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Original Communication

Metabolic stress and shell thinning in *Pomacea canaliculata* (Caenogastropoda, Ampullaridae) in rice agroecosystems of Argentina

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ABSTRACT

Pomacea canaliculata is a freshwater snail native to South America that has become a serious pest of rice crops. Insecticides are used to control other pests in paddy fields and *P. canaliculata* is collaterally exposed. Here a *P. canaliculata* population from a rice field (RF) where the insecticides bifenthrin and imidacloprid (IMC) were applied was compared with a reference site (RS). The activities of three metabolic endpoints, namely acetylcholinesterase (AChE), carboxylesterase (CbE) and glutathione-S-transferase (GST) were measured to test metabolic stress. Intestine and midgut-gland of snails after 7 and 30 days of insecticide application were studied. A possible effect of IMC on shell thickness was also explored. The results indicate an inhibition of CbE and GST activities in the intestine of RF snails relative to RS snails at 7 days, while AChE and GST increased in the intestine after 30 days of IMC exposure. GST in the midgut-gland of RF snails was inhibited with respect to RS snails at 7 and 30 days, whereas CbE increased at 30 days in RF snails. Thinner shells with lower proportion of ash content were observed in snails from RF. Exposure to pesticides exerts neurotoxic resistance and oxidative stress in *P. canaliculata* and is probably responsible for the alteration in the shell development.

KEYWORDS: rice field, *Pomacea canaliculata*, enzymes, shell thickness, pesticides.

INTRODUCTION

The golden apple snail *Pomacea canaliculata* (Lamarck) is native to South America and is distributed from Colombia and Guiana to the south of Buenos Aires province, Argentina, in La Plata and Amazon river basins [1]. This species was introduced to Asia in the early 1980s (India, Sri Lanka, southern China, Indonesia, Philippines, and Taiwan up to Japan), where it has found a suitable tropical climate, abundant food in rice fields and wetlands, with no natural enemies [2] and has became a pest. Due to its rapid reproduction and growth [3], it is considered one of the 100 most harmful invasive species worldwide [4]. In paddy fields the snails cut the base of the rice plants with its radula and chew on the sheaths of rice, causing considerable damage and economic losses [5]. It is well demonstrated that this species also predates on taro (*Colocasia esculenta*), a starchy plant of importance for human consumption and ecologically similar to rice [6]. In addition, *P. canaliculata* is an important host of the parasite *Angiostrongylus cantonensis*, which

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causes eosinophilic meningo-encephalitis [7] and has become an important threat to human health.

In mid-eastern Argentina, the area of highest rice production of this country, rice is a highly valuable crop cultivated under submerged conditions and requires the use of high pesticide concentrations [8]. Neonicotinoids are among the most effective insecticides for the control of sucking insect pests such as aphids, whiteflies, leaf- and plant hoppers, some lepidopterans and a number of coleopteran herbivores [9]. Insecticides of the neonicotinoid class (e.g., imidacloprid, thiamethoxam) are synthetic derivatives of nicotine, an alkaloid compound that is found in the leaves of many plants [10, 11]. These insecticides exert toxic effects *via* interactions with nicotinic acetylcholine receptors of the insect nervous system; they are used extensively for the management of insect pests of agricultural importance [10]. Although specific mollusquicides like metaldehyde is frequently used [12] for the control of snails the effect of insecticides (IMC) on them remains unknown.

Several enzymatic biomarkers are used to establish the ecotoxicity of some contaminants in aquatic organisms [13-16]. B-type esterases participate in the detoxification of different pesticides [17] and are considered important biomarkers to identify exposure, resistance or oxidative stress of pesticides in mollusk species [18, 19, 12]. Aldridge [20] classified these hydrolase enzymes as A and B esterases; the cholinesterases (ChEs: acetylcholinesterase AChE, EC 3.1.1.7 and butyrylcholinesterase BChE, EC 3.1.1.8) and carboxylesterases (CbEs, EC 3.1.1.1). CbEs consist of multiple isozymes that vary with tissue and organism. CbEs play a protective role, since they remove a significant amount of pesticides by stoichiometric binding before reaching AChE and also hydrolyzing carboxyl esters present in some pesticides [21] and endogenous esters. Thus, they are involved in physiological in addition to toxicological processes [22]. It has been repeatedly proposed that the combined monitoring of ChE and CbE activities on aquatic invertebrates provide a useful indication of exposure to agrochemicals [23, 24]. Furthermore, CbEs have been studied not only as biomarkers of exposure but also as indicators of insect resistance to different insecticides, with resistant insects exhibiting higher enzyme levels [25].

Moreover, the antioxidant system is composed of a group of enzymes, such as glutathione-S-transferase (GST; EC 2.5.1.18). GST is an important phase II detoxification enzyme that not only eliminates the products of phase I metabolism by conjugation, but also acts as an antioxidant and participates in cellular protection against oxidative stress [26]. In addition, the potential damage of free radicals and hyperoxides, and the possible direct effect of several oxidants on cell components of cell defense systems have gained attention of toxicologists [26].

Mollusks in agroecosystems are under pressure of contaminants (e.g. pesticides). Márquez *et al*. [27] demonstrated that changes in shell thickness can potentially reflect environmental pollution. A few studies on *P. canaliculata* found effects of copper on growth rates [28] and of paraquat and glyphosate on survivorship [29]. In addition, *P. canaliculata* bioconcentrates different classes of chemicals in their tissues, such as brain, kidneys, and particularly the midgut gland [30]. Apple snails have the potential to become a toxicity test organism; however only a few studies have reported on their biological response to different pollutants [31]. On the other hand shell thickness of *P. canaliculata* is also affected by epigenetic factors [32] like food availability [33].

In the present study, we evaluated the response of *Pomacea canaliculata* to agrochemicals in their native range. Measurements of AChE, CbE, and GST activities and shell thickness were selected as stress biomarkers in this abundant species that occur in rice crops where IMC is frequently applied due to the harmful herbivorous effects of several species on rice plants in mid-eastern Argentina. In addition, we investigated bifenthrin and imidacloprid residues in the sediment at each sampling site.

MATERIALS AND METHODS

Study area

The study area was situated in Santa Fe province, mid-eastern Argentina. The area of highest rice production is located between Romang (San Javier department) and Colonia San Joaquín (Garay department), to the north and south of the locality of San Javier, with a total cultivated area of 30,000 ha [34]. Rice crops are planted in low deforested lands and are delimited by the Parana River to the east.

Two sites (Figure 1), a reference site (RS) and rice field (RF), were selected. RS was located in the Capital department (31°38'12.22"S - 60°40'32.22"W, Santa Fe province, Argentina). This site is free of contamination and no treatment with chemical or biological pesticides has been applied in the nearby area. RF is a rice (*Oryza sativa*) plantation located in San Javier department (30°05'13.56"S - 59°53'19.98"W). On 30 December 2016, the insecticide MAGIC® (IMC) composed of bifenthrin and imidacloprid (2 and 10 g, respectively) was applied in the RF by aircraft spraying at a proportion of 500 cm^3/ha.

Sediment pesticide residue

To analyze bifenthrin and imidacloprid (IMC) residues in the sediment from RF and RS, samples were collected from a sprayed area at the beginning of the study. Each sediment sample was transported in a plastic container to the laboratory and frozen at -20 °C. Pesticide residue was extracted from a 10 g soil sample, first with n-hexane and then with methanol. Gas chromatographic determination of IMC was performed using ultra high efficiency liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS).

Animal sampling

Only males were selected for the enzymatic study in order to avoid differences in enzyme activities between sexes [35]; the criterion for male identification was the lack of the albumen gland combined with the presence of the penis sheath to avoid misclassification in the case of imposex [36]. A total of N = 35 adult males were collected by hand from the sampling sites on two dates: after 7 days of pesticide application (7 January 2016; N_{RS} = 8 and N_{RF} = 9) and after 30 days (30 January 2016; N_{RS} = 9 and N_{RF} = 9). The two observation dates were selected based on previous work [8]. The snail samples collected before insecticide application were not considered for enzyme activities because no statistical difference was observed in the enzyme activities. After capture, animals were rapidly transported to the laboratory in darkened buckets (approximately 10 cm deep) containing water and aquatic plants to minimize stress. At the laboratory, snails were instant euthanized at -20 ºC. Then they

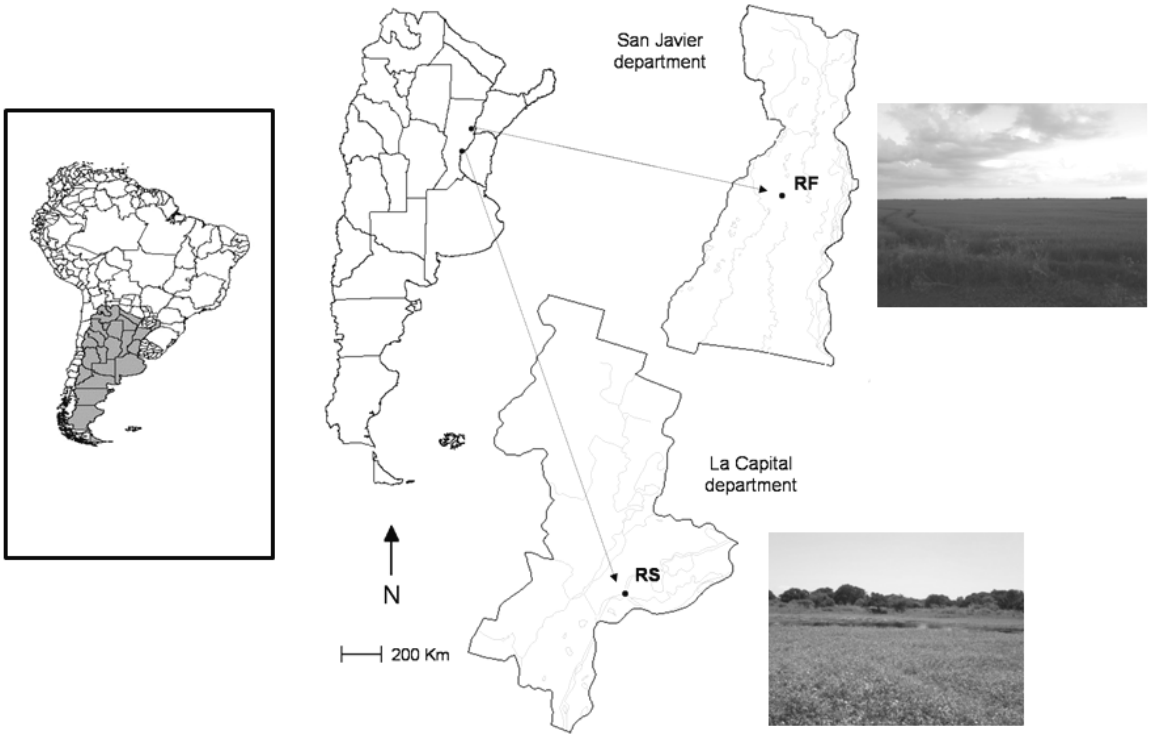

Figure 1. Location of sampling sites in mid-eastern Argentina. RS: reference site and RF: rice field.

were dissected along the mid-ventral line by making a longitudinal incision, and digestive organs (intestine, and midgut gland) were removed. Digestive organs contribute mainly to pesticide detoxification [12, 37]. Tissues were washed with distilled water and placed on a filter paper to remove excess fluids. Tissue samples were stored at -80 °C until biochemical analyses were performed.

For shell thickness study eleven snails (size > 25mm) from each site (RS and RF) were randomly selected after 30 days of pesticide application. They were euthanized by immersion in water at 80 °C and maintained at -25 °C for laboratory analysis. One specimen from each population was discarded to achieve the same sexual ratio for both samples.

Voucher specimens from each population were deposited at Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN-41139 and MACN-41140, Buenos Aires, Argentina).

Enzyme assays

Intestine and midgut gland were individually homogenized (on ice) in 20% (w/v) buffer containing 0.1% t-octylphenox-polyethoxy ethanol (triton X-100) in 25 mM tris (hydroxymethyl) amino methane hydrochloride (pH 8.0) using a polytron. The homogenates were centrifuged at 10,000 rpm at 4 °C for 15 min, and the supernatant was collected and frozen at -80 °C until assayed for enzymatic determination.

Enzyme assays: B-esterases

AChE activity was determined colorimetrically following the procedure of Ellman *et al.* [38]. The reaction mixture (final volume [F.V. = 930 µl]) consisted of 25 mM Tris-HCl containing 1 mM $CaCl_2$ (pH = 7.6), 10 µl 20 mM acetylthiocholine iodide (AcSCh), and 50 µl DTNB (3×10^{-4} M, final concentration). Variation in optical density was measured in duplicate at 410 nm and 25 ºC for 1 min using the Jenway 6405 UV-VIS spectrophotometer. AChE activity was expressed as nmol of hydrolyzed substrate per minute per milligram of protein using a molar extinction coefficient of 13.6×10^3 M^{-1} cm^{-1}. Carboxylesterase (CbE) was determined using the α-naphthyl acetate (α-NA) substrate and specific enzyme activity was expressed as nmol min^{-1} mg^{-1} protein. The hydrolysis of α-NA by CbE was measured as described by Gomori [39] and adapted by Bunyan *et al.* [40]. The reaction medium (F.V. = 1950 µl) contained 25 mM Tris-HCl, 1 mM $CaCl_2$ (pH = 7.6) and the sample. The reaction was initiated by adding 50 µl α-naphthyl acetate (1.04 mg ml^{-1} in acetone) after a preincubation period to 10 min at 25 ºC. The formation of naphthol was stopped after 10 min by adding 500 µl of 2.5% sodium dodecyl sulfate and subsequently 0.1% of Fast Red ITR in 2.5% Triton X-100 in deionizer water (prepared immediately before use). The samples were allowed to develop in the dark for 30 min, and the absorbance of the complex was read at 530 nm (using a molar extinction coefficient of 33.225×10^3 M^{-1} cm^{-1}). Total protein concentrations in the supernatants were determined using the Biuret method [41].

Enzyme assays: Oxidative stress

GST activity was determined spectrophotometrically using the method described by Habig *et al.* [42] and adapted by Habdous *et al.* [43] for mammal serum GST activity. The enzyme assay was performed at 340 nm in 100 mM Na–phosphate buffer (pH 6.5) [F.V. = 920 µl], 20 µl of 0.2 mM 1-chloro-2, 4-dinitrobenzene, 50 µl of 5 mM reduced glutathione, and the sample. Enzyme kinetics assays were performed at 25 °C and whole GST activity was expressed as nmol min^{-1} mg^{-1} protein using a molar extinction coefficient of $9.6 \times 10^3 M^{-1}$ cm^{-1}

Shell thickness

Total weight (TW) of snails was recorded using an analytical scale (± 0.001 g), and shell length (SL; from the apex to the lower margin of the aperture) was measured with a caliper (± 0.05mm). Shell thickness was measured with a caliper (± 0.05mm) 2 mm inwards of the external margin of the aperture. The body of the snails was extracted by dissection and the anatomy was carefully inspected. Shell volume (V, mm^3) was estimated as the difference between the weight of the shell filled with distilled water with a micropipette (considering a density of 1 $g.cm^{-3}$) and that of the empty shell [33]. The shells were dried at 80 °C for 48 h and weighed; then they were burned at 600 °C and weighed again to estimate the ash weight. Two indices were calculated: overall shell density (OSD), i.e. shell dry weight relative to shell internal volume (V), and shell ash content (SAC), i.e. shell ash weight relative to shell

dry weight. Small pieces of shell were fractured to exposure a face perpendicular to the direction of shell growth. The fractured edges of the shell of two specimens from each site were inspected under scanning electron microscope (LEO, EVO 40-XVP).

Statistical analysis

Variables related to enzymatic activity and shell thickness are shown in Figures 2-4 as means ± standard error (SE). Differences between sampling sites (RF and RS) and sampling periods (7 and

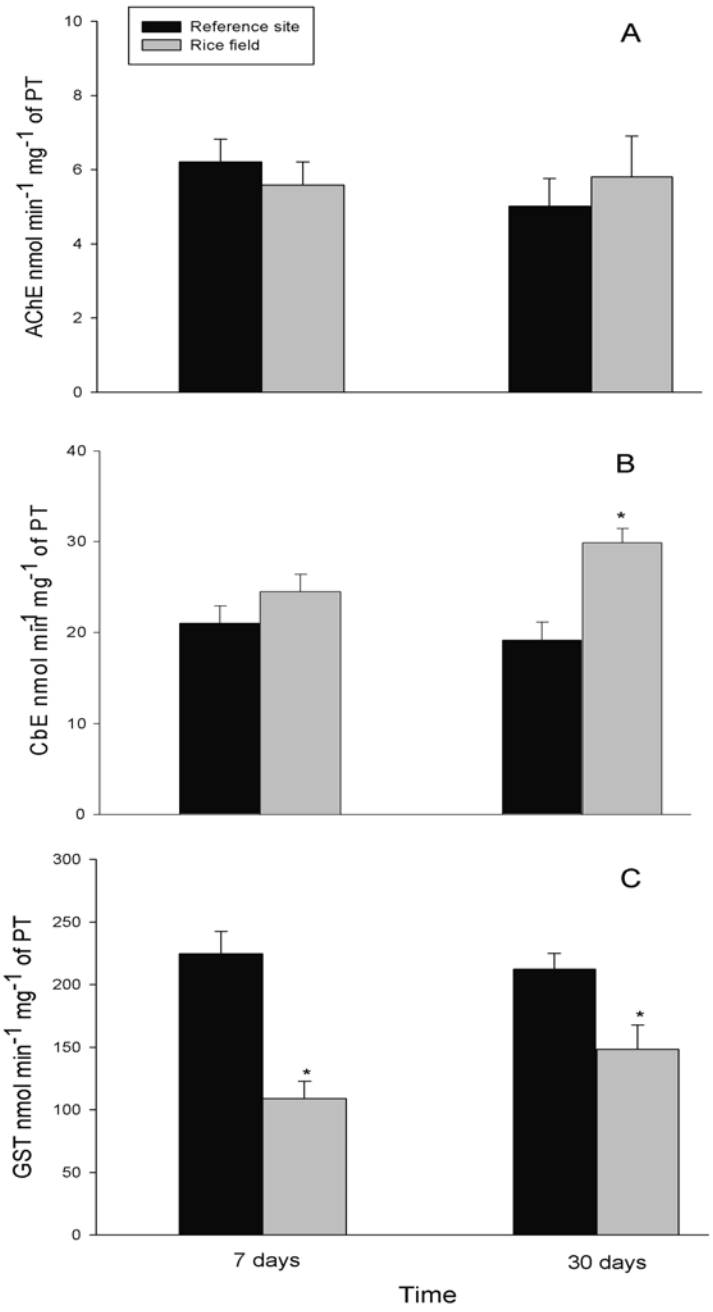

Figure 2. Comparative values of enzymatic activities in the midgut gland of *P. canaliculata*. **A**: acetylcholinesterase (AChE). **B**: carboxylesterase (CbE) and **C**: glutathione-S-transferase (GST). Bars represent the mean ± SE. *When mean values of rice field differ ($p < 0.05$) significantly from those of reference site (at 7 and 30 days).

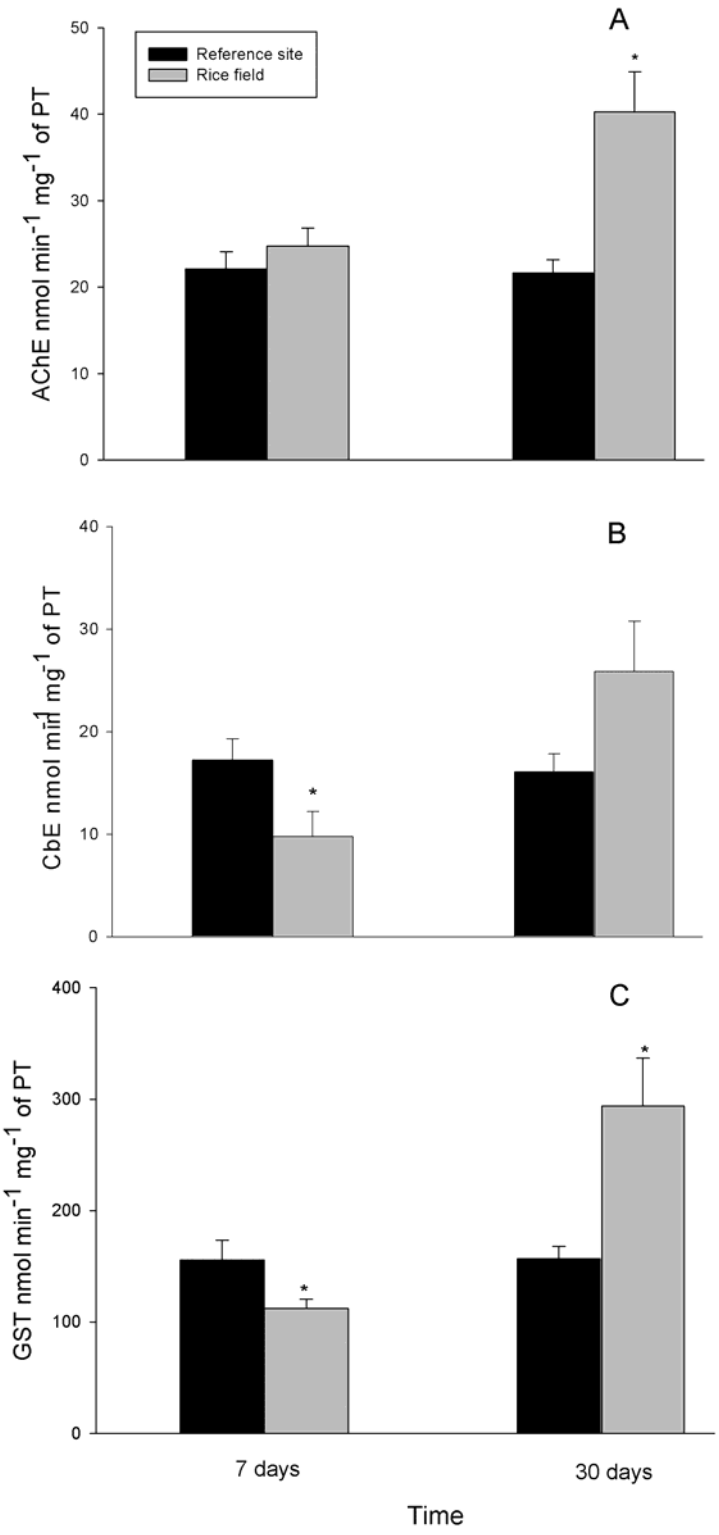

Figure 3. Comparative values of enzymatic activities in the intestine of *P. canaliculata*. **A**: acetylcholinesterase (AChE). **B**: carboxylesterase (CbE) and **C**: glutathione-S-transferase (GST). Bars represent the mean ± SE. *When mean values of rice field differ ($p < 0.05$) significantly from those of reference site (at 7 and 30 days).

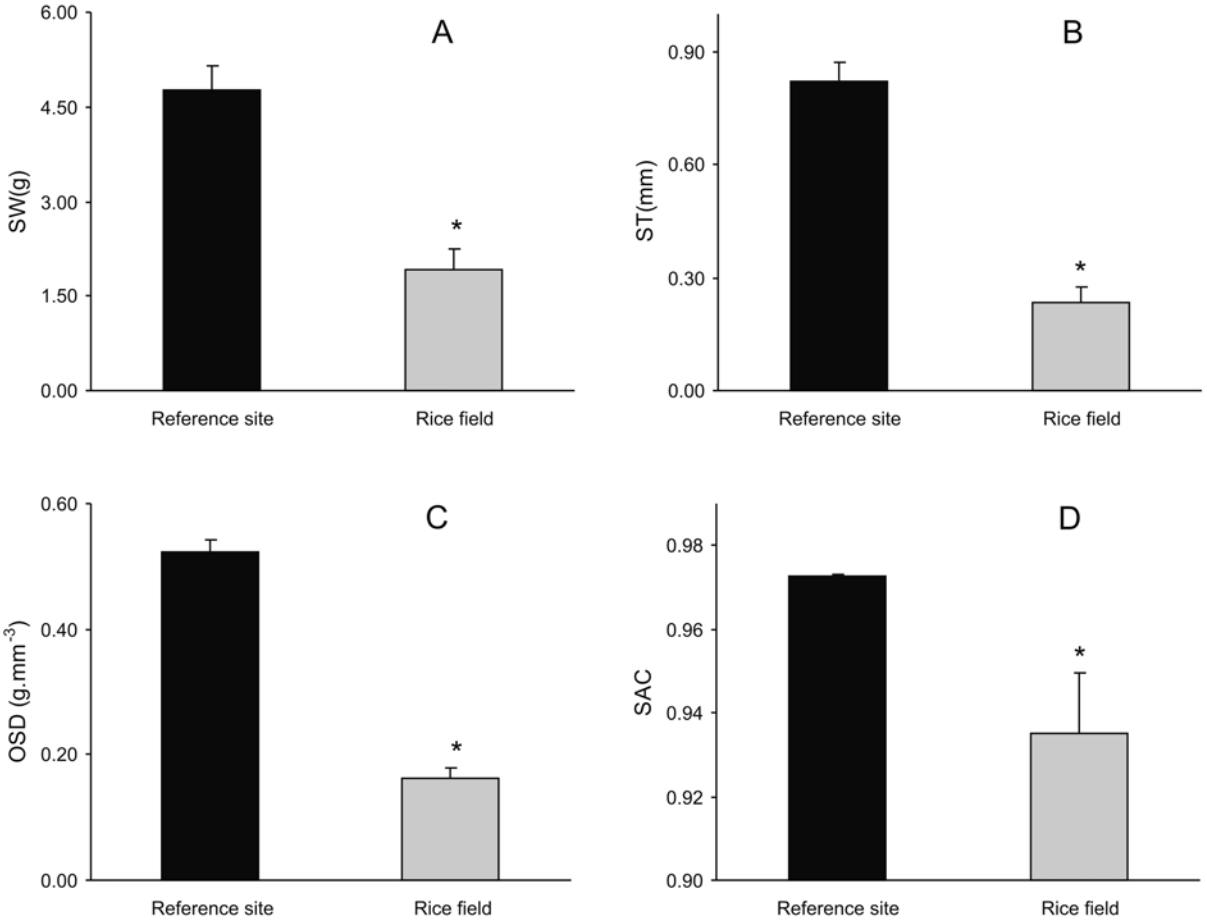

Figure 4. Conchological variables of *P. canaliculata* snails (mean ± SE) from the rice field and the reference site. **A**: Shell weight (SW; g), **B**: Shell thickness (ST; mm), **C**: Overall shell density (OSD g.mm^{-3}), **D**: Shell ash content (SAC). *P < 0.05 compared with the reference site.

30 days) in terms of the enzyme (AChE, CbE, and GST) activity were analyzed using t-tests for midgut gland and intestine tissues. Differences between sampling sites in the variables relative to snail size (SL, SW, V) and shell thickness (ST, OSD, SAC) were analyzed with the same test. Normality and homogeneity of variances were verified using the Kolmogorov-Smirnov and Levene tests, respectively. When homogeneity of variances was rejected unequal variance (Welch) test-t was performed. Statistical analyses were performed using InFostat software. The criterion for significance was $p < 0.05$.

RESULTS

Sediment pesticide residue

Imidacloprid was detected in sediment samples from RF, at concentrations of 9 ± 3 ng Kg^{-1}, but not bifenthrin (detection limit; 0.01 ng Kg^{-1}) after application. As expected, neither of these pesticides was detected in RS.

Midgut gland

Reference values of AChE activity measured in the midgut gland of snails from RS were 6.21 ± 0.61 nmol. min^{-1} mg^{-1} of protein at 7 days and 6.38 ± 0.55 nmol min $^{-1}$ mg^{-1} of protein at 30 days. AChE activity was similar in snails collected from RF and RS at 7 and 30 days (t = 0.72 and t = 0.74 p = 0.49 and p = 0.51; Figure 2 A). Reference values of CbE activity measured in RS snails were 21.12 ± 1.93 nmol min^{-1} mg^{-1} of protein at 7 days and 19.13 ± 2.01 nmol min^{-1} mg^{-1} of protein at 30 days. CbE activity was significantly lower in the midgut gland of snails from RF compared to RS at 30 days (t = -3.87, p = 0.003; Figure 2B). Reference values of

GST activity in the midgut gland of snails from RS were 224.69 ± 17.99 nmol min^{-1} mg^{-1} of protein at 7 days and 212.50 ± 12.50 nmol min^{-1} mg^{-1} of protein at 30 days. GST activity was significantly lower in snails collected from RF compared to those from RS at 7 and 30 days (t = 5.30, p = 0.0002 and t = -2.84, p = 0.0174).

Intestine

Mean values of the AChE activity in the reference site (RS) snails were 22.12 ± 2.28 nmol. min^{-1} mg^{-1} of protein at 7 days and 21.62 ± 1.59 nmol min^{-1} mg^{-1} of protein at 30 days. AChE activity was significantly higher in snails collected from RF at 30 days (t = 3.81, p = 0.008) than in RS snails. Reference values of CbE activity measured in RS were 19.13 ± 2.78 nmol min^{-1} mg^{-1} of protein at 7 days and 16.08 ± 1.76 nmol min^{-1} mg^{-1} of protein at 30 days. CbE activity was higher in the intestine of snails from RF than those from RS at 7 days (t = -2.22, p = 0.044; Figure 3B). Reference values of GST activity in the midgut gland of snails from RS was 155. 58 ± 18.05 nmol min^{-1} mg^{-1} of protein at 7 days and 156.55± 11.02 nmol min^{-1} mg^{-1} of protein at 30 days. GST activity was significantly lower in snails collected from RF than those from RS at 7 days (t = -2.44, p = 0.033 Figure 3 C), and higher at 30 days (t = 3.09, p = 0.029; Figure 3 C).

Shell thickness

The anatomical analysis revealed no unusual condition, such as deformations or imposex. No significant differences in mean values of shell

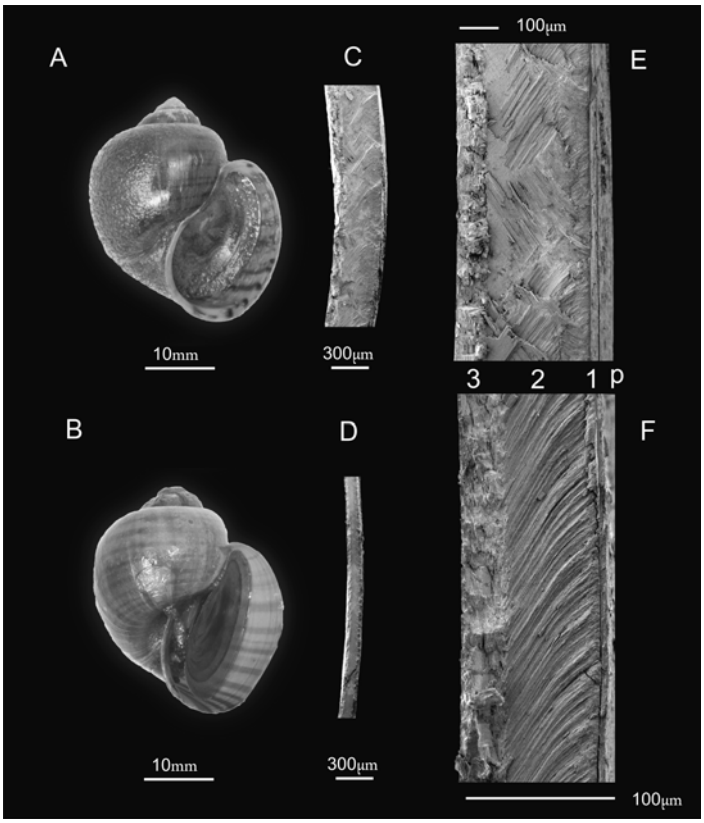

Figure 5. Shells of *P. canaliculata* from the reference site (**A**) and from the rice field population (**B**). Scanning electron micrographs of *P. canaliculata* shells showing differences in thickness between the reference site (**C**) and the rice field (**D**) at the same magnification. The observed fractured edges are perpendicular to the direction of shell growth and a few millimeters to the interior of the shell aperture. Details of the shell microstructure of snails from the reference site (**E**) and rice field (**F**) at 350x and 1200x of magnification, respectively. p = periostracum; 1 = external layer; 2 = central cross lamellar layer; 3 = internal laminar layer.

length (SL) (t = -0.763, p = 0.455), total weight (TW) (t = 0.009, p = 0.993) or shell volume (V) (t = -1.44 p = 0.168) were observed between snails from RS and those from RF. Lower mean values for shell weight (SW) (t = 5.75, p < 0.001 Figure 4A), shell thickness (ST) (t = 8.76, p = 0.001 Figure 4B), overall shell density (OSD) (t = 14.8, p < 0.001; Figure 4C) and shell ash content (SAC) (unequal variance test t = 2.622, p = 0.028; Figure 4D) were detected in snails collected from RF. The difference in shell thickness between RS and RF snails was clearly noticeable under the scanning electron microscope (Figure 5).

DISCUSSION

In the present investigation, the activity of AChE, CbE and GST was measured in the midgut gland and intestine of *P. canaliculata* snails on two dates after insecticide application in a paddy field. Levels of pesticides on sediments confirm that the reference site was non-fumigated. Basal levels of AChE and CbE activity observed here in non-exposed snails were similar to those reported in the literature in other species of freshwater gastropod [44, 16]. For example, in the bloodfluke planorb, *Biomphalaria glabrata*, AChE activity was 45 nmol min^{-1} mg^{-1} protein [13], whereas in the murex, *Hexaplex trunculus*, AChE activity was 60 nmol min^{-1} mg^{-1} protein [45]. In addition, CbE activity of *Planorbarius corneus* snails showed a mean of 200 nmol min^{-1} mg^{-1} protein [44]. A significant induction of AChE activity was detected only in the intestine of snails from RF at 30 days with respect to RS snails. Indeed, Dondero *et al*. [46] showed that thiacloprid (an insecticide of the neonicotinoid class) significantly increased AChE activity in marine mussels after exposure to a concentration of 1 mg L^{-1}, whereas IMC significantly inhibited enzyme activity after exposure to the same concentration. Increased AChE activity has also been detected in response to exposure to neonicotinoids in other invertebrates [47, 48]. Neonicotinoids are agonist of nAChRs [49] and did not show a direct inhibition of AChE activity compared to a known group of organophosphate inhibitors [50, 51]. Nevertheless, as indicated in previous reports, AChE responses seem to depend on a number on factors, such as concentration, toxicity, field application rate, and type of neonicotinoid insecticides [52]. It is also probable that inhibition occurred in the first days of exposure, which might have gone unnoticed in our sampling, and that what we did observe was a return to AChE basal levels.

CbE activity was significantly reduced in the intestine of snails from RF at 7 days and showed an increase in the midgut gland at 30 days. CbE activity increased in the intestine at 30 days, but this activity was not statistically significant compared to RS individuals. More than 20 years of ecotoxicological research in CbE has demonstrated that this enzyme is a suitable biomarker in a wide range of aquatic and terrestrial organisms exposed to various types of pollutants, including insecticides [53, 15] and that the high levels of CbE activity can contribute to the pesticide resistance in chronically exposed organisms [54, 18]. Pan *et al*. [55] concluded that esterase expression levels were significantly up-regulated in the resistant strain compared to the susceptible strain of the cotton aphid. Otero and Kristoff [56] showed an increment in CbE activities at 21 days in pesticide water compared to control values in the snail *Planorbarius corneus*. Some studies have reported a rebound effect in B-esterase activities during the recovery period after exposure to insecticides [19]. This increase suggests that the resistance mechanism is due to increase in ester hydrolysis caused by higher levels of carboxylesterase [57, 58]. These results are in agreement with previous reports on the mechanism of thiamethoxam resistance in different insect pests. For example, in *Frankliniella occidentalis* [59] and *Bemisia tabaci* [60], thiamethoxam resistance has been found due to increased activities of CbE. Likewise, El-Saleh *et al*. [61] observed an increase in CbE activity in *Spodoptera littoralis* larvae exposed to different neonicotinoid pesticides. Insecticide resistance mechanisms involve the increase in metabolism of esterases (carboxylesterases), the so called metabolic or physiological esterases; however, there is also evidence of behavioral resistance [62, 25]. Accordingly, *P. canaliculata* snails might remain inactive during agrochemical application, thus avoiding close contact with the pesticide. However, this hypothesis should be tested by analyzing exposure to different agrochemicals over different periods in the laboratory, and confirmed *via* native

gel electrophoresis. Besides conducting metabolic studies, behavior studies should be performed.

Moreover, GST activity decreased in the midgut gland of *P. canaliculata* at 7 days and 30 days of insecticide application and in the intestine at 7 days, but increased in the intestine at 30 days when compared with RS snails This enzyme catalyzes the conjugation of electrophilic xenobiotics to glutathione [63] and plays an important role in protecting tissues from oxidative stress [64], and its inhibition could lead to an imbalance of the cellular redox status (ROS). Similarly, Torres and Mason [65] reported the inhibition of GST activity (*in vitro*) in the snail *Helix aspersa* exposed to 10 μM of tributyltin. By contrast, induction of GST activity has also been reported in other snail species treated with IMC. The data obtained here are comparable to those reported by Radwan and Mohamed [66] who indicated that imidacloprid at a sublethal dose significantly increased GST activity, indicating detoxification of this pesticide with GST, since this enzyme is involved in the I-phase of xenobiotic biotransformation together with CYP450-dependent monooxygenases. Our results demonstrate that snails inhabiting RF when exposed to IMC regulate antioxidant defense systems to remove or transform ROS, thus reducing oxidative damage. Accordingly, enzyme changes observed both in GST and in esterase would be caused by exposure to IMC.

In addition, snails from RF have lighter shells, as indicated by absolute (SW) and relative (OSD) values, than snails from RS. Shells from RF are thinner (ST) and have relatively lower ash content (SAC) than RS snails. Results of both shell thickness measurements were similar; ST variable provides information about the last growth period and OSD provides a general measurement of shell thickness. Allometric effects can be discarded, since the range of sizes analyzed is similar for both populations. The observed values of SW and OSD are within the range reported for *P. canaliculata* elsewhere [32, 33]. Water chemistry, especially the content of Na^+, K^+ and Mg^{++}, affects the potential occurrence of *P. canaliculata* in the environment [67]; but the presence of these elements is weakly related to shell thickness [32, 68]. Although genetic causes do not usually explain differences in shell thickness between populations [32], differences in growth rate caused by epigenetic factors [33] cannot be discarded. Snails from RS and RF are statically similar in size (SL, TW, and V); therefore, differences in shell thickness related to age would be plausible only if both populations differed in growth rates. Unfortunately, food availability and growth rate are not easy to estimate in natural environments [69]. Formation of the eggshell of birds and the shell in mollusks involves carbonic anhydrase [70, 71]. Decreases in egg-shell thickness in birds [72] in contaminated areas are well documented and are explained at least partly by reductions in the expression of carbonic anhydrase in the shell gland [73, 74]. In fact carbonic anhydrase [71] has been proposed as a biomarker [75]. In the case of *P. canaliculata*, there is very little information available about the relationship between contaminants and shell thickness.

CONCLUSION

Overall, our results showed changes in the enzymes activity of *P. canaliculata* snails after IMC insecticide exposure. At 7 days the levels of enzymatic activity in exposed snails was equal or lower than those of snails from non-fumigated population. At 30 days an increment of enzymatic activity was detected in some cases. *P. canaliculata* snails from the population that is usually fumigated have thinner shells with a lower content of ash. Although no massive mortality of snail was visualized in the field during sampling, IMC application produced metabolic stress and affected the shell development, possibly allowing to diminish the damage of the snail to the crop. The use of enzyme activities and shell thickness of *P. canaliculata* as biomarkers are of great importance in understanding the impacts of pesticides on molluscs occurring in rice agroecosystems.

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CONFLICT OF INTEREST STATEMENT

The authors have no potential conflict of interest to report.

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