Seed dressing pesticides on springtails in two ecotoxicological laboratory tests

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ARTICLE INFO
Article history:
Received 27 January 2014
Received in revised form
3 April 2014
Accepted 9 April 2014

Keywords:
Seed treatment
Collembola
Folsomia candida
Environmental risk assessment
Soil fauna
Soil ecotoxicology

ABSTRACT
Terrestrial ecotoxicological tests are powerful tools for assessing the ecological risks that pesticides pose to soil invertebrates, but they are rarely used to evaluate seed dressing pesticides. This study investigated the effects of seed dressing pesticides on survival and reproduction of Folsomia candida (Collembola), using standardized ecotoxicological tests (after ISO guidelines with few adaptations for tropical conditions). Commercial formulations of five seed dressing pesticides were tested individually in Tropical Artificial Soil (TAS): the insecticides imidacloprid, fipronil, thiametoxam, and the fungicides captan and carboxin + thiram. Thiametoxam, captan, and carboxin + thiram were only lethal to F. candida at the highest concentration tested (1000 mg of active ingredient kg 

1 soil d.w, respectively). Imidacloprid and fipronil were lethal at lower concentrations (100 and 10 mg a.i. kg 

1 soil d.w, respectively), however, these concentrations were much higher than those predicted (PEC) for soil. Imidacloprid and fipronil were the most toxic pesticides in both tests, reducing significantly collembolan reproduction (EC20 = 0.02 and 0.12 mg a.i. kg 

1 soil d.w, respectively). Further studies under more realistic conditions are needed, since imidacloprid and fipronil reduced collembolan reproduction at concentrations below or close to their respective PECs.

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1. Introduction

Treating seeds with pesticides is an important and increasingly common practice in agriculture. According to FAO (2012), in the last twenty years we have seen a significant increase worldwide in the use of seed dressing pesticides. The method is considered one of the most efficient available for preventing or minimizing damage by pests and pathogens that attack seeds and seedlings early in the crop cycle (Munkvold et al., 2006). Because the method is simple to apply and has a low cost-benefit ratio, the Brazilian market for seed dressing fungicides has more than doubled in size over the last decade (Menten and Moraes, 2010), and there is evidence that nearly 100 percent of soybean seeds are now treated with fungicides and 30 percent with insecticides (Baudet and Peske, 2006).

Although treating seeds with pesticides contributes indirectly to higher crop productivity, it also generates residues that can be toxic to non-target organisms. These residues can cause poisoning in mammals, phytotoxicity in plants, impacts on aquatic and soil communities, and leave pesticide traces in food products (Paulsrud et al., 2001). In this context, increased amounts of pesticide residues in agricultural soils imply a greater threat to soil fauna. In turn, impacts on the soil fauna may impair the processes they drive, including those related to organic matter decomposition, nutrient cycling, and maintenance of soil structure (Lavelle et al., 2006).

In the European Union, the assessment of pesticide impacts on soils is regulated by specific guidelines for approving the sale of plant-protection products (EC, 2013). These guidelines include standardized methods to assess the toxic effects of pesticides on living non-target organisms. In terrestrial environments, three invertebrate species are mostly recommended for ecotoxicological assays: Eisenia fetida/andrei (Lumbricidae), Folsomia candida (Collembola), and Enchytraeus albidus crypticus (Enchytraeidae) (Jänsch et al., 2006). In line with these international guidelines, Brazil has developed specific laws to manage polluted sites, and Brazilian legislation now includes several criteria for assessing soil quality via the use of live organisms. These include the establishment of ecological risk guidance values for pesticides in soil.
which are obtained via traditional methods of terrestrial ecotoxicology (CONAMA, 2009).

While springtails account for a small proportion of soil biomass and respiration (Coleman et al., 2004; Jänsch et al., 2005), these arthropods have a significant influence on microbial ecology and soil fertility, via their role in regulating processes of decomposition and nutrient cycling (Culik and Zeppelini, 2003).

This is one reason why several collembolan species have been used as bioindicators of pesticide toxicity in soils (Achazi et al., 2000; Greenslade and Vaughan, 2003; Heupel, 2002). F. candida is the collembolan species most commonly used in standard ecotoxicological tests (ISO, 1999). This species has a short generation time and reproduces by parthenogenesis, which makes it especially appropriate for tests that require analyses both at individual and population level in a single assay (Jänsch et al., 2005). In addition, Frampton et al. (2006) have argued that in laboratory toxicity studies F. candida is more sensitive to a broad range of pesticide modes of action (including biocide, fungicide, herbicide, and insecticide) than the earthworm E. fetida. More recently, Daam et al. (2011), when evaluating the sensitivity of pesticides in several groups of soil invertebrates when compared to the sensitivity of Eisenia species, found that collembolans are much more sensitive towards insecticides. Although earthworms (soft bodied organisms with major uptake routes being via the direct contact with soil solution through the skin and via ingestion of soil particles) may be more susceptible to the uptake of pesticides than collembolans (hard-bodied organisms with an exoskeleton and uptaking water via specialized organs) (Pejnenburg et al., 2012), the toxicity of these substances is driven not only by exposure but mainly by their mode of action. Most insecticides have relatively higher accessibility to insect’s nervous system (or other metabolic pathways, depending on the invertebrate class), so are expected to cause higher toxicity on collembolans than on earthworms (Mars and Ballantyne, 2004). For these reasons, the use of collembolans in ecotoxicological laboratory tests has become increasingly common over the last decade (Heupel, 2002; Liu et al., 2012; Natal-da-Luz et al., 2009; Santos et al., 2010) and is now mandatory in the new data requirements for pesticide risk assessment when these products are applied directly to the soil (EC, 2013). To date, the ecotoxicological studies that have investigated standard invertebrate species under tropical conditions have used earthworms (De Silva and Van Gestel, 2009; De Silva et al., 2010; García et al., 2008; Nunes and Espíndola, 2012) and only very few used collembolans (Chelinho et al., 2012; Niemeyer et al., 2010). Likewise, the ecological risks posed by seed dressing pesticides have not been widely studied in tropical regions (Alves et al., 2013).

Among the various formulations of pesticides used to treat seeds, the insecticides Gauchos®, Cropguard® and Standak®, with the active ingredients (a.i.) imidacloprid, thiametoxam and fipronil, respectively, and the fungicides Captain® (a.i. captan), and Vitavax® (a.i. carboxin + thiaram), are widely used in agriculture and their effects on earthworms were already studied in laboratory ecotoxicological assays by Alves et al. (2013). According to the literature, these active ingredients can also be toxic to collembolans (EFSF, 2010; Heijbroek and Huijbregts, 1995; Peck, 2009; Rather and Shah, 2010; Reynolds, 2008; San Miguel et al., 2008) and other non-target soil organisms like predatory mites, coleopteran larvae and spiders (Jackson and Ford, 1973; Moser and Obrycki, 2009; Tingle et al., 2000). In order to increase the understanding of the ecotoxicological effects of seed dressing pesticides on soil fauna, survival and reproduction tests with F. candida (Collembola) were performed with soils contaminated with five seed dressing pesticides. The usefulness of either survival or reproduction tests for the evaluation of the potential environmental risks of these products was also discussed.

2. Material and Methods

2.1. Test organisms and test conditions

A laboratory culture of F. candida (Collembola) of European origin was established, following methods adapted from ISO standard 11268-2 (ISO, 1999). The organisms were cultured in cylindrical plastic vials containing a mixture of activated charcoal (dust), water, and plaster of Paris in the proportion 1:7:1 (w:w:w). Granulated dry yeast (Saccharomyces cerevisiae) was supplied weekly as food. F. candida of ten to twelve days old, taken from synchronized cultures, were used in the ecotoxicological assays. The laboratory cultures and all bioassays were carried out in a climate-controlled room with a temperature of 23 ± 2 °C and a 12:12 h light/dark photoperiod [slightly modified ISO (1999)], trying to mirror tropical conditions.

2.2. Artificial soil and test contaminants

The ecotoxicological assays were carried out with Tropical Artificial Soil (TAS), an adaptation of OECD artificial soil (OECD, 1984) for tropical conditions, also used by De Silva and Van Gestel (2009) and Rombke et al. (2007). The TAS consisted of fine sand (> 50 percent of particles measuring between 0.05 and 0.2 mm), kaolinitic clay (powdered kaolinite) and powdered coconut husk in the proportion of 7:2:1 (w:w:w). The pH of TAS was corrected to 6.0 ± 0.5 with the addition of calcium carbonate (CaCO₃). The water-holding capacity (WHC) of TAS was determined following ISO (1999). Before the beginning of the tests, soil moisture was corrected to a mean value of 60 percent of the WHC, using water for the control and diluted pesticide solutions for the treatments. At the start (after the application of pesticide solutions/suspensions) and at the end of each bioassay, the soil pH was measured (1 M KCl, 1:5, w/v) following ISO (1999).

The formulations of seed insecticides imidacloprid, thiametoxam and fipronil, and of the fungicides captan and carboxin + thiram, were chosen based on their widespread use to treat seeds in Brazilian crops:

- Gauchos® (Bayser AG), a neonicotinoid insecticide with the active ingredient (a.i.) imidacloprid (IUPAC: (E)-1-(6-chloro-3-pyridinylmethyl)-N-nitromethylazolidin- 2-ylideneamine), is active against sucking insects because of its unique plant systemic and transaminlar properties (Mars and Ballantyne, 2004). It causes irreversible blockage of the acetylcholine receptors of insect’s nervous system, which leads to an accumulation of this neurotransmitter, resulting in paralysis and sometimes death (Kidd and James, 1991).

- Cruiser® (Syngenta), the second-generation neonicotinoid insecticide, belonging to the sub-class of thianicotyril, with the a.i. thiametoxam (IUPAC: (E)-3-(2-chloro-3-thiazol-5-ylmethy)-5-methyl-1,3,5-oxadiazinan-4-ylide (nitroamine) (Maeniffich et al., 2001). It has a broad-spectrum insecticidal mode of action, focused on the nicotinic acetylcholine receptors of insects. The molecule mimics the chemical messengers acetylcholine and binds to its receptor site, causing irreversible harm to the nervous system that at high intensities can cause death in invertebrates (NRA, 2001).

- The insecticide Standak® (BASF) is a phenylpyrazole insecticide with the a.i. fipronil (IUPAC: 5-amino-1-(2,6-dichloro-4-oxothiazole)-3-trifluoro-4-trifluoro-methylphenylfuran-2-carbonitrile). It has a broad-spectrum activity and acts directly on the central nervous system of organisms, where it inhibits the gamma aminoobutyric acid (GABA) receptor, a neurotransmitter responsible for neuronal excitability and preventing excessive nerve stimulation. This inhibition causes death in sensitive individuals (Costinio et al., 2005).

- Captain® (Milenia AgroSciences) is a non-systemic thiocarbamothioamide fungicide which has protective and curative action, with the a.i. captan (IUPAC: N-(trichloromethylthio)cyclohex-4-ene-1,2-dicarbamoxide). Its mode of action is linked to an intracellular interaction with the sulphydryl, hydroxy, and amino enzyme groups, leading to an inhibition of some metabolic processes (Waxman, 1995; Leitner, 1996). Captain® is a mixture of the a.i. carboxin (IUPAC: 5,6-dihydro-2- methyl-1,4-oxathione-3-carbonilide) + thiram (IUPAC: tetramethylthiuram disulfide), which are in the oxathiin and dietheracarbamate fungicide classes, respectively. Carboxin is systemic and inhibits the dehydrogenation of succinic acid to fumaric acid, an important step in the tricarboxylic acid cycle (Stenersen, 2004). Thiram has contact action and inhibits the alcohol dehydrogenase enzymes, which can lead to toxicity by co-exposure to ethanol (Mars and Ballantyne, 2004).

The predicted environmental concentrations (PEC) of these tested pesticides (Table 1) were estimated based on the calculation of the volume of each pesticide residue that would occur in the natural environment, with the use of the respective rates, according to Alves et al. (2013). The commercial formulations used were diluted in deionized water and the pesticide solutions (test treatments with pesticide contamination based on active ingredients), or deionized water (control), were applied to the TAS, such that the solutions/suspensions were distributed evenly throughout the soil. Increasing concentrations of the a.i. (0; 1.0; 10; 500; 1000 mg a.i. kg⁻¹ soil dry weight [DW]) were used in the acute toxicity tests. For chronic toxicity assays, the increasing sub-lethal concentrations used (0; 0.06; 0.12; 0.25; 0.50; 1.0 mg a.i. kg⁻¹ soil DW) were based on the results obtained in the acute toxicity tests and on PEC values (Table 1).
2.3. Acute toxicity test

The lethal potential of the pesticides to *F. candida* was assessed via an acute ecotoxicity test (ISO, 1999). Cylindrical glass containers (15 x 115 mm, diameter/height) were filled with 30 g of artificial soil (fresh weight – FW) containing the pesticide concentrations (treatments) or deionized water (control). Six replicates per pesticide concentration were prepared (five of them containing springtails and one extra for pH and moisture determination at the end of the assay). At the start of the test, ten collembolans were introduced in each experimental unit that was immediately closed with a hermetic seal. At the start of the tests about 2 mg of granulated dry yeast was added as food in each container. After seven days the containers were opened to allow gas-exchange. After fourteen days of exposure, the content of each container was carefully transferred to a larger container, which was filled with water, such that the surviving individuals floated on the water surface. Drops of black ink were added to the water to increase the visual contrast between collembolans and the liquid. Collembolans were counted visually to calculate the average percentage survival in each treatment.

2.4. Chronic toxicity test

The effects of the pesticides on *F. candida* reproduction were assessed using the chronic toxicity tests, based on the methods described in the ISO guideline 11267 (ISO, 1999). Six replicates per pesticide concentration were prepared, as explained above. The procedures were all identical to those explained in acute toxicity test, except for the duration, that was of 28 days, after when the collembolans were visualized by flotation, as in the mortality test. In this case, however, the number of juveniles was determined by photographing the water surface for each replicate. The images were analyzed with UTHSCSA Image Tool 3.0 software to quantify the juveniles in each treatment.

2.5. Data analysis

Results of the acute toxicity tests were expressed as the average percentage of surviving organisms, while the average number of juveniles was counted in the chronic test. Significant differences between treatments were tested through analysis of variance (ANOVA, \( p < 0.05 \)) in both acute and chronic toxicity tests. Treatments were compared with the control through post-hoc Dunnett’s test, using the R software package, version 2.5.1. These differences were used to establish NOEC (No observed effect concentration) and LOEC (Lowest observed effect concentration) values for the toxicity tests.

In addition, PriProbit® 1.63 software was used to estimate the LC50 (lethal concentration of 50 percent) values in the acute toxicity tests. For the chronic toxicity tests, non-linear regressions using logistic, exponential, or hormetic models were performed to estimate the EC20 and the EC50 values (the concentration that reduces collembolan reproduction by 20 and 50 percent, respectively) using Statistica® 7.0 software.

3. Results

3.1. Test validation

*F. candida* mortality was < twenty percent in the controls of the acute and chronic toxicity tests, fulfilling the validity criteria required in ISO (1999). In chronic toxicity assays, the number of juveniles was ≥ 100 individuals in all replicates, and the coefficient of variation (CV) was < 30 percent for the control.

3.2. Acute toxicity responses

All the pesticides tested were lethal to *F. candida*, but the toxicity level obtained was dependent on the a.i. (Table 2). Only the insecticides imidacloprid and fipronil yielded mortality levels that were sufficient to estimate the 50 percent lethal concentration (LC50=20.96 and 59.62 mg a.i. kg\(^{-1}\) DW, respectively) for collembolans. Therefore, these pesticides were the most lethal ones for *F. candida*. The insecticide thiametoxam and the fungicides captan and carboxin+thiram were only lethal at the highest concentration tested (1000 mg a.i. kg\(^{-1}\) DW).

3.3. Chronic toxicity responses

Concentrations of captan, carboxin+thiram and thiametoxam in TAS had no significant effect on the reproductive performance of *F. candida*, while the insecticides imidacloprid and fipronil caused a significant reduction in the number of juveniles produced by the collembolans in concentrations from 0.06 to 0.12 mg a.i. kg\(^{-1}\) DW, respectively (the LOEC values; Fig. 1). In spite of the toxicity caused by these insecticides, in the highest tested concentration the reduction in reproduction was always < 50 percent of that observed in the

<table>
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<th>Table 1</th>
<th>Commercial names, manufacturers and active ingredients (a.i.) of the pesticides studied, and their predicted environmental concentrations (PEC) at the commercially recommended doses for soybean crops (Adapted from Alves et al., 2013).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial name</td>
<td>manufacturer</td>
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<tr>
<td>Gaucho® 600 FS</td>
<td>Bayer AG</td>
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<tr>
<td>Standak® 250 SC</td>
<td>BASF</td>
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<tr>
<td>Cruiser® 350 FS</td>
<td>Syngenta</td>
</tr>
<tr>
<td>Captan® 480 SC</td>
<td>Milenia Agrosciences</td>
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<td>Vitavax® 200 SC</td>
<td>Chemtura</td>
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<th>Table 2</th>
<th>Toxic values estimated based on pesticide effects on <em>F. candida</em> in acute (LC50) and chronic toxicity tests (LOEC, NOEC and EC20).</th>
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</thead>
<tbody>
<tr>
<td>Test</td>
<td>Parameter</td>
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<tr>
<td>Acute</td>
<td>NOEC</td>
</tr>
<tr>
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</tr>
<tr>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Upper limits (95 percent)</td>
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</tr>
<tr>
<td>LC50</td>
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</tr>
<tr>
<td>Lower limits (95 percent)</td>
<td>9.51</td>
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<tr>
<td>Chronic</td>
<td>NOEC</td>
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<tr>
<td></td>
<td>0.06</td>
</tr>
<tr>
<td>Upper limits (95 percent)</td>
<td>0.021</td>
</tr>
<tr>
<td>EC20</td>
<td>0.030</td>
</tr>
<tr>
<td>Lower limits (95 percent)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

n.d. – data do not allow estimation of a 95 percent confidence interval.
control, which did not allow estimation of the EC50 values and therefore we estimate the EC20 values (Table 2).

4. Discussion

4.1. Fipronil

While fipronil was the most lethal pesticide tested for *F. candida* (Table 2), the NOEC value (1 mg kg\(^{-1}\) soil DW) of this insecticide in the acute toxicity test is ten times higher than its PECsoil (0.096 mg a.i. kg\(^{-1}\) soil DW; Table 1). Similar results have been reported by San Miguel et al. (2008), who obtained a NOEC of 0.5 mg kg\(^{-1}\) soil DW for *F. candida*, almost five times the PEC we estimated (Table 1). However, there may be other factors that influence fipronil’s lethality to *F. candida*, such as the stage of development of the collembolans. San Miguel et al. (2008) argued that toxicity may be stronger in juveniles than in adults. While our results suggest a low lethal potential of fipronil for adult collembolans, we did not set up an acute toxicity test with juveniles in order to quantify these effects on the various developmental stages present in the soil. Fipronil is also known to be lethal to earthworms with LC50 of 25.53 mg a.i. kg\(^{-1}\) DW, that is much lower than that estimated in our study (20.96 mg a.i. kg\(^{-1}\) DW). According to Diogo et al. (2007) the sensitivity of *F. candida* towards contaminants (e.g. pesticides) is dependent on the strain of the individuals. In any case, the PEC of fipronil (Table 1) was below the estimated LC50 value. It has been reported that fipronil kills other non-target soil invertebrates, including earthworms with LC50 of 25.53 mg a.i. kg\(^{-1}\) DW.
(Alves et al., 2013) and predatory coleopterans with LC50 of 11.54 ppm of the a.i. (He et al., 2012). The different LC50 values found for imidacloprid for different components of the soil fauna have been attributed in part to different test methods and/or conditions, but are primarily associated with the selective toxicity of the neonicotinoid for different species/strains (Tomizawa and Casida, 2005).

Imidacloprid was the most toxic pesticide in chronic toxicity tests (Table 2). The EC20 obtained for this insecticide (0.01 mg kg\(^{-1}\) DW) was more than twenty times lower than the soil concentration expected after using treated seeds (PEC=0.23 mg a.i. kg\(^{-1}\) DW). This suggests that springtail populations in fields where seeds treated with this compound may be at risk, as well as the biological processes in which they play a role (Rusek, 1998). These considerations would corroborate the results of Peck (2009), who reports significant suppression on the abundance of Entomobryomorpha (same order as F. candida) over three years, when using 0.37 kg of imidacloprid ha\(^{-1}\) (dose in the recommended range of application of Merit 0.5G) in field plots. Kreutzweiser et al. (2009) also reported that sub-lethal doses of imidacloprid can cause adverse effects on non-target decomposer invertebrates, what is prone to affect the decomposition process of leaf litter.

4.3. Thiametoxam

Thiametoxam caused mortality to F. candida only at 1000 mg a.i. kg\(^{-1}\) DW. The low toxicity found in the acute test agrees with the outcome of the chronic reproduction test, in which no statistical differences were found between collembolan’s reproduction in treatments and control (Table 1).

This concentration also greatly exceeds the PEC of this pesticide (0.201 mg a.i. kg\(^{-1}\) DW; Table 1). In spite of the low toxicity of thiametoxam found for F. candida in acute tests, this insecticide is known to be lethal for isopterans at 0.41–0.64 ppm of a.i. (Acda, 2007) and for larvae of the predaceous coccinellid at 0.250 mg a.i. kg\(^{-1}\) DW (Moser and Obrycki, 2009).

4.4. Fungicides

Only the highest concentration of captan killed the springtails in the acute toxicity test. When applying captan (0.3 percent) on apple orchards, Rather and Shah (2010) also reported a short lived effect on collembolan populations in the field. Furthermore, other studies reported that captan is not lethal to earthworms (Anton et al., 1990), even at 1000 mg a.i. kg\(^{-1}\) DW in TAS (Alves et al., 2013). We also did not find toxicity of captan on reproduction of F. candida at the highest concentration tested (NOEC=1.0 mg a.i. kg\(^{-1}\) soil DW). Although Frampton et al. (2006) and Daam et al. (2011) observed higher sensitivity of collembolans towards fungicides than earthworms, concentrations higher than those of our study reduced the reproduction of earthworms at 200 mg kg\(^{-1}\) soil DW (Alves et al., 2013). These concentrations were above the PEC, suggesting that the use of seed dressing with captan offers a reduction earthworm reproduction (LOEC=25 mg kg\(^{-1}\) soil DW).

Both fungicides showed low potential risk for F. candida, since there were no effects on reproduction and the impacts on survival only occurred at concentrations higher than their PEC (Table 1). There are other reports of non-toxic effects of the fungicides epoxiconazole + piraclostrobin (1 and 2 L ha\(^{-1}\)) and epoxiconazole (0.75 and 1.5 L ha\(^{-1}\)) on F. candida (Antonioli et al., 2013). However, Idinger (2002) testing the effects of the fungicide Euparen M WG50 (with the a.i. tolylfluazifuran) on this species, found the NOEC only at concentrations 80 times below the highest recommended field rate. This suggests that even though the species F. candida is not highly sensitive to some fungicides, its susceptibility is dependent on the mode of action of the active ingredient.

4.5. Using ecotoxicological tests for risk prediction

Mortality is one of the factors responsible for a reduction in the number of soil organisms. Declines in collembolan abundance have been observed by several authors (Frampton, 2002; Fountain et al., 2007; Peck, 2009), some of whom have argued that they are associated with a limitation in the decomposition of organic matter (Cortet et al., 2002). However, population reductions are not always associated with lethal effects. San Miguel et al. (2008) reported that under natural conditions some insecticides may pose a low lethal risk to collembolans, but that lower abundance may result from organisms’ ability to avoid areas that are seriously contaminated with these substances. Declines in collembolan abundance may also be linked to impacts on reproduction, including the malformation of gamesters and embryos, inability to hatch and teratogenic effects. In our study, while all the tested pesticides caused mortality, the effects were significant at concentrations at least 100 times higher (Table 2) than the PEC values (Table 1). This reduces the possibility that population declines of F. candida in the field are due to mortality caused by treating seeds with these products. Similar results were found by Coja et al. (2006), who assessed the lethal effect of pesticides on F. candida and observed that the concentrations that caused such effects were higher than those normally found in soils and, therefore, were considered not to offer direct risk to these organisms.

The results of our chronic toxicity assays corroborate those of Frampton et al. (2006) and Daam et al. (2011), who report that collembolans were less sensitive to fungicides than to insecticides. However, there are also reports of lower reproduction in collembolans in the presence of fungicides (EFSA, 2010; Idinger, 2002; Jänsch et al., 2006). Reproduction in F. candida (EFSA, 2010) was also less sensitive to carboxin + thiram than reproduction of E. andrei earthworms (Alves et al., 2013). This difference in sensitivity is likely an exception and should be attributed to the different susceptibility of the organisms to the modes of action of the fungicides. Thus, Frampton et al. (2006) and Daam et al. (2011) reviewed several classes of pesticides and highlights that collembolans were more sensitive than earthworms in acute and chronic tests of a wide variety of pesticides (including biocides, fungicides, herbicides, and insecticides), as we observed in this study.

Although chronic toxicity tests were the most sensitive, detecting effects at concentrations close to those expected in the field (Table 1), the acute toxicity assays represent the endpoint of the worst expected situation and can be useful in the initial screening.
of concentrations for the other laboratory tests (e.g., avoidance and reproduction assays). Therefore the use of different/complementary analytical criteria (i.e., more than one type of ecotoxicological test) in the first stage of a pesticide risk assessment for soils increases its sensitivity, since the exposure of organisms to different levels of stress reduces uncertainty regarding the risks these substances pose to soil organisms.

5. Conclusions

All the studied pesticides caused mortality in F. candida, but at concentrations exceeding those predicted in the field (PEC). Fipronil and imidacloprid were the most lethal. In the chronic toxicity assays, imidacloprid was the most toxic, followed by fipronil. These pesticides reduced collembolan reproduction at concentrations below and close to their respective PECs. The other pesticides did not interfere with F. candida reproduction. The intensity of these effects increased with increasing pesticide concentrations in the TAS. While these results increase our understanding of the toxicity of seed dressing pesticides they must be carefully compared with results obtained for other soil organisms, as well as studies under more realistic conditions (e.g. natural soils), in order to effectively assess the risks posed by these products to the soil fauna. In any case, these results are useful for the revision of the risk assessment of these compounds on the light of the new data requirements for pesticide risk assessment.

Acknowledgments

P.R.L.A. thanks the Graduate Course in Agronomy of UEL (State University of Londrina) and the Soil Microbiology Laboratory of the Soil Science Department at ESAQ/USP (University of São Paulo) for the research opportunity, and wants to express his gratefulness to the Agrisus Foundation for Sustainable Agriculture for a research opportunity, and wants to express his gratefulness for the technical assistance in the manuscript.

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