Behavioral, Histological, and Physiological Evaluation of the Effect of Imidacloprid on the Spider *Misumenops maculissparsus*

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Abstract: The aim of this study was to evaluate the effects of the neonicotinoid insecticide imidacloprid (commercial formulation) on juveniles of the spider *Misumenops maculissparsus* (Keyserling, 1891). We first analyzed whether spiders recognized the presence of the insecticide on surfaces and in drinking water (in the form of droplets). Afterwards, we investigated if the insecticide generated histologic, physiologic and/or biochemical alterations. We observed that spiders do not detect the insecticide on a surface (*e. g.*, paper) or in the form of droplets. After the imidacloprid ingestion by

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droplet intake, most spiders exhibited a paralysis that reverted after 48 h. Consequently, we observed histopathologic damage (*i. e.*, pigment accumulation, necrosis, and cuticle detachment), and an increased catalase activity and total-protein concentration in the individuals treated. The activities of glutathione-S-transferase, glutathione peroxidase, glutathione reductase, and superoxide dismutase, however, did not undergo significant variations. The results obtained emphasize the need to consider different classes of biomarkers like catalase and other proteins to identify and evaluate the histologic, biologic, and biochemical effects of imidacloprid, one of the most widely used insecticides.

Keywords: behavioral toxicology, biomarkers, histopathology, insecticide, soil invertebrates, Aranae, oxidative stress, catalasa

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INTRODUCTION

Since the neonicotinoids are very effective in controlling the pests on crops, ornamental plants, and gardens and in forests (Bonmatin et al. 2005; Wang et al. 2018); use of that pesticides have increased worldwide (Simon-Delso et al. 2015; Cressey2017). Because of their widespread application and permanence those compounds may accumulate in soils and irrigation channels for over two years after the initial application (Krupke 2012; Bonmatin et al. 2015) neonicotinoids represent a danger to human health (Pimentel et al. 1992; Aktar et al. 2009; Phua et al. 2009; Lin et al. 2013) and the

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environment (Thompson et al. 2020). Among the arthropods, the pollinators (Stanley et al. 2015; Tsvetkov et al. 2017) and predators (Prabhaker et al. 2011; He et al. 2012) are the most severely affected.

Spiders are one of the main biologic controllers of crop pests (Pekár 2012) owing to their abundance (Nyffeler et al. 1994), distribution (Sunderland et al. 1986; Nentwig 1988), and generalist feeding habit (Marc et al. 1999). Nevertheless, little attention has been paid to these beneficial arachnids (Theiling and Croft 1988; Korenko et al. 2019, 2020; Sentenská et al. 2021), especially with respect to the direct and indirect effects of insecticides (Pekár 2012).

Thomisidae is a very common family in rural areas (Gabellone, 2019). For instance, that family of spiders is one of the most abundant in a cotton plantation in Africa (Dippenaar-Schoeman et al. 1999) and the second most abundant in a corn plantation in Europe (Whitford et al. 1987).

Studies performed on samples of the Thomisidae with pesticides revealed that the representatives of this family became more susceptible than other spider taxa against certain pesticides *e. g.*, flucycloxuron and hexaflumuron (Pekár 1997). Imidacloprid showed an effect on spider abundance in the short term (Marquini et al. 2002), but not in the long term (6 years) (Peck 2009).

Imidacloprid is a systemic insecticide belonging to the group of neonicotinoids, whose principal mechanism of action is agonism of acetylcholine receptors that mediate synaptic responses in the central nervous system, thus causing death (Jeschke et al. 2011). The toxic effect of this insecticide was recently analyzed in the laboratory in juveniles and adults of the spider *Pardosa saltans* of the Lycosidae family. Variations in the

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enzyme activity of the intracocoons antioxidant system in juveniles were observed along with the mother's behavior with respect to cocoons contaminated with imidacloprid (Laino et al. 2021). As an indirect effect, imidacloprid decreases the prey-capture relationship in *Pardosa lugubris* (Řezáč et al. 2019) and *Pardosa pseudoannulata* (Widiarta et al. 2001).

When insecticides are internalized, in addition to their effect on the target organ, they cause histopathologic alterations to a greater or lesser extent (Hinton et al. 1992). In addition, some compounds cause many metabolic variations that include changes in energy reserve for example, alterations in the protein content (Drobne et al. 2008; Dutra et al. 2009; Sancho et al. 2009), activation of detoxifying enzymes like glutathione-S-transferase (GST), and redox imbalance with increased production of reactive oxygen species ROSs (Wilczek et al. 2013). Which may generate oxidation and the breakdown of internal macromolecules (*e. g.*, proteins, lipids, DNA) to provoke an irreversible impairment of the organism's metabolism (Juan et al., 2021). To avoid these damages, enzymatic antioxidant-defense systems exist, which include, among other enzymes, superoxide dismutase (SOD,), catalase (CAT,), glutathione reductase (GR), and glutathione peroxidase (GPx; Nielsen et al. 1999; Wilczek et al. 2013).

Because insecticides affect non-target organisms when they are sprayed on agricultural systems, the main objective of the present study was to evaluate for the first time the effect of imidacloprid in field concentrations (CASAFE 2022) on juveniles of *Misumenops maculissparsus* (Keyserling 1891) as a consequence of surface contact and/or droplet ingestion. We thus performed a combination of behavioral observations plus histological, physiological, and biochemical analyses.

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MATERIALS AND METHODS

Specimen collection and maintenance

The sampling was done in the periurban area of La Plata (34° 59' 55.4" S, 57° 55' 47.4" W, Buenos Aires, Argentina) in March and April 2020 and 2021, in areas without pre-exposure to neonicotinoids Spiders were collected with an entomological net and immediately stored individually in plastic tubes (1.5 ml). In the laboratory, all the specimens of *M. maculissparsus* were housed individually in transparent plastic Petri dishes (1.5 cm in height x 5.5 cm in diameter) with a 1cm^2 sponge containing water. Juveniles (J3) were used for all the assays analyzed (behavioral, histological, physiological, and biochemical) with a postemergence-development time of between 30–35 days, an average weight of 3 mg, a body length of 3 mm, and (at that stage of development) a high dispersion mobility and ability to hunt large preys.

In order to achieve a more comprehensive behavioral test, other juveniles were used (heretofore called J2) which were characterized by a postemergent-development time of less than 18–22 days, an average weight of 2 mg, a body length of 2 mm, and little mobility. J2 and J3 juveniles were collected in the same region and at the same time.

All the Petri dishes were kept under controlled conditions of temperature (25 ± 2 °C), relative humidity (75 ± 5%), and photoperiodic cycle (16:8 light: dark). All the spiders were checked and fed once a week with *Drosophila melanogaster* (Diptera: Drosophilidae). To carry out all the tests, the spiders were fasted before treatment (14 consecutive days) and without water (4 consecutive days).

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Insecticide

Imidacloprid was used in its commercial formulation Matrero 35™ (Nufarm SA) in a field dose concentration of 175 µg/ml. To carry out the behavioral tests, the surface contact was performed with filter papers (Whatman N° 2) treated with the field dose, and for histological and physiological analysis the individuals were offered a 5-µl droplet of the imidacloprid solution (0.875 µg).

Behavioral assays

The behavioral assays were performed to evaluate the way the spiders selected surfaces treated with either imidacloprid (1.47 ug/cm^2) or water (as control). Whether or not the insecticide elicits a repulsion was analyzed in both J3s and J2s. To maximize the effect, a 10 times of the field concentration was also used, with 40 J3s involved at that concentration plus 40 J3s at the field concentration. For the J2s, 80 individuals were used to analyze their behavior upon surface contact (with both the field concentration and the 10 times of the field concentration).

To carry out the analysis, 5.5 cm diameter Petri dishes of 1.5 cm height were used. One half of the dishes contained a paper (11.9 cm^2) with the insecticide, while in the other half had a paper with the same volume of water, with both papers having been dried for 20 min. The Petri dishes were contained in an opaque box 58.3 cm long, 36 cm wide, and 36 cm high with red light. This arrangement had the objective of isolating the individuals from the distraction of external visual stimuli. The displacements and movements of the spiders were recorded for 30 min through the use of a 4k Noblex Acn4k1 camera and Debut Professional v 6.67 (NCH) video-capture software with subsequent processing with tracker software (video analysis and modelling tool).

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Toxicity assays

Our main objective was to observe whether spiders in drinking could discriminate droplets with and without imidacloprid. Of the J3s, 380 were used for the present analysis. Once a spider ingested the drop (with imidacloprid), its level of involvement was recorded both immediately and after 24, 48, and 72 h. The resulting pathologic symptoms under consideration were a poor coordination of the legs, a dragging of the hind legs, a complete immobility, involuntary movements, and a closed position with leg flexure.

Histological analysis

For histological study, four specimens of *M. maculissparus* that had drunk imidacloprid and four that had drunk only water were fixed in 4% (v/v) aqueous formaldehyde. The opisthosoma of each individual was dehydrated by means of an ascending series of ethanol concentrations and then immersed in an infiltration solution of glycol-methacrylate resin (Leica Historesin®) plus 96% (v/v) aqueous ethanol (1:1) for 2 h. Thereafter the dehydrated, fixed specimens were placed in infiltration solution for 24 h at 4 ºC before a final embedding in molding cups containing the infiltration solution and hardener. Blocks were cut at a thickness of 5 µm with an electronic microtome (Leica® RM 2155), stained with hematoxylin-eosin, and observed with a light microscope (AXIOPLAN 2 Zeiss®). The resulting images were recorded in an image-analysis system (AxiovisionRel 4.4).

Enzymatic activity assays

Forty-eight hours after the intake of an insecticide-containing droplet, the affected spiders were divided into 4 independent pools of 25 individuals each, as were the control

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groups. The pools were weighed on an analytical scale (Mettler-Toledo New Classic MS-204), homogenized with a protease-inhibitor cocktail at 8 μl/mg wet weight (Sigma-Aldrich Chemicals, St. Louis, MO, USA) in 50 mM potassium-phosphate buffer pH 7.4, and then centrifuged for 20 min at 10,000 g. The protein concentration of the homogenates was determined colorimetrically (Lowry et al. 1951). SOD activity assayed (Misra and Fridovich 1972) by following the effect of SOD on the autoxidation of epinephrine in 50 mM glycine buffer (pH 10.2). One SOD unit was defined as the amount of enzyme necessary to inhibit the rate of autocatalytic adrenochrome formation by 50%. CAT activity was measured according to Aebi (1984) by following the decrease in the absorbance at 240 nm of H_2O_2 in a reaction mixture containing 50 mM potassium phosphate buffer (pH 7) and 10 mM H_2O_2. One CAT unit was defined as the amount of enzyme required to catalyze the degradation of 1 pmol of H_2O_2 per min.

GST activity was assayed after Habig et al. (1974) with 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. The reaction mix contains 1 mM CDNB and 1 mM reduced glutathione (GSH). One GST unit represented the amount of enzyme required to conjugate GSH with 1 μmol of CDNB per min determined at 340 nm. GR activity after Calberg and Mannervik (1985) was measured from the reduction of oxidized glutathione affecting nicotnamide-adenine-diphosphate-nucleotide (NADPH) oxidation. A unit of GR activity was defined as the amount of enzyme that catalyzed the reduction of 1 μmol of NADPH per min. GPx activity was measured after Flohé and Günzler (1984). To calculate the enzymatic activities (nmoles of NADPH per min), we considered $\varepsilon = 6.22\ mM^{-1}.cm^{-1}$ (at $A_{340\ nm}$).

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Statistical analyses

At least three separate experiments were performed for each study. Differences between treated and controls were analyzed using Student's t-test. Data were analyzed using the Stats graphics Centurion XVI v. 16.2.04 statistical software. Results were considered significant at ($p < 0.05$).

RESULTS

In the study of the time spiders were recorded on surfaces with and without imidacloprid, no significant changes were observed when was analyzed by Student's t-test. In the J3 juveniles for the surface with the field concentration, the spiders remained for 40 ± 32% of the time on the control and 60% ± 32% on the imidacloprid surface, ($p = 0.972$). For the 10 times of the field dose, the spiders remained 53.8 ± 29.6% of the time on control and 46.2 ± 29.6% on imidacloprid surface ($p = 0.167$). When the same paradigm was studied with the J2 juveniles, those spiders stayed 46.7% +/- 36.4% of the time on the control and 53.3 ± 36.4% on the imidacloprid surface containing the field dose ($p = 0.543$). Those spiders stayed 51 ± 27.1% of the time on the control surface and 49 ± 27.1% on the 10 times of the field concentration ($p = 0.319$).

The distance the J3 juveniles travelled on surfaces with and without imidacloprid (at both the field dose and the 10 times of field dose) was similar at values of 199 ± 70 mm for control surface and 203 ± 96 mm for imidacloprid surface with the field dose (Student's t-test $p = 0.15$) as well as, in a separate determination, at values of 194 ± 91 mm for the control surface and 201 ± 64 mm for the imidacloprid surface containing the 10 times of field dose ($p = 0.30$). Similarly, the J2 juveniles travelled equally on the surfaces with and without imidacloprid at values of 67.2 ± 97 mm for the control surface

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and 253.2 ± 152 mm for the imidacloprid surface ($p = 0.90$) as well as, in a separate determination, at values of 172.2 ± 54 mm for the control surface and 147.9 ± 49 mm for the 10 times of field concentration -of imidacloprid ($p = 0.84$). In the complementary material, fig. S1 illustrates an example the path taken in the four analyses performed.

The *right inset* in Fig. 1 depicts how, when spiders had two exclusive options (two droplets of water, one with and the other without imidacloprid), 61 ± 6.6 % selected the water without the imidacloprid and 39 ± 6.6 % the water with the imidacloprid. The response of the spiders that drank water with the insecticide is illustrated in Fig. 1. The unaffected condition (100% at 0 hours) decreased to 25.6 ± 1.6 % at 24 h through 72 h. The number of spiders affected (spiders in which there is a dragging of the hind legs, or complete immobility, or involuntary movements or a closed position with flexion of the legs) increased to 72 % at 1 h, then decreased significantly at 48 and 72 h (to 19 and 6 %, respectively). The percent recovering was very low at 24 h (2 %), but became higher at 48 and 72 h (48 and 50 %, respectively). Finally, a small percentage of spiders died at 48 h (10 %), which number increased at 72 h (20 %).

In the opisthosoma of control spiders, numerous midgut diverticula and several large silk glands were observed (Fig. 2, Panel A). The midgut diverticula (main organ of detoxification) were formed by digestive and secretory cells (Fig. 2, panels A and B). The digestive cells were more abundant than the secretory cells and contained cytoplasmic vacuoles. The secretory cells were characterized by high amounts of deeply stained cytoplasmic granules (Fig. 2, Panel B). The most prominent silk glands observed were the ampullate and aggregate glands. The ampullate glands were located anteriorly, near the book lung, and were composed of tall columnar cells with cytoplasmic droplets of

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various sizes and a wide lumen full of secretory material (Fig. 2, Panel A). In the posterior part of the opisthosoma, the aggregate glands were observed with the epithelium composed of cuboidal cells with moderate nuclei (Fig. 2, panels A and B). The cuticle of control spiders was divided into layers: epicuticle, endocuticle, and epidermis (Fig. 2, Panel B). The epicuticle was thin and acellular, forming the outermost layer. The endocuticle lay just beneath the epicuticle and was thin, eosinophilic, and acellular. The epidermis was formed by a single layer of columnar cells with the midgut diverticula below. Small pigment deposits were observed within the epidermis (Fig. 2, Panel B).

Histological examination of the spiders exposed to imidacloprid revealed morphologic alterations in the midgut diverticula (Fig. 2, panels C and D). A loss of tissue integrity and necrosis was observed in the midgut diverticula, along with a decrease in the number of secretory cells (Fig. 2, Panel D). The overall gland structure of spiders exposed to 0.875 μg of imidacloprid was impaired, as manifested in a disintegration of the epithelia (Fig. 2, panels C and D). Microscopic cuticle damage was observed in the exposed spiders in the form of a separation of the epidermis from the midgut diverticula (Fig. 2, Panels C and D). In addition, a marked aggregation of pigment deposits in the epidermis was observed (Fig. 2, Panel D).

The activity of SOD (Fig. 3, Panel A) was unaffected by the insecticide, remaining 361 ± 109 mU/mg of protein in spiders exposed to imidacloprid and 193 ± 12.2 mU/mg of protein in spider not exposed to imidacloprid ($p = 0.30$). CAT activity (Fig. 3, Panel B), however, was significantly increased by 30% with insecticide exposure, with 13.2 ± 1.4 mU/mg of protein observed in the presence of imidacloprid and 10.7 ± 1

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.28 mU/mg of protein in its absence ($p = 0.042$). The values of GST, GR, and GPX in the spiders exposed to imidacloprid manifested no statistically significant differences from the respective values of the control groups. The activity of GST (Fig. 3, Panel C) was between 19.1 ± 3 and 21.2 ± 2.7 mU/mg of protein ($p = 0.35$), while that of GR (Fig. 3, Panel D) was between 22.9 ± 6.4 and 21.1 ± 11.8 mU/mg of protein ($p = 0.8$) and of GPX (Fig. 3, Panel E) between 19.6 ± 2.65 and 14.1 ± 2.8 mU/mg ($p = 0.72$).

Finally, imidacloprid caused a significant increase in the total amount of total protein (Fig. 3 Panel F). In the exposed spiders, a value of 61.2 ± 3.4 ug/mg wet weight was observed, about 17 µg/mg wet weight above the value of the spiders not exposed to the insecticide (44.3 ± 11, 1 µg/mg wet weight) ($p = 0.030$).

DISCUSSION

The sensitivity of nontarget organisms to insecticide exposure is extremely variable and depends on several conditions (Pekár 2012; Balabanidou et al. 2019; Gunstone et al. 2021), though two fundamental considerations must be made: first, how insecticides reach and/or enter the organisms (*i. e.*, orally or via residues, administered topically) and, second, how organisms respond physiologically to maintain homeostasis *i. e.*, via detoxification mechanisms. The mean half-life of insecticides in ecosystems becomes of vital significance since those agents can be found as residues on surfaces (maintaining the toxicity for a longer time) or in the form of droplets that may be drunk by the nontarget organisms like spiders. Indeed, imidacloprid has a mean 40-day life after its application in soil (Rouchaud et al. 1996; Sarkar et al. 2001).

Spiders have a chemosensitive sensilla on their first legs and pedipalps (Kronestedt 1979; Foelix 2011; Trabalon 2013) capable of recognizing residues of

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organophosphate, pyrethroid (Pekár and Haddad 2005), and neonicotinoid pesticides (Easton and Goulson 2013). The spider *Pardosa saltans* (Lycosidae) recognizes surfaces with imidacloprid, with a resulting decrease in the time of contact (Laino et al. 2021). The parasitoid *Encarsia formosa* (Hymenoptera: Aphelinidae) also avoids residues of imidacloprid after 16 weeks of application (Ritchter et al. 2003). In the present study, however, juveniles of *M. maculissparsus* did not seem to recognize residues of the imidacloprid on surfaces with a field dose or even a 10 times offield dose (14.7 ug/cm^2). A similar effect had been observed in the spider *Pardosa pseudoannulata* (Lycosidae) through the use of preys impregnated with imidacloprid (Widiarta et al. 2001).

The movement behavior of the juveniles (the pattern of exploration and the path taken) remained unchanged on surfaces with imidacloprid unlike that of *Pardosa milvina* (Lycosidae), which species had increased its locomotion on surfaces with herbicides (Griesinger et al. 2011); while *Oxyopes javanus* (Oxyopidae) had exhibited an increase in prey capture after exposure to imidacloprid (Butt et al. 2019).

Imidacloprid, as other neonicotinoids (thiacloprid and acetamiprid), caused a temporary paralysis, as had been observed in several species of Linyphidae (Rezac et al. 2019). With *M. maculissparsus*, the reaction occurred at 24 h in approximately 74% of the individuals treated, but then decreased to 19% at 48 hours, and finally to 6% at 72 h. This time of paralysis is of major significance, since that condition exposes spiders to predators, leaving them unable to protect themselves. A similar situation was observed in Linyphiid and Erigonid spiders after exposure to a pyrethroid insecticide (Everts et al. 1991). At 48 h a decrease occurred in the spiders that were affected and, consequently, a great increase in the ones that recovered perhaps because of the physiologic changes

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analyzed at 48 h (*vide infra*) along with a low percent mortality. Finally, spiders failing to recover by 72 hours appeared to no longer be able to do so because a value of 20% mortality resulted by the end of the experiment (Fig. 1).

Imidacloprid had caused histopathologic changes in vertebrates (Toor et al. 2013; Arfat et al. 2014) and in certain invertebrates (Dittbrenner et al. 2011; Shan et al. 2020), but no reports were available on spiders. In the present work, we determined that the insecticide provoked a loss of tissue integrity, a separation of the epidermis, necrosis, and an aggregation of pigments in *M. maculissparsus* after consuming imidacloprid at the field dose for agricultural use. The aggregation of pigment deposits was a clear biomarker of exposure to toxic agents, as had been documented in vertebrates (Schwindt et al. 2006; Mela et al. 2007; Marchand et al. 2009; Pascoli et al. 2011) and invertebrates (Marigómez et al. 2013; Lavarías et al. 2021). In histological sections, melanin is difficult to distinguish from other pigments like lipofuscin and ceroids (Agius and Roberts 2003; Thorsen et al. 2006). Yet, spiders may possibly accumulate melanin when exposed to a xenobiotic (like imidacloprid) since a protection against cytotoxic damage is one of that pigment's functions (Pérez-Iglesias et al. 2016), similar to what had been observed in vertebrates (Passantino et al. 2014). Nevertheless, we cannot rule out the hypothesis that the accumulation of “melanin” was generated in response to increased oxidative stress, because melanins can scavenge free radicals, inactivate ROSs and bind prooxidant cations of transition valence to form inactive complexes (Potts and Au 1976; Riley 1997; Nappi and Christensen 2005; Dontsov 2014; Ushakova et al. 2019). New works are necessary to understand how imidacloprid can affect the synthesis of pigments in spiders. Imidacloprid like other neonicotinoids affects acetylcholine receptors, mediating the

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synaptic response in the central nervous system (Jeschke et al. 2011). This compound, as other chemical contaminants, is a major inducer of ROS in living organisms (Valavanidis et al. 2006) that can alter the cellular redox balance by different mechanisms (Franco et al. 2009). A ROS excess is counteracted by the antioxidant enzymes to avoid oxidative stress and cellular damage. One of the enzymes acting against free radicals is SOD and later CAT. This double induction was recently observed in the spider *Polybetes pythagoricus* (Sparassidae) treated with cypermethrin (Laino and García. 2020) and in *Xerolycosa nemoralis* (Lycosidae) exposed to dimethoate (Wilczek et al. 2013). In the example of insects, this induction had been observed in *Lymantria dispar* (Lepidoptera: Erebidae) after exposure to the organophosphate malondialdehyde (Aslanturk et al. 2011) and in *Oxya chinensis* (Orthoptera: Acrididae) after exposure to chlorpyrifos and malathion (Wu et al. 2011). In the present work, imidacloprid evoked a different response in *M. maculissparsus*, since the spider did not manifest an increase in SOD activity, but did so in CAT activity. The immediate presence of H_2O_2 that promoted the CAT induction was probably due to other sources of peroxide like the system of cytochrome P450 (Krest et al. 2013; Albertolle and Guengerich 2018). The non induction of SOD and the induction of CAT by imidacloprid had recently been observed in juveniles of *Pardosa saltans* (Laino et al. 2021), in the insect *Rhopalosiphum padi* (Hemiptera: Aphididae) (Li et al. 2018), and in the crustacean *Daphnia magna* (Cladocera: Daphniidae) (Qi et al. 2018).

GPx and GR are key enzymes for the detoxification of ROS. GPx removes H_2O_2 through the conversion of GSH from its reduced state to the oxidized form, while GR maintains the levels of reduced GSH through the oxidation of NADPH. GSTs represent a

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major family of phase-II detoxifying enzymes for catalyzing the conjugation of electrophilic compounds with glutathione (GSH). The products of the conjugation are less active and more water-soluble, thus facilitating their excretion (Clark 1989, Strange et al. 2000). When *M. maculissparsus* was exposed to imidacloprid, the activities of GPx and GST did not increase as had been recently observed in *P. saltans* for this insecticide (Laino et al. 2021). The activity of GR remained similar to control values; which observation does not coincide with the finding in juveniles of *P. saltans,* where imidacloprid was seen to increase that activity (Laino et al. 2021), or in females of *P. pythagoricus* upon exposure to cypermethrin (Laino and García 2020).

The enzymes associated with glutathione (GPx, GR, and especially GST) are very frequently used as biomarkers of contamination by insecticides (Lagadic et al. 1994). The activities of those enzymes, however, constitute highly variable parameters and can also decrease or be affected upon exposure to a toxic agent, even within the same species for different times of exposure (Domingues et al. 2010; Paskerová et al. 2012). In spiders the inactivation of GR, GST, and SOD is probably related to the presence of determined classes of ROS, as has occurred in other organisms (Lushchak et al. 2009). For instance, both GST and GR are known to be sensitive to products of the Haber-Weiss reaction (Gutierrez-Correa and Stoppani 1997; Hermes-Lima 2004), and SOD can be inactivated by hydrogen peroxide (Bray et al. 1974).

We need to emphasize that at 48 h of treatment with imidacloprid the only enzyme that exhibited a significant increase in activity was CAT, coinciding (except for the afore mentioned GR) with that described for juveniles of *P. saltans* (Laino et al. 2021). In *M. maculissparsus,* imidacloprid induces CAT activity, which enzyme turned

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out to be the most sensitive antioxidant and also one with the greatest protective activity against oxidative damage, thus coinciding with reports for other spiders such as *P. saltans* (Lushchak et al. 2009; Kumar et al. 2011).

When imidacloprid enters the spider's body, this induces a significant increase in proteins similar to that described for the same insecticide in juveniles of *Porcellios caber* (Isopoda: Porcellionidae); (Drobne et al. 2008), in *Helix aspersa* (Pulmonata: Helicidae) (Radwan and Mohamed 2013), and in *Nilaparvata lugens* (Hemiptera: Delphacidae); (Ge et al. 2009).

The results obtained emphasize the need to consider different classes of biomarkers (CAT, proteins, and histopathologies) in order to identify and evaluate the biologic and biochemical effects of one of the most widely used insecticides within the terrestrial environment along with its plethora of effects on nontarget organisms.

Supporting Information—The Supporting information are available on the Wiley Online Library at DOI: 10.1002/etc.xxxx.

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Data availability—Data, associated metadata, and calculation tools are available from the corresponding author (cfgarcia1123@yahoo.com.ar). Most data is included within the

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manuscript. The full data is not in a repository or included as supporting information because they are basic mathematical and biochemical analyzes common to this type of study.

Author contributions statement—Gabellone, Molina and Arrighetti. Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing Review & Editing. Laino A., Investigation, Methodology, Formal analysis, Writing Review & Editing. Garcia C. F. Conceptualization, Funding acquisition, Writing Original Draft, Writing Review & Editing.

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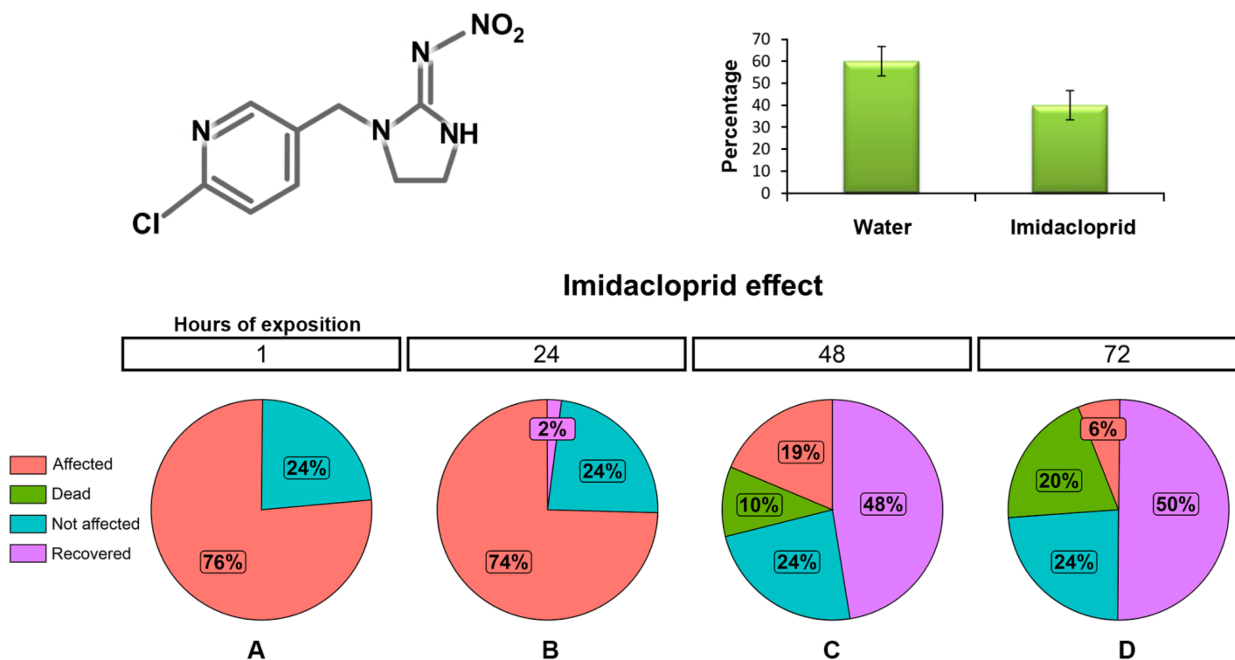

FIGURE 1. Effect of imidacloprid on *Misumenops maculissparsus* in the drinking water. The pie plots summarize the state of juveniles (unaffected, blue; affected, red; recovered, violet; dead, green) at different times A, B, C, D (1, 24, 48, and 72 h demarcated above) after drinking water with imidacloprid (n = 300). *Left inset*: The chemical structure of imidacloprid. *Rightinset*: In the bar graph, the percent of spiders choosing the water with or without imidacloprid (n = 80) is plotted on the *ordinate* with the two choices being indicated on the *abscissa*.

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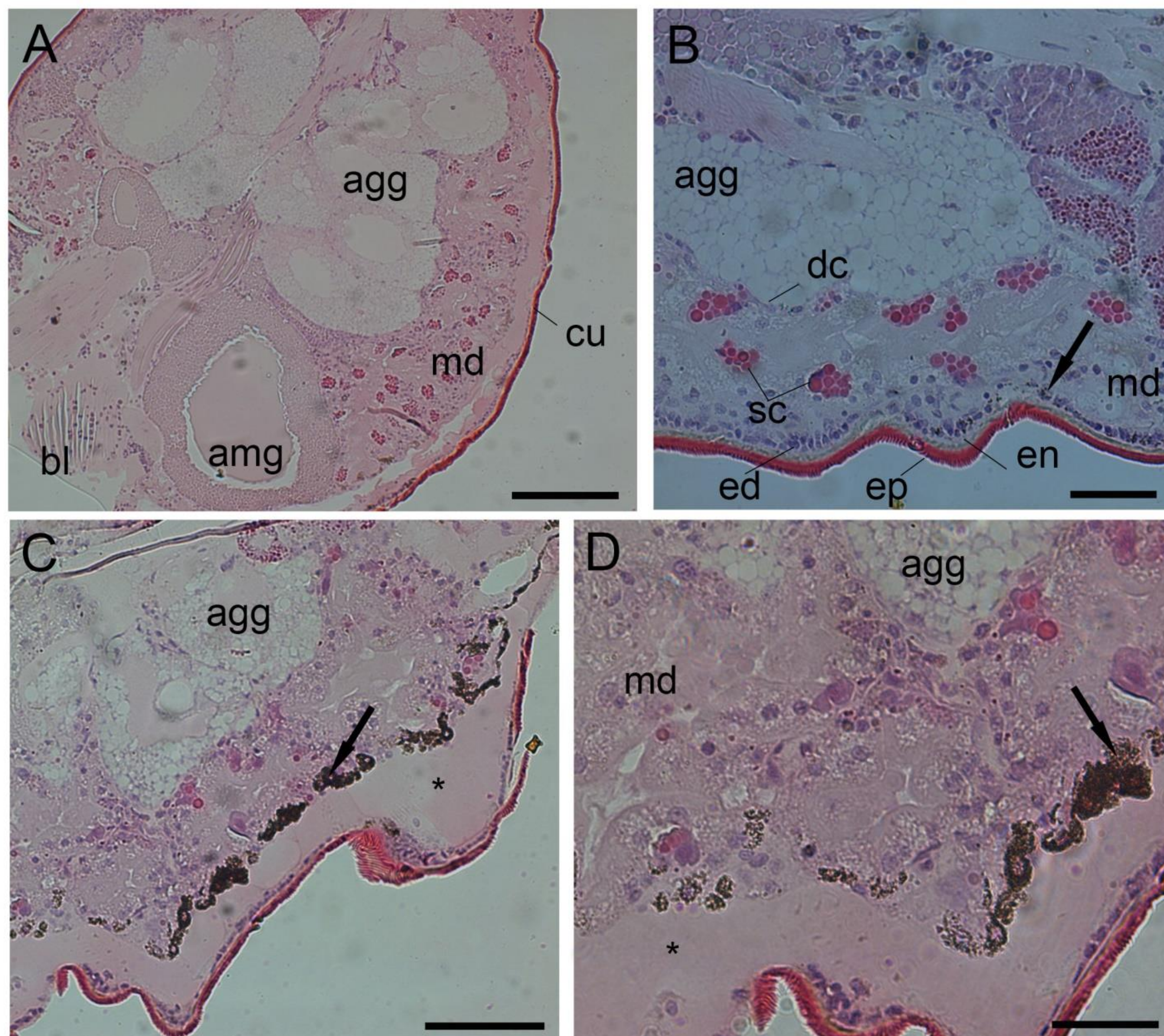

FIGURE 2. Histological analysis of *Misumenops maculissparsus*. Panels A and B: Cross sections of the opisthosoma in control spiders. Panel A: General view of the ampullate glands near the book lung (bl), the aggregate glands (agg), and the midgut diverticula (md) beneath the cuticle (cu). Panel B: Detail of the midgut diverticula revealing digestive and secretory cells (dc). Cuticle with the three layers: epicuticle (ep), endocuticle (en), and epidermis (ed) with small pigment deposits (thin black arrow). Panels C and D: Cross sections of the opisthosoma in spiders exposed to 0.875 µg of imidacloprid (5-µl drop) illustrating a separation of the cuticle from the midgut

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diverticula (small black asterisk). Considerable aggregation of pigment deposits (thin black arrow) and a disintegrated midgut diverticula. The aggregate glands exhibited signs of necrosis. Scale bar: (A) = 200 μm; (B), (C) = 50 μm; (D) = 100 μm.

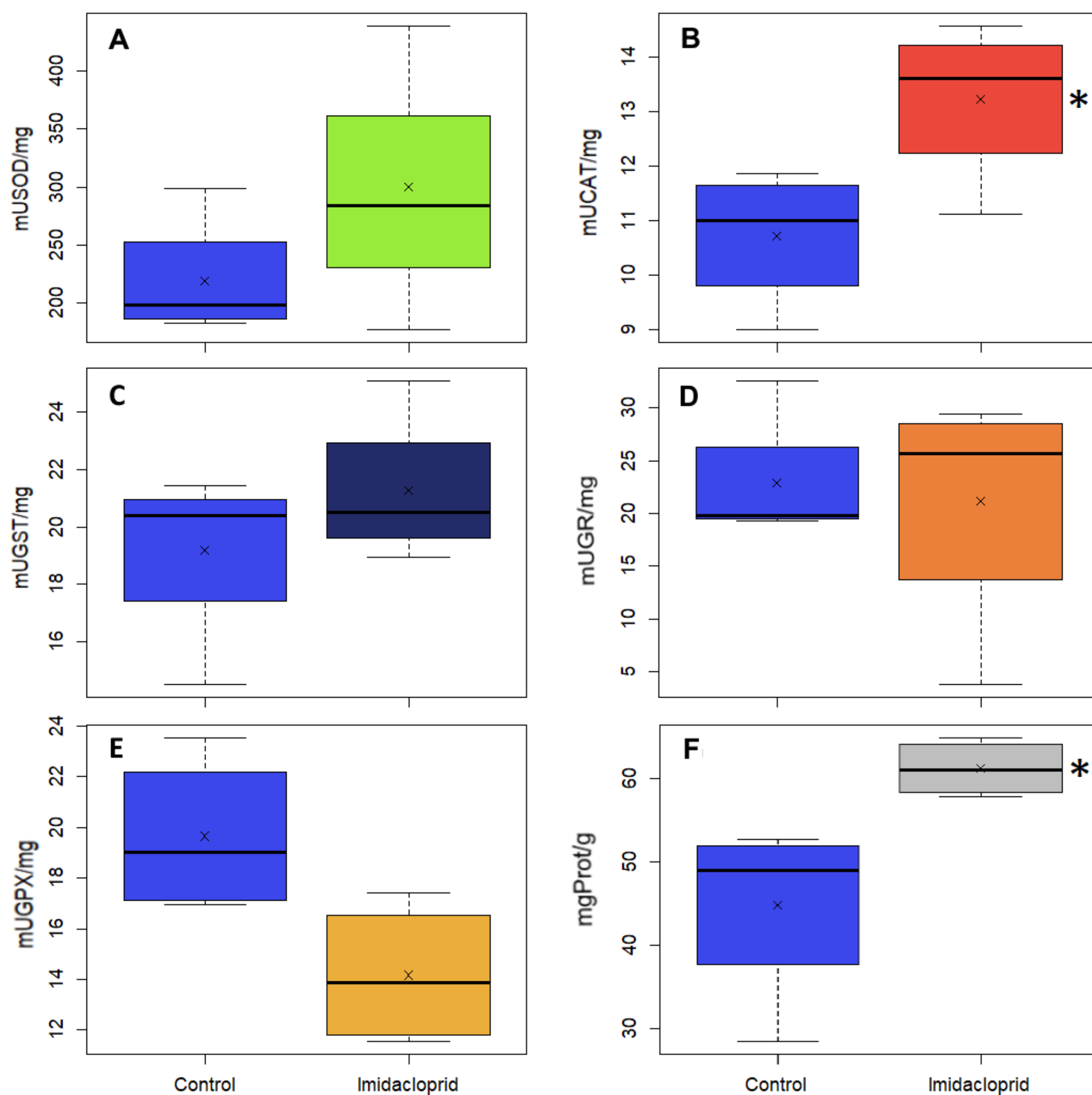

FIGURE 3. Effect of exposure of *Misumenops maculissparsus* to field doses of imidacloprid on antioxidant activity. After exposure of J3 juveniles to the insecticide, the activity in enzyme units of SOD (Panel A), CAT (Panel B), GST (Panel C), GR (Panel D), GPX (Panel E) and total body proteins (Panel F) were assayed. The values represent

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the mean of 4 independent determinations ± SD (*, $p < 0.05$; $n = 30$ for each group). In each of the panels, the activity in the control spiders (left) and those exposed to imidacloprid (right) is plotted on the *ordinate*. In the box plots, the upper and lower borders represent the quartiles of the data, the solid line the median, and the upper and lower outriders (the whiskers) the respective maximum and minimum values.

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