



Effects of the insecticides methoxyfenozide and cypermethrin on non-target arthropods: a field experiment

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Abstract

The adverse effect of pesticides on non-target organisms and the environment is an issue of great social concern. Currently, there are a variety of available insecticides, which are claimed to be safer for beneficial organisms than broad-spectrum insecticides. We conducted a field experiment to compare the effect of applications of methoxyfenozide (an insect growth regulator) and cypermethrin (a conventional insecticide) on the arthropod community in a commercial willow plantation. We used a one-way Anova design with insecticide as the treatment factor, each individual tree as the experimental unit, and the number of dead arthropods collected per sampling unit as the response variable. Results showed that the number of dead arthropods collected 72 h after treatment with methoxyfenozide (1274 individuals) was 66% lower than that collected underneath cypermethrin-treated trees (3408 individuals). The only groups not recorded from boxes placed underneath methoxyfenozide-treated trees, but yes underneath cypermethrin-treated trees, were Dicyoptera, Pseudoscorpionida and Isopoda. Methoxyfenozide was lethal to a lower number of families of dipterans, coleopterans and hymenopterans than the cypermethrin during the first 72 h after treatment. The number of taxonomic groups and abundance of dead predators, herbivores and parasitoids was also lower in methoxyfenozide than in cypermethrin-treated trees. Our results obtained from a field experiment suggest that methoxyfenozide provides a less toxic alternative for control of pests in willow plantations due to a reduced effect on non-target organisms.

Key words functional groups, Paraná River Delta, Salicaceae, tree plantations.

INTRODUCTION

Pesticide application is usually perceived as a powerful tool in crop protection. Although pesticides are typically effective for pest control in the short term, they may have direct or indirect negative effects on non-target and beneficial organisms. That is especially relevant because arthropods play key roles in ecosystem functioning and provide ecological services (Pimentel 2005). Losey and Vaughan (2006), for example, estimated annual economic losses of approximately 7.9 billion dollars for the United States if beneficial insects were not undertaking functions at their current level in ecological services such as dung burial, pest control, and pollination. At the community level, insect diversity in cropping systems has been negatively associated with pesticide use (Crowder *et al.* 2010; Trichard *et al.* 2013). Although the consequences of human activities that change community diversity are not fully understood, there is consensus in that the loss in species richness and changes in evenness affect ecosystem processes (Hooper *et al.* 2005; Cardinale *et al.* 2006; Hillebrand *et al.* 2008). Thus, it is crucial to implement agricultural and silvicultural practices that conserve ecosystem integrity and ensures the supply of ecosystem functions and services (Tilman *et al.* 2002). It is then necessary to develop safe and environmentally friendly pest

management strategies, for example, replacing conventional broad-spectrum insecticides with more selective products.

Recently, the agrochemical industry has produced a range of insecticides with alternative mechanisms of action compared to the conventional broad-spectrum insecticides. These newer compounds are considered to have relatively low adverse effects on humans and the environment (Ishaaya *et al.* 2007), and are often referred to as biorational or selective insecticides (e.g., Carlson 2000; Wang *et al.* 2005; Rimoldi *et al.* 2008; Saber & Abedi 2013). Although the biorational insecticides (e.g., spinosyns, insect growth regulators, avermectins) are claimed to be safer for non-target organisms than conventional products, there is evidence of negative effects on many species (e.g., Cisneros *et al.* 2002; Schneider *et al.* 2008; Gradish *et al.* 2012; Saber & Abedi 2013).

The effects of pesticides on non-target organisms are usually assessed under laboratory conditions using acute toxicity assays and different indicator species (Desneux *et al.* 2007). Although laboratory studies provide useful information, field studies provide more realistic scenarios because the effects of pesticide applications on insects depend on natural environmental conditions (Relyea & Hoverman 2006). Thus, the impact of insecticides on non-target arthropods is more realistically assessed in experiments conducted under field conditions.

The Delta of the Paraná River in Argentina is a freshwater wetland of high environmental heterogeneity and biodiversity (Kandus *et al.* 2006). It includes one of the largest areas used

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for commercial willow and poplar plantations in America (CIA 2004). At those plantations, the invasive willow sawfly *Nematus oligospilus* Förster (Hymenoptera: Tenthredinidae) often becomes a serious pest and chemical control is usually conducted by applying cypermethrin sprays. Cypermethrin induces neurotoxicity by acting on the sodium channels in neuronal membranes, which increases the duration of neuronal excitation (Ware & Whitacre 2004). It usually kills a wide range of pest and non-target arthropods (Cloyd 2012; Talebi *et al.* 2008), and has been classified as moderately toxic for mammals (WHO 2010) and as potentially carcinogenic for humans (US EPA 2008). Methoxyfenozide is a diacylhydrazine, which acts as insect growth regulator (Ware & Whitacre 2004). The compound is an agonist of the 20-hydroxyecdysone (molting hormone) that binds to the hormone receptors thereby stimulating a lethal premature molt. Ecdysone agonists are considered selective insecticides (Dhadialla *et al.* 1998). Methoxyfenozide is used in the control of lepidopteran pests such as *Epiphyas postvittana* (Walker), *Ennomos subsignaria* (Hübner) and *Lymantria dispar* (L.) (Smaghe *et al.* 2012); and it is considered an option to manage the codling moth *Cydia pomonella* (L.) and the oriental fruit moth *Grapholita molesta* (Busck) (Smaghe *et al.* 2012). Resistance for methoxyfenozide has already been reported (Knight *et al.* 2001). In Argentina, methoxyfenozide is a product admitted for commercial use as phytosanitary by National Service of Health and agro-Food Quality (SENASA). Thus, its use in silviculture will be determined by foresters. Given that methoxyfenozide is considered selectively toxic to lepidopterans and highly safe for non-target organisms (Dhadialla *et al.* 1998), it is considered a safer alternative than conventionally applied pyrethroids. Our objective was to quantify and compare the mortality of non-target arthropods exposed to methoxyfenozide and cypermethrin under field conditions, providing empirical evidence to help selecting more environmentally friendly options for pest control.

MATERIALS AND METHODS

The study was conducted in private willow tree plantations “Las Animas” from the forest company Papel Prensa S.A., located in the Lower Delta of the Paraná River, Argentina (33°37′72″S, 58°37′40″W). The Lower Delta is the most southern part of the Delta of the Paraná River basin, one of the longest rivers in South America. The Delta extends from 32°5′ S, 58°30′ W to 34°29′ S, 60°48′ W, and is a heterogeneous mosaic of wetlands that support high biodiversity (Malvárez 1999). Part of the Lower Delta was declared Biosphere Reserve by the UNESCO in 2000. The Lower Delta is characterized by pan-shaped islands dominated by marshes, with extended highlands bordering lowlands areas either temporarily or permanently flooded. The climate is temperate and humid, with mean annual temperature ranging 16.7°C and 18°C and mean annual precipitation of 1000 mm. Originally, the highlands were dominated by multistratified forest and rich plant communities, and marshes were composed by different species of bulrush such as *Scirpus giganteus* Kunth and *Schoenoplectus californicus* (C.A. Mey.)

Soják. Currently, most of the original forest is degraded due to human activities (Malvárez 1999, Kandus *et al.* 2006). Since 1950, willow and poplar plantations became a primary commercial activity in the Lower Delta, and now it stands out as the major center for the production of Salicaceae in Argentina, with about 60000 ha planted (Quintana & Bó 2010; MAGP 2012). Periodic floods are part of the natural regime but wetlands were converted into dryer lands through dam constructions to protect plantations (Kandus & Minotti 2010).

The experiment was conducted in a 9.1 ha willow tree stand containing stump sprouts (= trees) of hybrid willow *S. babylonica* × *S. alba* (“Ragonese 131–27 INTA”). Trees were 1.6 m height in average. We used a one-way Anova design with insecticide as the treatment factor, each individual tree as an experimental unit or replicate, and the number of dead arthropods collected per sampling unit as the response variable. We randomly selected 20 trees that were at least 8 m apart from each other to prevent treatment interference. Each tree was randomly assigned to treatment with either methoxyfenozide or cypermethrin (10 replicates or trees per treatment). Insecticide was applied to each single tree or experimental unit. After the insecticide application, trees were covered with a fine mesh to avoid the loss of small arthropods. Given that our objective was to compare mortality of arthropods between the new growth regulator methoxyfenozide and the currently used cypermethrin, the treatment with cypermethrin acted as control for the treatment with methoxyfenozide.

Insecticides were applied with a manual backpack sprayer. The insecticide spray was applied until the drip point was reached. The commercial formulation of methoxyfenozide (Intrepid*SC®, Dow AgroSciences Argentina S.A.) was applied at the recommended for lepidopteran pests of citrus (30 ml methoxyfenozide in 100 l water). We used the cypermethrin dose of 200 ml cypermethrin in 100 l water (Cipermetrina 25 EC, Daargusciper Reopen S.A.), which is commonly used by forest companies in the study area, and applied approximately 600 ml of solution per tree.

Dead arthropods were collected in four cardboard boxes (68.5 cm in length × 36.5 cm in width × 4 cm in height) placed under each tree. The four boxes were considered the sampling unit. Prior to insecticide application, cardboard boxes were placed on the ground and arranged in a cross design centered in the tree. Boxes were checked 72 h after the insecticide application, and arthropods were collected and preserved in 80% ethanol for later identification. In short-term laboratory assays, effects of methoxyfenozide were often monitored over 1 to 4 days (e.g., Kim *et al.* 2006; Stara *et al.* 2011). Given that the experiment was set up in the field, the potential of flooding associate with rainfall may interfere with the preservation of insects collected in boxes. Thus, the selection of 72 hours as the time for collection was a compromise between weather conditions and the time often used in previous studies.

Most collected arthropods were identified to family level using taxonomic keys (CSIRO 1991; Entomología 1998). After identification, some taxa were classified into functional groups based on feeding habits (i.e., predators, herbivores, detritivores,

hematophagous) or their role in ecosystem processes and services (i.e., pollinators, parasitoids) (CSIRO 1991; Marinoni 1997; Cassis & Gross 2002; Loíacono *et al.* 2002; Scudder & Cannings 2005; Loíacono *et al.* 2006; Nihei & Domínguez 2008; Díaz *et al.* 2011). In assigning families or orders to functional groups, we considered only those taxa that could be assigned to a single functional group. Taxa included in each of the considered functional group are listed in Appendix I.

Rarefaction curves were used to compare the number of families of dead arthropods between treatments. Calculations used 100 multiple random orderings of the samples in steps of 10 individuals, and were conducted using EstimateS (version 9.0, R. K. Colwell, <http://purl.oclc.org/estimates>). Rarefaction curves were rescaled to the accumulated number of individuals to compare family richness between treatments independently of the number of individuals. Differences between treatments in the abundance, number of families and number of taxa within functional groups were tested with the Student's *t*-test for two independent samples. Data were square root transformed when required to meet the Student's *t*-test assumptions of normality and homogeneity of variance, and the Wilcoxon test was used if data transformation was unsuccessful (Zar 1996). In either case, the Bonferroni correction was applied to adjust significance levels for multiple comparisons (Zar 1996).

RESULTS

We collected a total of 4682 arthropods representing 16 Orders. The number of identified families was 31 Diptera, 30 Coleoptera, 22 Hymenoptera, 8 Homoptera, 6 Heteroptera, 4 Arthropleona, 2 Thysanoptera, 2 Acarina, 1 Symphypleona, 1 Lepidoptera, 1 Dytioptera, 1 Psocoptera, 1 Amphipoda and 1 Isopoda. Araneae and Pseudoscorpionida were identified only to Order level. Dytioptera, Pseudoscorpionida and Isopoda were not recorded from boxes placed underneath methoxyfenozide-treated trees (Appendix II). Approximately 73% of the affected families were Coleoptera, Diptera or Hymenoptera in both treatments.

The number of dead arthropods collected underneath the cypermethrin-treated trees (3408 individuals) was approximately three times higher than that collected underneath the methoxyfenozide-treated trees (1274 individuals) 72 h after the

insecticide application. Rarefaction analysis based on samples showed that the number of families affected in cypermethrin-treated trees was always higher than in methoxyfenozide-treated trees (Fig. 1a). Individual-based rarefaction also showed the number of affected families was significantly higher in cypermethrin than in methoxyfenozide applications (Fig. 1b), indicating that the cypermethrin applications killed more arthropod families than the methoxyfenozide. Significantly fewer dead individuals of Araneae ($t=5.789$, $P<0.05$), Coleoptera ($t=6.062$, $P<0.05$), Hymenoptera ($t=3.073$, $P<0.05$), Homoptera ($t=3.224$, $P<0.05$) and Diptera ($t=3.889$, $P<0.05$) were collected underneath methoxyfenozide-treated trees than under the cypermethrin-treated trees (Fig. 2a). The number of families also showed significant differences between treatments: methoxyfenozide affected a lower number of families of Coleoptera ($t=5.854$, $P<0.05$), Hymenoptera ($t=3.108$, $P<0.05$), Homoptera ($t=4.638$, $P<0.05$) and Diptera ($t=4.069$, $P<0.05$) than the cypermethrin (Fig. 2 b).

All functional groups considered here were affected by both insecticides 72 h after treatment. Most arthropods were herbivores, detritivores, parasitoids or predators. Methoxyfenozide killed significantly fewer taxa of predators ($t=4.919$, $P<0.05$), herbivores ($t=6.151$, $P<0.05$) and parasitoids ($W=147.0$, $P<0.05$) than the cypermethrin application (Fig. 3a). Similarly, there were significant differences in the abundance of dead predators ($W=155.0$, $P<0.05$), herbivores ($W=151.0$, $P<0.05$) and parasitoids ($t=3.980$, $P<0.05$) between treatments, with the lethal effect of methoxyfenozide being significantly lower than that of cypermethrin. No differences were found between treatments in the abundance of detritivores ($t=1.620$, $P>0.05$), pollinators ($t=1.030$, $P>0.05$) and hematophagous ($t=1.100$, $P>0.05$) arthropods (Fig. 3b).

DISCUSSION

Our results indicated that the acute toxicity of methoxyfenozide was lower than that of cypermethrin 72 h after treatment. We expected such a difference because cypermethrin is a broad-spectrum pyrethroid, whose neurotoxic action usually kills a wide range of pest and non-target arthropods (Talebi *et al.* 2008; Cloyd 2012), whereas ecdysone agonists are considered

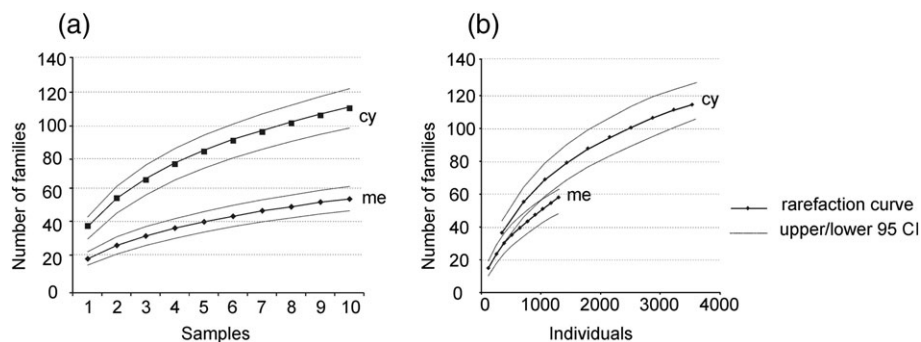


Fig. 1. Rarefaction curves of arthropod families represented in boxes placed underneath willow trees to collect dead arthropods after 72 h of treatment with methoxyfenozide (me) or cypermethrin (cy). (a) Family density (sampled-based rarefaction), (b) Family richness (individual-based rarefaction).

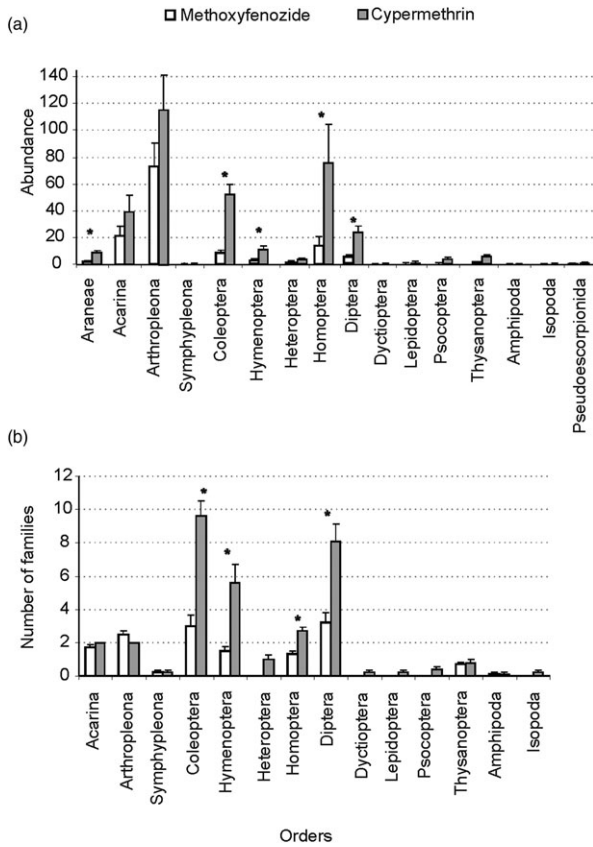


Fig. 2. Number of dead arthropods collected underneath willow trees (four boxes per tree) 72 h after treatment with methoxyfenozide ($n = 10$) or cypermethrin ($n = 10$). (a) Mean number (+ SE) of individuals. (b) Mean number (+ SE) of families. Within each order, asterisk indicates significant difference between treatments (Student's t -test; $P < 0.05$).

more selective insecticides (Dhadialla *et al.* 1998). Such selectivity has been related to differences in structure and biochemical properties of ecdysteroid receptors among insect species (Carlson 2000). However, differences in speed of action of insecticides cannot be ruled out as a possible source of bias. Insecticides that disrupt the hormonal system, like ecdysone agonists and others IGRs, generally act more slowly than neurotoxic insecticides and do not kill insects immediately (Biddinger *et al.* 2006). Some previous studies conducted in controlled laboratory conditions monitored the effects of methoxyfenozide for 1 to 4 days (e.g., Kim *et al.* 2006; Stara *et al.* 2011), indicating that the 72 hours we monitored were likely enough to kill most of the affected insects. However, it is possible that we missed some further mortality that may have occurred.

Although almost all arthropod orders affected by cypermethrin were also affected by methoxyfenozide (13 out of 16 orders), the lower number of families and abundance of dipterans, coleopterans and hymenopterans, among others, affected by the methoxyfenozide suggest that it has a narrower activity spectrum than the cypermethrin. Moreover, both insecticides affected the same functional groups, but the number of taxa and abundance of predators, herbivores and parasitoids was lower in methoxyfenozide-treated trees than in the

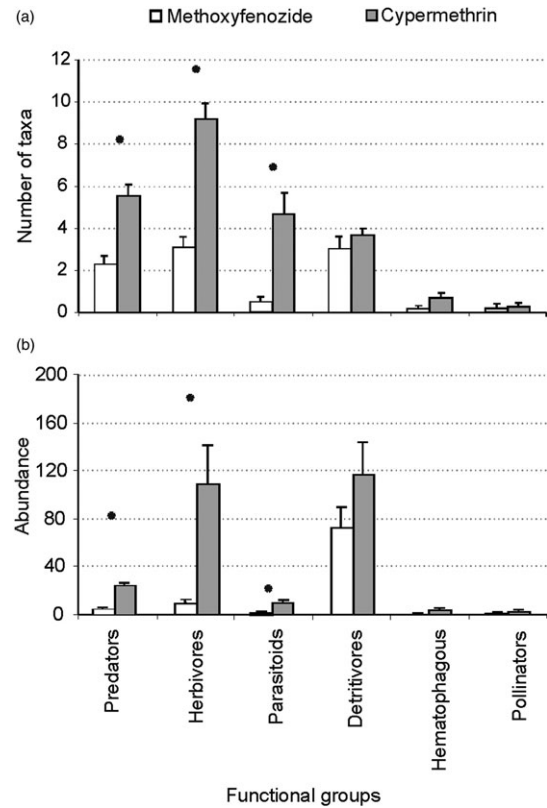


Fig. 3. Functional groups of arthropods recorded underneath willow trees (four boxes per tree) 72 h after treatment with methoxyfenozide ($n = 10$) or cypermethrin ($n = 10$). (a) Number of collected taxa (mean + SE). (b) Number of collected individuals (mean + SE). Within each functional group, asterisk indicates significant difference between treatments ($P < 0.05$).

cypermethrin-treated trees. Low toxicity to beneficial arthropods is a desirable trait for insecticides. The key role of beneficial arthropods such as predators, parasitoids and pathogens to limit damage of most pests is widely accepted (Symondson *et al.* 2002; Bianchi *et al.* 2006). This is therefore crucial to keep a balance between the effectiveness of applying insecticides and the preservation of natural enemies in pest management strategies (Kogan 1998). Methoxyfenozide was previously shown to have relatively low acute toxicity to different predator and parasitoid species. For example, Rimoldi *et al.* (2008) found no effects on immature survivorship after exposure of *Chrysoperla externa* eggs to methoxyfenozide. Kim *et al.* (2006) reported no acute toxicity to adults of the predator *Deraeocoris brevis* (Uhler) 24, 48, and 96 h after spray application. Similarly, Schneider *et al.* (2008) found no harmful effects on adult survival of the parasitoid *Hyposoter didymator* five days after exposure. According to Stara *et al.* (2011), methoxyfenozide can be classified as harmless to the parasitic wasp *Aphidius colemani* Viereck 24 h after treatment, although mortality increased after 48 h. Low acute toxicity has also been reported on preimaginal and adult stages of the parasitoid wasp *Habrobracon hebetor* Say (Saber & Abedi 2013). However, sublethal effects were also reported, i.e. behavioral or physiological effects on individuals that survive exposure to a pesticide (Desneux *et al.* 2007). For example,

Saber and Abedi (2013) observed reduction of fertility and fecundity, and sex ratio alteration in the parasitoid wasp *Habrobracon hebetor* Say after exposure to methoxyfenozide. Kim *et al.* (2006) observed increased development time and reduced fecundity in the predator *Deraeocoris brevis* (Uhler). Reduction in fecundity was also reported by Stara *et al.* (2011) in the parasitic wasp *Aphidius colemani* Viereck.

Our results indicated that methoxyfenozide is a better option than cypermethrin with regards to short-term lethal effects on non-target arthropods. However, our results could be influenced by the monitoring time. Although they are conclusive about arthropods killed within the time frame examined (72 h), methoxyfenozide (a growth regulator) could require a longer time period to show acute toxicity; thus the absence of dead individuals of a given taxon do not necessarily imply that the insecticide has no effect on the taxon. Moreover, sublethal effects need to be included in the assessment to make a more complete evaluation of the ecological impact of methoxyfenozide use in forestry plantations. Synergic interactions among pesticides, abiotic factors and biotic stressors may occur under field conditions influencing the effect of insecticides on non-target organisms (Relyea & Hoverman 2006). Therefore, it is recommended to assess insecticide selectivity at the geographical location where it is applied. We hope our study will help to encourage the use of more realistic and holistic approaches to study the effects of insecticides on non-target organisms.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Appendix I Classification into functional groups of arthropod taxa collected dead from boxes placed underneath willow trees after 72 h of treatment with methoxyfenozide or cypermethrin.

Appendix II Presence of arthropods collected underneath willow trees 72 h after treatment with methoxyfenozide or cypermethrin.