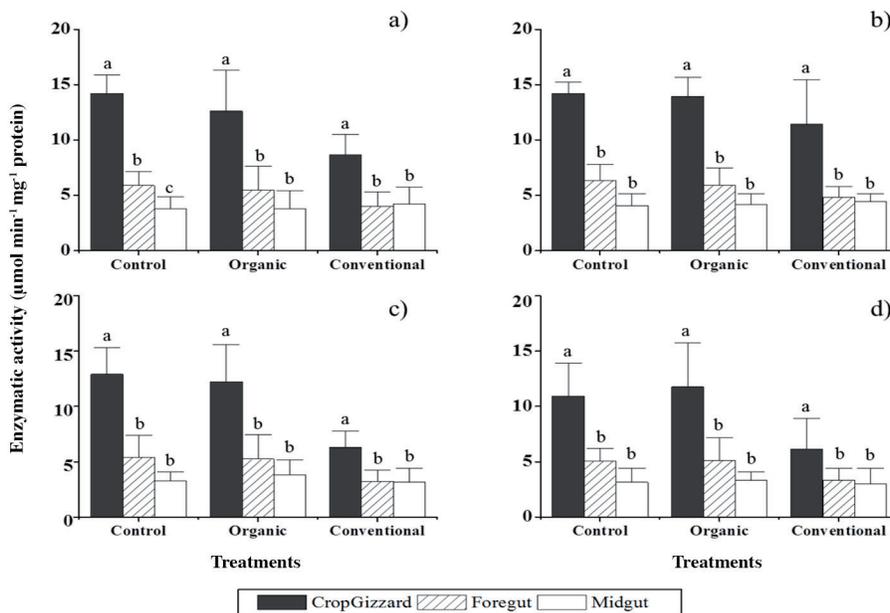
A three-way ANOVA was performed where soil type and season factors were in a factorial arrangement and the different gut tissue evaluations were considered as repeated measures because they were carried out on the same specimen (individual). Another factorial ANOVA was used to analyze the effects of the crop-gizzard tissue response. Tukey's multiple comparison test was used a posteriori. The level of significance was 0.05. Results were analyzed with the statistical software SPSS v22.0 (IBM Corporation, Armonk, New York, USA). Evaluated variables were expressed as mean and standard deviation, and standard error was used in the graphs.

## RESULTS

Enzymatic activity of CbE in autumn (Figure 1a) revealed a 31% difference in crop-gizzard tissue in conventional ( $8.69 \pm 1.81 \mu$ mol min<sup>-1</sup> mg<sup>-1</sup>) vs. organic ( $12.61 \pm 3.72 \mu$ mol min<sup>-1</sup> mg<sup>-1</sup>) management of soil, while the control ( $13.17 \pm 3.26 \mu$ mol min<sup>-1</sup> mg<sup>-1</sup>) was 34% higher than conventional management. When comparing the different tissues in autumn, the highest activity was in the crop-gizzard in the control ( $13.17 \pm 3.26 \mu$ mol min<sup>-1</sup> mg<sup>-1</sup>) while the lowest activity was found in the foregut ( $3.77 \pm 1.62 \mu$ mol min<sup>-1</sup> mg<sup>-1</sup>) in organically managed soil.

Enzymatic activity of CbE in winter (Figure 1b) showed a 22% difference in crop-gizzard tissue between conventional ( $11.43 \pm 4.03 \mu$ mol min<sup>-1</sup> mg<sup>-1</sup>) and organic ( $13.93 \pm 1.74 \mu$ mol min<sup>-1</sup> mg<sup>-1</sup>) management soil while the control ( $14.22 \pm 1.00 \mu$ mol min<sup>-1</sup> mg<sup>-1</sup>) was 24% higher than under

**Figure 1. Carboxylesterase enzymatic activity in autumn (a), winter (b), spring (c), and summer (d) for soil management treatments and in the crop-gizzard, foregut, and midgut tissues of *Lumbricus terrestris*.**



Different letters over the bars indicate significant differences according to Tukey's test ( $P \leq 0.05$ ); vertical lines over the bars indicate standard error.

conventional management. When comparing these different tissues in winter, the highest crop-gizzard activity was in the control and the lowest activity in the midgut in soil under conventional management ( $3.10 \pm 1.25 \mu\text{mol min}^{-1} \text{mg}^{-1}$ ).

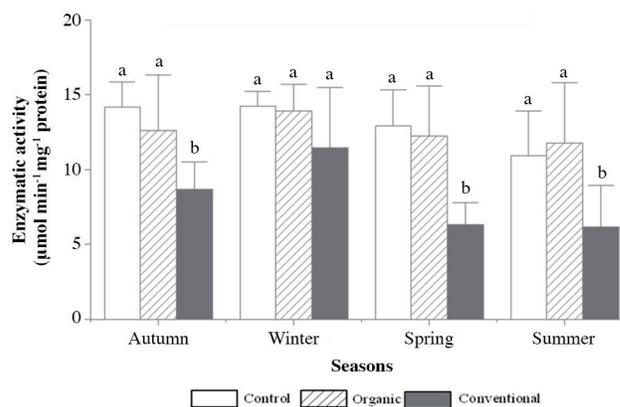
Enzymatic activity of CbE in spring (Figure 1c) showed the same behavior as in the other seasons where crop-gizzard tissue in soil under conventional management had lower activity ( $6.31 \pm 1.48 \mu\text{mol min}^{-1} \text{mg}^{-1}$ ) than soil under organic management ( $12.23 \pm 3.34 \mu\text{mol min}^{-1} \text{mg}^{-1}$ ); the difference between organic and conventional management was 94%. The CbE activity in the control was 87% higher than under conventional management and had a value of  $11.77 \pm 6.64 \mu\text{mol min}^{-1} \text{mg}^{-1}$ . When comparing tissues in spring, the highest activity was in the crop-gizzard with organic soil management, unlike winter and autumn when the maximum activity was found in the control and the lowest activity occurred in the midgut ( $3.21 \pm 1.01 \mu\text{mol min}^{-1} \text{mg}^{-1}$ ).

Enzymatic activity of CbE in summer (Figure 1d) was similar to autumn and winter in which crop-gizzard exhibited greater differences (92%) when comparing activity with conventional ( $6.15 \pm 2.77 \mu\text{mol min}^{-1} \text{mg}^{-1}$ ) and organic ( $11.78 \pm 3.99 \mu\text{mol min}^{-1} \text{mg}^{-1}$ ) soil management. The control treatment ( $10.93 \pm 2.96 \mu\text{mol min}^{-1} \text{mg}^{-1}$ ) was 78% higher than conventional management. The highest activity was in the crop-gizzard with organic soil management ( $11.78 \pm 3.99 \mu\text{mol min}^{-1} \text{mg}^{-1}$ ) when comparing tissues in summer, which was similar to spring observations; however, it was the lowest activity for this tissue among seasons. The lowest activity in tissues was found in midgut ( $3.01 \pm 1.40 \mu\text{mol min}^{-1} \text{mg}^{-1}$ ).

These results allow us to establish that crop-gizzard as the earthworm's internal tissue that indicated a better trend

with higher values in CbE activity; it also exhibited greater differences among treatments under different soil management systems regarding the foregut and midgut. The latter two tissues had lower enzymatic activity, values that were similar between soil management systems. Therefore, crop-gizzard activity was used as an indicator tissue to study the effect of soil management on the enzymatic activity in different seasons (Figure 2). The CbE activity in the crop-gizzard was only different in conventionally managed soil for all the seasons. The highest activity was in winter with values of  $11.43 \pm 4.03 \mu\text{mol min}^{-1} \text{mg}^{-1}$  and the lowest activity was recorded in summer with values of  $6.15 \pm 2.77 \mu\text{mol min}^{-1} \text{mg}^{-1}$ . The CbE activity in autumn ( $8.69 \pm 1.82 \mu\text{mol min}^{-1} \text{mg}^{-1}$ )

**Figure 2. Seasonal distribution of carboxylesterase enzyme activity in the crop-gizzard tissue of *Lumbricus terrestris* exposed to different soil management systems.**



Different letters over the bars indicate significant differences according to Tukey's test ( $P \leq 0.05$ ); vertical lines over the bars indicate standard error.

was higher than in spring ( $6.31 \pm 1.48 \mu\text{mol min}^{-1} \text{mg}^{-1}$ ). Organically managed soil was not different among seasons and activity varied between  $13.93 \pm 1.74$  and  $11.78 \pm 2.96 \mu\text{mol min}^{-1} \text{mg}^{-1}$  in winter and summer, respectively. These crop-gizzard CbE activity values are similar to those of control, which varied between  $14.22 \pm 1.00$  and  $10.93 \pm 2.96 \mu\text{mol min}^{-1} \text{mg}^{-1}$  in winter and summer, respectively.

## DISCUSSION

Carboxylesterase activity in the crop-gizzard, foregut, and midgut tissues is consistent with findings by Sanchez-Hernandez and Wheelock (2009) in an *in vitro* study indicating that determination of CbE activity is well represented in the gastrointestinal tract of *L. terrestris* species, and it is a good indicator to evaluate the effects of pesticides.

Results of the present study indicate that crop-gizzard tissue had higher CbE activity values than foregut and midgut tissues. It was also observed that CbE activity in the latter two tissues decreased as the ingested soil was processed through the gastrointestinal tract (Sanchez-Hernandez et al., 2009). This is why no differences in foregut and midgut were found among soil management treatments with higher or lower pesticide application, which could be due to the gut's digestive function (Brown and Doube, 2004). However, according to González et al. (2010), high enzymatic activity in the crop-gizzard would be explained because CbE activity in this organ could contribute to chlorpyrifos retention and thus decrease the amount of pesticides passing to subsequent organs and reduce intestinal absorption; as a consequence, enzyme inhibition is lower.

The CbE activity found in the four seasons had a similar behavior among soil treatments, that is, control treatment and organically managed soil had higher activity and conventionally managed soil with pesticides always had lower activity.

The highest soil CbE activity was found in winter under organic management and the lowest CbE activity occurred in summer under conventional management, which was well identified in the crop-gizzard tissue. These results could be related to the greater use of organophosphorus pesticides and carbamates applied in spring and summer in apple orchards under conventional management, where pesticides were retained by soil OM at a rate varying between 10.2% and 10.7%; this is an important factor for pesticide retention in soil (Boivin et al., 2005; Alvarez et al., 2013). There are also authors who maintain that pesticides can be biodegraded in soil by organisms found in OM (Sorensen and Aamand, 2001). These contrasting effects are not only related to pesticide properties but also to the nature of OM.

The CbE activity exhibited a slow recovery rate in autumn and winter. Some authors (Aamodt et al., 2007; Rault et al., 2008; and Collange et al., 2010), have indicated that cholinesterase activity usually shows slow recovery rates until it reaches normal levels when exposed to organophosphates. This CbE activity in intestinal tissue of *L. terrestris* remains inhibited for a long period of

time, more than 1-mo, after applying organophosphorus (González et al., 2010); that is, enzymatic activity is not recovered either by the spontaneous reactivation of the phosphorylated enzyme by the organophosphorus pesticide (Rodríguez-Castellanos and Sanchez-Hernandez, 2007) or the synthesis of the new enzyme during this inhibition period. This slow activity allows the stability of this response to organophosphorus pesticides and makes it a useful enzyme for biochemical assessment in monitoring exposure to agrochemicals.

This slow CbE recovery in the earthworm makes it a suitable biomarker for monitoring soils exposed to organophosphorus that can be detected several weeks after being applied. Some studies have concluded that CbE activity in other earthworm species, such as *Allolobophora caliginosa*, can be influenced by factors such as temperature and the nutritional and reproductive status (Lowe and Butt, 2007). Furthermore, the hydrolytic activity can be altered by ingesting lipids because CbEs metabolize them. Enzymatic CbE activity can be affected by the availability of the food substrate for the earthworm; according to González et al. (2010), the *A. caliginosa* earthworm had a high variation during 35-d periods with no access to food. In the present study, earthworms had access to a sufficient food substrate despite being enclosed in polyethylene containers; they were under fruit trees so CbE activity was not affected by nutritional deficiency.

The toxic effects of pesticides on earthworms are different for dermal exposure or ingestion. Carboxylesterases in the gastrointestinal tract of *L. terrestris* offer an efficient chemical barrier against pesticide absorption because CbE intestinal activity is sensitive to pesticides when they are applied to soils. This was demonstrated by Henson-Ramsey et al. (2007) when exposed *L. terrestris* to malathion through skin and by ingestion; they observed that dermal exposure is more tolerant than exposure by soil ingestion. In fact, Yu et al. (2006) indicated that high chlorpyrifos absorption by the earthworm *A. caliginosa* was due to active ingestion of the pesticide found in the soil, while chlorpyrifos absorption through the skin surface was nonsignificant. *Lumbricus terrestris* was a good exposure indicator in soils where pesticides were applied because they are resistant to the conditions of an agroecosystem with a high pesticide load; all the specimens (100%) in the present experiment survived during the four different seasons, which allowed us to collect them from the polyethylene container to determine enzymatic activity.

## CONCLUSIONS

The use of carboxylesterase enzymes of *Lumbricus terrestris* provides evidence that they are good indicators of exposure to pesticides such as organophosphorus and carbamates; this was reflected by lower enzymatic activity in spring and summer, which coincided with the highest pesticide application in the orchard under conventional management. Enzymatic activity contrasted more in crop-

gizzard tissue than in foregut and midgut, which showed no differences in activity in soils under conventional and organic management.

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