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THUANNE BRAÚLIO HENNIG

INFLUÊNCIAS DO TIPO DE SOLO, REGIME DE UMIDADE E TEMPERATURA NO POTENCIAL TÓXICO DE FIPRONIL SOBRE A FAUNA EDÁFICA

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Dissertação apresentada ao Curso de Pós-graduação em Ciência do Solo, do Centro de Ciências Agroveterinárias da Universidade do Estado de Santa Catarina, como requisito parcial para obtenção do grau de Mestra em Ciência do Solo.

Orientador: Dr. Dilmar Baretta

Co-orientador: Dr. Paulo Roger Lopes Alves

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BANCA EXAMINADORA

Orientador Prof. Dr. Dilmar Baretta

Universidade do Estado de Santa Catarina

Membros:

Prof. Dr. Osmar Klauberg Filho

Universidade do Estado de Santa Catarina

Dra. Maria Edna Tenório Nunes

Lages, 18 de fevereiro de 2021.

Dedico aos meus pais, Oberti e Almerinda, e à minha irmã, Tainá, com todo o meu amor.

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“Os rios sabem disto: não é preciso ter
pressa. Haveremos de chegar lá um dia.”

Alan Milne

RESUMO

HENNIG, Thuanne Braúlio**. Influências do tipo de solo, regime de umidade e temperatura no potencial tóxico de fipronil sobre a fauna edáfica**. 2021. 110 p. Dissertação (Mestrado em Ciência do Solo) – Universidade do Estado de Santa Catarina. Programa de Pós-Graduação em Ciência do solo, Lages, 2021.

O fipronil é um inseticida amplamente utilizado na agricultura, principalmente no tratamento químico de sementes. Apesar deste ingrediente ativo (i.a.) ser considerado eficiente no controle de pragas agrícolas, atuando diretamente sobre o sistema nervoso de invertebrados, pode representar risco também para os organismos não-alvo do solo, os quais são responsáveis por diversos serviços ecossistêmicos. Embora os efeitos ecotoxicológicos do fipronil para alguns invertebrados do solo sejam conhecidos, são escassos os estudos que relacionam a influência do tipo de solo e de fatores climáticos no seu potencial tóxico. Neste sentido, e considerando as previsões de mudanças no clima global, o objetivo deste projeto foi avaliar como o potencial tóxico do fipronil para invertebrados não-alvo do solo é afetado por: a) tipos de solos subtropicais brasileiros; b) redução da umidade do solo; e c) aumento da temperatura atmosférica. Para responder a essas questões, foram realizados três experimentos independentes. No primeiro experimento, colêmbolos (*Folsomia candida*) foram expostos a três tipos de solo (Solo Artificial Tropical – SAT, Latossolo e Neossolo) contaminados com concentrações crescentes do i.a., sob dois regimes de umidade do solo (60% e 30% ou 45% da capacidade de retenção de água do solo - CRA), sob a temperatura de 25 ºC. Neste experimento, a toxicidade de fipronil para colêmbolos foi maior em Neossolo comparado à exposição em SAT e Latossolo. Além disso, foi observado que a redução da umidade do solo causou aumento na toxicidade de fipronil para collembolos em SAT e em Latossolo, enquanto que em Neossolo o solo em condição de seca revelou uma menor toxicidade do i.a., em comparação com o solo úmido. No segundo experimento, os efeitos do fipronil sobre *F. candida* foram investigados sob três temperaturas crescentes (20, 25 e 27 ºC), na condição de umidade de 60 % da CRA. Para SAT e Latossolo o aumento da temperatura promoveu um aumento na toxicidade do i.a., enquanto que para Neossolo houve a alteração na toxicidade. No terceiro experimento, foi investigada a toxicidade de fipronil para minhocas *Eisenia andrei* em Neossolo, sob as condições de umidade de 30 e 60% CRA, e em Latossolo à 60% da CRA, sob três temperaturas crescentes (20, 25 e 27 °C). Foi observado um aumento na toxicidade do i.a. com o aumento da temperatura em Latossolo e em Neossolo a 30% CRA, enquanto que em Neossolo 60% CRA não houve clara influência da temperatura. A combinação entre a redução da umidade em Neossolo e a temperatura de 27 ºC também causou aumento na toxicidade do i.a. para as minhocas. Os resultados deste estudo indicam que a toxicidade de fipronil, no geral, aumenta para os invertebrados do solo em solos com fracas propriedades adsorptivas, como solos arenosos. Além disso, reduções na umidade do solo e aumentos na temperatura, decorrentes das mudanças climáticas, podem aumentar o efeito tóxico e o risco de fipronil para a fauna edáfica, dependendo do tipo de solo.

Palavras-chave: Mudanças climáticas. Solos tropicais. Invertebrados do solo. Avaliação ecotoxicológica. Avaliação de risco ecológico.

ABSTRACT

HENNIG, Thuanne Braúlio. **Influences of soil type, soil moisture regime and temperature on the toxic potential of fipronil on edaphic fauna.** 2021. 110 p. Dissertation (Master in Soil Science) - State University of Santa Catarina. Graduate Program in Soil Science, Lages, 2021.

Fipronil is an insecticide widely used in agriculture, mainly through the chemical treatment of seeds. Although this active ingredient (a.i.) is considered efficient in the control of agricultural pests, acting directly on the central nervous system of invertebrates, representing a risk also for non-target organisms in the soil, which are responsible for several ecosystem services. Although the ecotoxicological effects of fipronil on some soil invertebrates are known in the scientific literature, few studies relate the influence of soil type and climatic factors on its toxic potential. In this sense, and considering that there are numerous predictions of changes in the global climate, the objective of this project was to evaluate how the toxic potential of fipronil for non-target soil invertebrates is affected by a) Brazilian tropical soil types; b) the reduction of soil moisture and; c) the increase in atmospheric temperature. To answer these research questions, three independent experiments were carried out.In the first experiment, collembolans (*Folsomia candida*) were exposed to three types of soil (Tropical Artificial Soil - TAS, Entisol and Oxisol) contaminated with increasing concentrations of a.i., under two soil moisture regimes (60% and 30% or 45% soil water holding capacity - WHC), at a temperature of 25ºC. In this experiment, fipronil toxicity for collembolans was higher in Entisol compared to exposure in TAS and Oxisol. In addition, was observed that the reduction in soil moisture caused an increase in the toxicity of fipronil to collembolans in TAS and Oxisol, whereas in Entisol the dry soil showed lower toxicity of a.i., compared to wet soil. In the second experiment, the effects of fipronil on *F. candida* were investigated under three increasing temperatures (20, 25 and 27 ºC), in the condition of soil moisture of 60% WHC. For TAS and Oxisol, the increase in temperature promoted an increase in a.i. toxicity for collembolans, while for Entisol there was a change in toxicity. In the third experiment, the toxicity of fipronil to earthworms *Eisenia andrei* was investigated in Entisol, under soil moisture regimes of 30 and 60% WHC and in Oxisol at 60% WHC, under three increasing temperatures (20, 25 and 27 °C). An increase in the toxicity of i.a. to earthworms was observed with increasing temperature in Oxisol, and in Entisol at 30% WHC, whereas in Neossolo 60% CRA there was no clear influence of temperature. The combination between the reduction of moisture in Entisol and the temperature of 27 ºC also caused an increase in the toxicity of the a.i. for earthworms. The results of this project indicate that the toxicity of fipronil increases for soil invertebrates in soils with poor adsorptive properties, such as sandy soils. In addition, reductions in soil moisture and increases in atmospheric temperature, resulting from climate change, may increase the toxic effect and the risk of fipronil for soil fauna, depending on the type of soil.

Key-words: Climate change. Tropical soils. Soil invertebrates. Ecotoxicological assessment. Ecological risk assessment.

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SUMMARY

1 INTRODUCTION

Brazil is one of the largest food producers worldwide, with direct impacts on the food distribution and security of several countries (BRAGA et al., 2020). To guarantee and increase agricultural productivity, Brazil adopted the use of agrochemicals and other techniques based on the Green Revolution (EMBRAPA, 2014). In this sense, one of the techniques most used is the chemical treatment of seeds with pesticides, which is used for pest control at the beginning of crops, favoring good seedling development and providing greater productivity (DOUGLAS and TOOKER, 2015).

In Brazil, the sale of active ingredients (a.i.) used as pesticides grew almost 40 times between the years 2000 and 2019, being sold in this last about 620 thousand tons (IBAMA, 2019). Among these, fipronil is widely used in the chemical treatment of seeds from several cultures, with about 2 thousand tons sold only in 2019 (IBAMA, 2019). This molecule is a broad spectrum phenylpyrazole pesticide, considered effective against a wide range of pests (MOHAPATRA et al., 2010), acting directly on the central nervous system of organisms, canceling the neuroregulatory effect of the neurotransmitters, causing neuronal hyperactivity and consequent death of organisms (TINGLE et al., 2003; MOHAPATRA et al., 2010). However, despite the benefits of this a.i. for crops, it can cause deleterious effects in non-target organisms, especially in soil invertebrates, that play important ecosystem services and are often in direct contact with the pesticide in the soil (ALVES et al., 2013, ALVES et al., 2014; SIMON-DELSON et al., 2015).

Terrestrial ecotoxicology is a science area that allows evaluating the effects of chemical substances on non-target organisms in the soil. In ecotoxicological assays, representative organisms of soil fauna are exposed to contaminants to assess its toxic effects and also to estimate safety values of exposure (ALVES and CARDOSO, 2016). Negative impacts of fipronil on the soil invertebrates' survival, reproduction and on the bioaccumulation of species (even in multi-generations) are reported in the literature (PEVELING et al., 2003; SAN MIGUEL et al., 2008; PISA et al., 2014; ALVES et al., 2014; QIN et al., 2014; ZORTÉA et al., 2018a; ZORTÉA et al., 2018b), indicating that this a.i. can cause unbalances in soil invertebrate populations. However, the majority of these studies have been carried out only on standard soil, disregarding intrinsic characteristics of natural soils, such as texture, pH, cation exchange capacity (CEC), clay and OM content, which are important soil attributes that drive the water availability, the pesticide bioavailability and toxicity to soil invertebrates (AMORIM et al., 2005).

Some studies reported that soil type and its properties can influence the ecotoxicology of chemical substances to soil invertebrates. Domene et al. (2012) evaluated that soil properties influence directly the toxicity of phenmedipham to *F. candida*, indicating that each soil type play a different role in the toxicity of substances. Amorim et al. (2005) also investigated the phenmedipan toxicity for collembolans *F. candida* and *Hypogastrura assimilis* and found that standard artificial soil OECD can not represent natural soils, once its high OM content (10%) can decreased the bioavailability and consequently, the toxicity of the substance. Zortéa et al. (2018a and 2018b) evaluated the influence of soil type on the toxicity of fipronil from veterinary medicine to *F. candida*, and found that Sandy soil (2018a) was more sensitive than Sandy Loam and Clay Loam soils (2018b). Similarly, Bandeira et al. (2019) evaluated the influence of soil type on toxicity of a neonicotinoid to collembolans *F. candida* and earthworms *E. andrei* and verified that Sandy soils demonstrate higher toxicity of pesticide when compared with Sandy Loam and Clay Loam soils. Although the influence of soil type on the ecotoxicological effect of some pesticides on soil invertebrates is known, studies in this perspective with fipronil in formulation for seed treatment have not yet been found.

In addition to the influence of soil properties, climatic factors also play an important influence on the behavior and dynamics of pesticides in soils, as well as affect the physiology, composition and diversity of soil invertebrates (NOYES et al., 2009). According to Intergovernmental Panel on Climate Change (IPCC), if emissions are not controlled, it is expected, in addition to the increase in the temperature of the oceans, melting of polar ice caps and consequent rise in sea level, an increase greater than on the global temperature, as well as changes in rainfall patterns over the years (IPCC, 2014; 2019). The reduction of soil moisture content (due to reduction in precipitation patterns) and the increase of atmospheric temperature, can cause changes in homeostasis (JEGEDE et al., 2017), life cycle, growth and reproduction (FAYOLLE et al., 1997; WILES and KROGH, 1998; JÄNSCH et al., 2005; BONMATIN et al., 2017) on soil invertebrates, however, these effects must be enhanced if combined with the presence of chemicals in the environment (DAAM et al., 2019).

Changes in soil moisture and the increase of temperature may influence the dynamics, fate of pesticides and, consequently, their toxicity to soil organisms. Where is expected the increase in the frequency of rainfall, is also expected increase in soil moisture contents, that can cause effects of dilution of pesticides (LIMA et al., 2011), while where is expected the reduction of precipitation regimes, is also expected the reduction on soil moisture content, that may increase the concentration of contaminants in the soil (BANDOW et al., 2014b). Hennig et al. (2020) found that reductions in soil moisture regime can enhance imidacloprid toxicity to collembolans in a soil with high clay content, while in sandy soil the soil moisture content did not influence imidacloprid toxicity. Some authors have found that toxicity of lambda-cyhalothrin toxicity (BANDOW et al., 2014a) increased with increasing soil moisture, while the toxicity of pyrimethanil (BANDOW et al., 2014b) decreased with the increasing of soil moisture regime, for collembolans *F. candida* and *Sinella curviseta*. Lima et al. (2011), assessing combined effects of soil moisture and carbaryl to *E. andrei,* found a synergistic interaction between carbaryl and drought conditions for earthworms, indicating the enhance of substance toxicity in this condition.

High temperatures also may play an additive effect on substances effect as well can cause an increase in degradation and volatilization rates (SHUNTHIRASINGHAM et al., 2010). These changes can result in shifts in rates of absorption, metabolism, bioaccumulation and the dynamics of excretion of pollutants and toxicity to the exposed species (SEELAND et al., 2012; DELCOUR et al., 2015; DAI et al., 2019). In this context, Dai et al. (2019) assessed synergistic effects of phenanthrene associated with heatwaves, that affected negatively the body mass of *F. candida* adults exposed, well as their reproduction, revealing that heat stress can potentiate the toxic effect of the chemical. Bandeira et al. (2020) found that imidacloprid toxicity increase with increasing temperature, what compromised the survival and reproduction of *F. candida* and *E. andrei*. Daam et al. (2020) evaluated the chronic and acute toxicity of carbendazim to *E. fetida* in tropical and temperate conditions and verified that tropical conditions, with a temperature of 28 ºC, reduced the toxicity of fungicide for earthworms in comparison with observed at 20 ºC, in temperate condition.

Although the studies report the influence of the type of soil, as well as the soil moisture and temperature regime on the bioavailability and toxicity of substances for soil organisms., Most of ecotoxicological studies carried out with fipronil have so far disregarded these factors that presume about realistic and predictive scenarios (DAAM et al., 2019). The main question about the contamination of natural soils with pesticides in the light of climate change is the uncertainty about how the factors will interact and affect the edaphic fauna (AMORIM et al., 2005; HOLMSTRUP et al., 2010). In this sense, know about the effects of different soil types and climate change on the toxicity of fipronil to earthworms and collembolans, is important for the creation of a database about the toxicity of pesticides for groups of edaphic fauna, for the projection of the long-term effects of a.i. on natural soils, and finally, for the estimation of safety values of a.i. concentration for the exposure of edaphic organisms in different climatic contexts.

1.1 HYPOTHESES

1. The toxic effects and risk of fipronil via seed dressing commercial formulation (Shelter®) vary in contrasting soil types, being higher in natural sandy soil;

2. The reduction of soil moisture content increases the fipronil toxicity to collembolans, depending on the tropical soil type;

3. The increase in atmospheric temperature increases the toxicity of fipronil to collembolans, but that this effect may vary with the type of soil used;

4. The increase in temperature and decrease in soil moisture content increases the toxicity of fipronil to *Eisenia andrei* in subtropical soils.

1.2 GENERAL OBJECTIVE

To assess how the type of soil and the effects of climate change influence the ecotoxicological potential of fipronil for different species of edaphic fauna.

1.3 SPECIFIC OBJECTIVES

1. To assess the influence of soil type and soil moisture content on the chronic toxicity and risk of fipronil to collembolans *Folsomia candida*;

2. To assess the influence of increasing atmospheric temperatures on the chronic toxicity and risk of fipronil to collembolans *Folsomia candida*;

3. To assess the influence of soil moisture content and of increasing atmospheric temperatures on the chronic toxicity and risk of fipronil to earthworms *Eisenia andrei*.

2 LITERATURE REVIEW

2.1 EDAPHIC FAUNA

The edaphic fauna corresponds to all organisms that use the soil as a habitat in at least one stage of life (SWIFT et al., 1979). Historically, the first published studies about edaphic fauna considered these organisms like pests. Only at the end of the 20th century the role of edaphic fauna in the trophic web, functions in the soil, as wellas bioindicators began to be recognized (LAVELLE, 1996). The organisms of the edaphic fauna have functions considered to be invaluable essential, related to the maintenance of the soil structure, nutrient cycling, in biological and biogeochemical cycles, functioning of microbial ecology, contributing, therefore, in a series of ecosystem services (LAVELLE, 1996; CULIK and ZEPPELINI, 2003).

About the functionality of edaphic fauna, the many functions play for them are activities like the formation of aggregates in the soil, control of drainage and erosion, control and move of SOM along with the soil profile, nutrient cycling through pasture and SOM decomposition (CORTET et al., 1999; LAVELLE et al., 2006).

Some groups of edaphic fauna, like earthworms, are considered ecosystem engineers, due to their fundamental role on dynamic between different ecological niches in the soil, by creating of habitats for other organisms. Besides that, earthworms' activity improves the soil structure, acting on the formation of aggregates, channels and galleries, which allow the circulation of water, air, SOM and other soil invertebrates (LAVELLE et al., 2006). The edaphic fauna organisms known as decomposers, which include arthropods such as collembolans, help in the fragmentation of SOM, facilitating the microbiota activity on the decomposition of this material (CULIK and ZEPPELINI, 2003). There are also micro predator organisms, which obtain energy through the consumption of microorganisms, contributing to the regulation of microbial biomass (AQUINO et al., 2005). In this way, due to their diverse contributions to the terrestrial ecosystem, these organisms can be considered fundamental to soil quality.

2.2 BIOINDICATORS OF SOIL QUALITY AND TERRESTRIAL ECOTOXICOLOGY

The terrestrial ecosystems are regulated by a series of physical, chemical and biological processes, and the way how this compartment is altered must reflect directly on environmental services (EMBRAPA, 2004).

The use of bioindicators of soil quality allows to measure and evaluate impacts on the soil ecosystem. Therefore, these indicators must have capacity and sensitivity to respond to the environmental conditions to which they are exposed (DUMANSKI and PIERI, 2000).

Some edaphic organismsare good bioindicators of soil quality because they present high ecological relevance, are abundant, are common in the most diverse ecosystems and of simple creation in the laboratory (ALVES and CARDOSO, 2016).

Collembolans and earthworms are the edaphic organisms most used as bioindicators of soil quality. Ease of maintenance, short life cycle and high sensitivity to pollutants, qualify them as being some of the most suitable groups to assess soil quality (JÄNSCH et al., 2005; CORTET et al., 1999; PAOLETTI, 1999). These organisms are widely recommended, including by international standards for ecotoxicological studies, by which toxicity parameters are obtained that allow to reduce uncertainty about risks and also to determine safe exposure limits for soil organisms (ALVES and CARDOSO, 2016).

Ecotoxicology is a “young” science that helps to determine the effects of chemical substances introduced into the environment from anthropic activities on the organisms from ecosystems (MARKERT et al., 2003), through ecotoxicological endpoints, like lethality, reproduction and behavior (TEREKHOVA, 2011). The knowledge of these effects allows the know the extent of environmental risk, as well as to develop techniques for control, tracking and remediation of environmental contaminations (AZEVEDO and CHASIN, 2004).

2.2.1 Collembolans

Collembolans are characterized as one of the most abundant and diverse groups present in the most different biomes (COLEMAN et al., 2004). These organisms can influence the soil fertility and microbial ecology, also regulating the processes of nutrient cycling together with other edaphic groups (PEIJNENBURG et al., 2012). Due mainly to its environmental function, morphological characteristics, behavioral, alimentary habits and the abundance in the soil, some authors list collembolans as the most representative group in the evaluation of contaminated soils (SMIT and VAN GESTEL, 1996; FOUNTAIN and HOPKIN, 2005; AN et al., 2013). The main routes of exposure of collembolans to contaminants are through contact and absorption of the soil solution, consumption of contaminated food (predominantly organic matter), or by inhaling the contaminated air that may be present in the soil pores (PEIJNENBURG et al., 2012).

Due to a short generation time and reproduction via parthenogenesis, which makes it especially suitable for tests that require an individual or population analysis in a single assay, the collembolans species *F. candida* is considered the most suitable for ecotoxicological tests (JÄNSCH et al., 2005) and is recommended by the International Organization for Standardization - ISO (ISO, 2014).

2.2.2 Earthworms

Earthworms are considered invertebrates that inhabit the soil widely recognized for their rolein soil formation, fertility and nutrient cycling (EDWARDS, 2004). The ecological relevance of these organisms, high biomass as well as sensivity to environmental disturbances, make them good bioindicators of on the environmental risk of substances presents in soil (REINECKE and REINECKE, 2007) and, because of this, they have been suggested as good indicators of soil quality (BERRY et al., 1996).

E. andrei is an earthworm considered as one of the best indicators of soil quality for ecotoxicological tests, recommended by the International Organization for Standardization - ISO (ISO, 2012). They are abundant and diverse in different types of soil and climate, and also have a key role in the soil food web. These organisms can suffer the effects of contamination when they ingest the chemicals together with the soil and when they are in direct contact with the contaminant that is available in the soil solution (BERRY, et al., 1996).

2.3 PESTICIDES IN BRAZIL

In Brazil, the production of pesticides started in the 1940s, with the industrial consolidation of the sector only established around 1970. At the same time, it started a series of programs and movements that encouraged the use and trade of these substances, such as the National Rural Credit System (SNCR) and the National Program for Agricultural Defensives (LONDRES, 2011).

Based on the Green Revolution, the predominant agricultural production model in Brazil is based on the intensive use of inputs and techniques to increase productivity (EMBRAPA, 2014). According to the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA), the sale of pesticides in Brazil has grown almost 40 times in the last 20 years, registering the sale of 620 thousand tons of a.i. in 2019 (IBAMA, 2019). Herbicides, followed by fungicides and, finally, insecticides, were the most sold. Concerning the class of hazard to the environment, 60% of the most sold pesticides are of class III (dangerous), 32.03% of class II (very dangerous), and approximately 1% of class I (highly dangerous) (IBAMA, 2019).

The intensive use of pesticides has caused concern about the environmental fate, behavior in the environment and effects on non-target organisms (DIVELY et al., 2015). The pesticides dynamics in the environment depend on a series of factors that affect the persistence and mobility of substances in different environmental compartments. In the soil, the climate and the many pedogenetic factors and processes of soil formation may be considered, since it is through these aspects that the soil acquires its intrinsic characteristics and properties, which directly influence how pesticides are available in the soil (EMBRAPA, 2008).

2.4 FIPRONIL

Fipronil (5 - amino - 1 - [2,6 - dichloro - 4 - (trifluoromethyl) phenyl] - 4 (trifluoromethyl sulfinyl) -1H –pyrazole - 3 - carbonitrile) is a broad spectrum i.a. belonging to the class of phenylpyrazoles (TINGLE et al., 2003). Developed around 1986 by the RhônePoulenc company, the active ingredient started to be marketed only in 1993 and is recommended for agricultural, veterinary and household cleaning purposes (TINGLE et al., 2003; PESTICIDE ACTION NETWORK, 2006).

The mode of action of fipronil occurs in the central nervous system of organisms. The direct action is on the neurotransmitters gamma-aminobutyric (GABA) and glutamate associated with the chloride channels of insects. The molecule acts by inhibiting neurotransmitters by blocking chloride channels, canceling the neuroregulatory effect of GABA, causing neuronal hyperactivity and consequent muscle paralysis and death of organisms (TINGLE et al., 2003).

Due to its low water solubility and high sorption coefficient, fipronil is characterized as a hydrophobic molecule, with high sorption capacity in solid fractions of the soil and with greater affinity for organic matrices and, therefore, persistent in the environment (BARCELÓ, 1997; GUNASEKARA et al., 2007).

The fipronil molecule is also known to generate metabolites from its degradation, which can be even more toxic and persistent than the original molecule (TINGLE et al., 2003). Fipronil can degrade via oxidation, reduction, hydrolysis (alkaline medium) and/or photolysis, generating the metabolites fipronil sulfone, fipronil sulfide, fipronil amide and fipronil desulfinyl, respectively. The latter is the most stable in the soil and the one that represents the greatest toxicity to organisms (HAINZL and CASIDA, 1996; BOBÉ et al., 1998; GUNASEKARA et al., 2007).

2.4.1 Effects of fipronil for non-target soil organisms

The physical and chemical characteristics of fipronil indicate that the molecule has a great potential to reach non-target organisms in soil and water, and may compromise the respective ecosystems (GIBBONS et al., 2014).

Fipronil is potentially toxic to bees, Coleoptera, Oligochaeta (PISA et al., 2014), Isoptera (PEVELING et al., 2003), Blattodea (ZHAO et al., 2004), mammals (TAVARES et al., 2015) and fishes (XU et al., 2018) among other organisms (WALKER et al., 2016), compromising their survival, development and reproduction. Peveling et al. (2003) reported that fipronil reduced a termite (Isoptera) community by 90%, as a result of its bioaccumulation and persistence.

A study evaluating the toxicity of phenylpyrazoles indicated that fipronil has high toxicity to collembolans, reducing their reproduction and causing physiological impacts on the generated juveniles (smaller size, reduction on the thickness of integument, and deficiencies on detoxification processes - SAN MIGUEL et al., 2008). Also, there was greater bioaccumulation of fipronil in collembolans, compared to the other tested phenylpyrazoles (SAN MIGUEL et al., 2008).

Alves et al. (2014) comparing the toxicity of imidacloprid, fipronil, thiamethoxam, captan and carboxin + thiram to collembolans, observed that fipronil was one of the a.i. that caused the highest mortality and lowest reproduction for collembolans, due to its mode of action on the central nervous system and their higher sensitivity.

Zortéa et al. (2017; 2018a; 2018b) evaluated that the presence of fipronil-based veterinary drugs in the soil may negatively affect the soil's invertebrate fauna. The authors found that fipronil and its metabolites have high toxicity, affecting the survival and reproduction of collembolans

Qin et al. (2014) evaluated the effects of fipronil on earthworms *Eisenia fetida* and did not observe mortality when exposed to the pesticide, however, a negative effect on the reproduction of the organisms was observed by reduction in generation of juveniles. In addition, according to the authors, earthworms have a high capacity to bioaccumulate substances such as fipronil, due to their affinity to organic compounds, therefore, contaminated earthworms can unbalance the food web (QIN et al., 2014).

2.5 INFLUENCE OF THE SOIL TYPE ON PESTICIDE TOXICITY TO SOIL FAUNA

In addition to influencing the formation of ecological niches for edaphic fauna, soil properties also influence the bioavailability of pesticides and other contaminants to soil organisms (AMORIM et al., 2005; DOMENE et al., 2012). The intrinsic properties of the soil define the balance of sorption of contaminants between the liquid:solid phases and, consequently, control the concentration of contaminants in the soil solution (DOMENE et al., 2012).

The bioavailability of pesticides in the soil depends on intrinsic soil properties like texture, cation exchange capacity (CEC), clay SOM content (AMORIM et al., 2005; CHELINHO et al., 2014; BANDEIRA et al., 2019). However, for each contaminant, the properties may have different influences (DOMENE et al., 2012).

Fine-textured soils are considered excellent adsorbents since they are composed of a high amount of clay, which has a high specific area and high cation exchange capacity (CEC), allow higher retention of organic pollutants (BHATTACHARYYA and GUPTA, 2008). The content and quality of SOM also play an important role in the retention of pesticides in the soil, due to their functional groups (carboxylic, hydroxylic and carbonyl sites) that have a high capacity to retain organic pollutants, as is the case of most pesticides (HUANG et al., 2003).

Zortéa et al. (2018a; 2018b) evaluated the toxicity of fipronil in veterinary drugs to collembolans in a TAS (5% SOM), Entisol (predominantly sandy) and Oxisol (predominantly clay), and verified higher toxicity in Entisol (ZORTÉA et al., 2018a) compared to TAS and Oxisol (ZORTÉA et al., 2018b). The authors explained their results based on clay and SOM contents (highly in Oxisol and TAS, respectively), that have higher adsorptive capacities, making a.i. less available to collembolans uptake in these soils, in contrast to what occurs in sandy soils.

Bandeira et al. (2019) observed that the toxicity of imidacloprid to collembolans was also lower in TAS and Oxisol compared to Entisol, while for earthworms the toxicity in Oxisol was lower compared to only TAS. In the same way as Zortéa et al. (2018a; 2018b), Bandeira et al. (2019) attributed their results to the levels of SOM and clay present in TAS and Oxisol, that favored the adsorption and, consequently, reduced the bioavailability and toxicity of a.i. for soil organisms.

Owojori et al. (2010) exposed earthworms in an OECD artificial soil (10% SOM) contaminated with copper and evaluated the effect of increasing amounts of kaolinitic clay (5%, 20% and 40%) on the soil composition. The authors noted that the increase in clay content reduced the copper toxicity and bioaccumulation in earthworms, demonstrating that clay plays a fundamental role in the adsorption of pesticides.

Even though the influence of soil type on the toxicity of contaminants to soil organisms is evident, so far few ecotoxicological studies related to soil type have been carried out in Brazil (ZORTÉA et al., 2018a; ZORTÉA et al., 2018b; BANDEIRA et al., 2019; BANDEIRA et al., 2020), therefore, ecotoxicological studies in more realistic conditions are scarce.

2.6 CLIMATE CHANGE AND THE USE OF PESTICIDES

The emission of greenhouse gases, originating from natural causes and mainly due to anthropic activities, has caused substantial changes in the Earth's climate exposing all forms of life to many climatic stressors (NADAL et al., 2015).

Historically, the world has undergone constant climate change at different scales. However, in recent decades an acceleration in the events of climate change has been observed, characterized by natural disasters that are increasingly recurring around the world (NADAL et al., 2015).

According to Intergovernmental Panel on Climate Change (IPCC), the anthropogenic activities already caused an increase of 1 ± 0.2 ºC above pre-industrial levels. Between 2030 and 2052 is expected that warming global reach 2 ºC if the greenhouse gases keep if greenhouse gas emissions and deforestation continue to increase at the current rate (IPCC, 2019). By these climate change, are expected increases in average temperature are expected for most of the globe, increases in extreme heat events in urban locations and, depending on the region, increase in the events of storms and intense rainfall or drought severe (IPCC, 2019).

South American countries, such as Brazil, must suffer not only from rising temperatures but also from the reduction of precipitation regimes by the end of this century (NOYES et al., 2009; PEREIRA et al., 2018). Although the consequences of climate changes may vary for different regions, an increase in atmospheric temperature by about 4 ºC and reductions in rainfall regimes by about 20% are expected until the end of the century to Brazilian territory (PBMC, 2014).

These climate changes can directly or indirectly influence the toxicity of pesticides in the soil. Direct effects of climate changes associated with temperature and precipitation should influence the environmental fate, soil dynamics, degradation and toxicity of pesticides (NOYES et al., 2009; KATTWINKEL et al., 2011). Among the indirect effects, there may be a change in the planting period of some crops (KATTWINKEL et al., 2011) due tochanges in regional precipitation regimes (BLOOMFIELD et al., 2006), in addition to the increase in the loss of pesticides due to the effects of degradation and volatility, requiring higher rates of application of the pesticide have to be adopted (NOYES et al., 2009).

2.6.1 Influence of soil moisture on the toxicity of pesticides to soil fauna

Soil moisture is considered one of the fundamental ecological factors for the balance of the terrestrial ecosystem and the maintenance of the vital functions of soil organisms (BRADY and WEIL, 2013). In a possible future scenario, the restriction of soil moisture, resulted from the reduction of precipitation regimes, should cause a decrease in reproduction and other negative biological effects on meso and macrofauna organisms (LIMA et al., 2011).

Moisture can influence the availability of toxic substances in the soil in different ways. When soil moisture is high, may be expected a fast degradation of pesticides (ATHANASOPOULOS et al., 2004; QIN et a., 2014). The reduction of soil moisture, as predicted by climate change in tropical countries (IPCC, 2014; PBMC, 2014), can increase the concentration of pesticides in the soil, making it easily available, representing a greater risk for different groups of non-target invertebrates (LONG et al., 2009; BANDOW, et al., 2014a; BANDOW, et al., 2014b; BARMENTLO, et al., 2017).

Bandow et al. (2014a; 2014b) evaluated the influence of soil moisture (30%, 50% and 70% of water holding capacity - WHC) on the toxicity of lambda-cyhalothrin and pyrimethanil to collembolans *F. candida* and *S. curviseta*. The lambda-cyhalothrin (BANDOW et al., 2014a) toxicity increased with the increase of soil moisture regime, whereas pyrimethanil (BANDOW et al., 2014b) toxicity decreases with the increase of soil moisture regime, revealing that soil moisture regime can influence in different ways the toxicity of the substances.

Hennig et al. (2020), evaluating the soil moisture influence in imidacloprid toxicity in two contrasting soils (sandy soil and clay soil) to collembolans *F. candida* found that the a.i. toxicity in clayey soils at 45% WHC was about 9 times higher in comparison to 60% WHC, while in sandy soil the influence of the moisture content was not clear.

Hackenberger et al. (2018) investigated the influence of soil moisture (30 and 50% WHC) associated with contamination by propiconazole and chlorantraniliprole on enzymatic markers of earthworms *E. fetida*. For both pesticides, the reduction of moisture in the test soil reduced enzymatic activities, affecting the behavior, reproduction and growth of earthworms.

Lima et al. (2011) investigated the combined effects of different soil moisture (10, 20, 40, 60, 80, 100 and 120% WHC) with carbaryl contamination on *E. andrei* and found an increase in toxicity as the soil moisture decreased, suggesting the occurrence of a synergistic effect of contamination with the reduction of soil moisture.

Long et al. (2009), studying the physiological effects of changes in soil moisture on fluorethane toxicity to earthworms *Lumbricus rubellus* found that in soil moisture below 15% of the optimum soil moisture (about 60% WHC), the exposed organisms had reduced oviposition.

Although the effect of soil moisture on the toxicity of contaminants in the soil is variable according to the substance and test species, the influence of soil moisture content is evident in the studies above mentioned, which makes it an important factor to be considered in ecotoxicological and risk assessments of pesticides (LIMA et al., 2011).

2.6.2 Influence of temperature on pesticide toxicity to soil fauna

Temperature is one of the main environmental factors able to alter the metabolism of edaphic organisms (JEGEDE et al., 2017). Some studies have indicated that, individually, the temperature can cause metabolic stress and compromise the reproduction, biological growth of soil invertebrates (GULLAN and CRANSTON, 2005; KANEDA and KANEKO, 2008; ALVES and CARDOSO, 2016).

In soils contaminated by pesticides, the increase in temperature may reduce the concentration of the contaminant by loss, due to the fast degradation via volatilization and/or microbial activity, and some times may generate metabolites even more toxic than the a.i. itself (NOYES et al., 2009; ZORTÉA et al., 2018a). It is also possible that some a.i. becomes more available in the soil, since the increase in the temperature can reduce the capacity of the soil to adsorb pesticides (BROZNIĆ and MILIN, 2012).

On the other hand, the increase in temperature associated with soil contamination by pesticides can result in synergistic effects, potentiating the toxic effects of a.i. for soil organisms (DELCOUR et al., 2015), as well as changes in temperature patterns can alter the metabolism of exposed organisms, affecting eating behavior, pollutant uptake and excretion processes (DONKER et al., 1998). Besides that, according to Nørhave et al. (2014), the temperature added to the effects of contamination can change the physiological state of the organisms, directly influencing their reproduction and generation time.

Some studies have reported the influence of temperature on the toxicity of pesticides to collembolans. Bandow et al. (2014a; 2014b), evaluated the toxicity of lambda-cyhalothrin and pyrimethanil at temperatures of 20 and 26 ºC for the species *F. candida* and *S. curviseta* and found that both species were more affected by pyrimethanil at a temperature of 26 ºC, while lambda-cyhalothrin represented greater toxicity for *F. candida* at 26 ºC and *S. curviseta* at 20 ºC. According to the authors, the effect observed for lambda-cyhalothrin probably occurred because the ideal physiological temperature for *F. candida* is 20 ºC and, for *S. curviseta*, it is close to 26 ºC (BANDOW et al., 2014a; BANDOW et al., 2014b).

Bandeira et al. (2020) found that for three tested soils the imidacloprid toxicity was significantly higher at 25 and 28 ºC to collembolans and earthworms in comparison with toxicity at 20 ºC. The authors also found that risk-based on the Toxicity-Exposure Ratio (TER) approach increased with the increase of temperature, for both species in all tested soils.

Jegede et al. (2017) observed that soils contaminated with chlorpyrifos and dimethoate under tropical temperatures (26 ºC and 28 ºC) represent greater toxicity to collembolans when compared to the effects observed in temperate climate temperatures (20 ºC). The authors also evaluated the toxicity of deltamethrin, but could not verify the influence of temperature on the toxic effects of a.i. to collembolans. This effect was attributed to the rapid degradation and volatilization of the a.i. when subjected to higher temperatures (JEGEDE et al., 2017).

Lima et al. (2015) observed that the increase in temperature increased the toxicity of carbaryl to earthworms *E. andrei*, due to the synergistic effects of temperature with the pesticide.

Hackenberger et al. (2018) evaluated the enzymatic activity of *E. fetida* earthworms exposed to propiconazole and chlorantraniliprole, under temperatures of 20 and 25 ºC, and the authors found that all enzyme markers tested were negatively affected at a temperature of 25 ºC when compared to a temperature of 20 ºC.

In the field, the exposure of soil organisms to contaminants is conditioned to regional climatic characteristics (SANCHEZ-BAYO and HYNE, 2011) that influence bioavailability and potential toxicity of substances, according to the literature above mentioned. Hackenberger et al. (2018) reported the importance of considering changes in temperature in the risk assessments of pesticides, mainly due to the increase in temperature expected in the coming years, which should change the dynamics and toxicity of these substances for soil organisms.

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3 TOXICITY OF FIPRONIL TO *Folsomia candida* IN CONTRASTING TROPICAL SOILS AND SOIL MOISTURE CONTENTS: EFFECTS ON THE REPRODUCTION AND GROWTH

ABSTRACT

This study assessed the influence of two tropical soil types and an artificial tropical soil under contrating soil moisture contents on the toxicity and risk of the insecticide fipronil to collembolans *Folsomia candida*. Chronic toxicity tests were performed in a Tropical Artificial Soil (TAS), an Oxisol and an Entisol spiked with increasing concentrations of fipronil to assess the effects on the reproduction and growth of the species. The soil moisture contents were kept at 60% (standard condition) and 30% or 45% (water restrictions) of their water holding capacity (WHC). The toxicity of fipronil on collembolans reproduction was about three times higher in Entisol compared to TAS or Oxisol. Higher toxicities were also found at the drier scenarios with TAS ($EC_{50\ 30\%\ WHC}$ = 0.20 *vs* $EC_{50\ 60\%\ WHC}$ = 0.70 mg kg^{-1}) and Oxisol ($EC_{50\ 45\%\ WHC}$ = 0.27 *vs* $EC_{50\ 60\%\ WHC}$ = 0.54 mg kg^{-1}), while in Entisol lower impacts were seen in the drier samples ($EC_{50\ 30\%\ WHC}$ = 0.41 *vs* $EC_{50\ 60\%\ WHC}$ = 0.24 mg kg^{-1}). For all tested soils, the size of generated collembolans was reduced by the fipronil concentrations, regardless of soil moisture. However, the drier condition further increased the effect on the growth in TAS and Entisol for some concentrations. A significant risk of exposure was found in TAS and Oxisol at drier conditions and, for Entisol, regardless of the soil moisture. The toxic effects and risk of fipronil on collembolans varied with the tropical soil type, being more intense in the natural sandy soils.The soil moisture content increase or decrease the toxicity of the insecticide for *F. candida*, depending on the tropical soil type.

Keywords: Climate change; Collembolans; Pesticides; Seed dressing; Soil ecotoxicology.

3.1 INTRODUCTION

The Brazilian territory is composed of a wide variety of soil types with different characteristics (EMBRAPA, 2006). These particular soil characteristics, such as texture, pH, cation exchange capacity (CEC), clay and OM content helps to regulate the water availability, and the pesticide bioavailability/toxicity to collembolans (AMORIM et al., 2005; CHELINHO et al., 2014; OGUNGBEMI and VAN GESTEL, 2018; BANDEIRA et al., 2019). In this line, some studies comparing the toxicity of organic pollutants in different types of soils indicate that the toxic effects may be enhanced in sandy soils when compared with fine-textured soils (AMORIM et al., 2005; DOMENE et al., 2012; SEGAT et al., 2015; MACCARI et al., 2016; BANDEIRA et al., 2019).

In addition to the influence of soil types, climatic factors also play an important influence on the behavior and dynamics of pesticides in soils. According to the Intergovernmental Panel on Climate Change (IPCC), an increase in drought events is expected for tropical countries in the next years, as consequence of climatic changes (IPCC, 2014). Although the consequences may vary for Brazilian regions, a 20% reduction in rainfall regimes is expected by the year 2040, 35% between 2041-2070, and 50% between 2071-2100, for five of the six biomes that constitute the Brazilian territory (PBMC, 2014).

Drought events can promote changes in the environmental fate of pesticides, to influence the life-cycle of soil fauna, as well as affect their responses to the toxicants (DOMENE et al., 2012; BONMATIN et al., 2015; DAAM et al., 2019). In this way, some studies have reported that reductions in soil moisture increased the toxicity of some organic pollutants to soil invertebrates (LIMA et al., 2011; BANDOW et al., 2014b; BANDOW et al., 2016; HØJER et al., 2001; HENNIG et al., 2020).

Fipronil (5 - amino - 1 - [2,6 - dichloro - 4 - (trifluoromethyl) phenyl] - 4 - (trifluoromethylsulfinyl) -1H –pyrazole - 3 - carbonitrile) is a broad-spectrum insecticide that belongs to the class of phenylpyrazoles, widely used in chemical seed treatment (MOHAPATRA et al., 2010; BONMATIN et al., 2015). Fipronil acts on the nervous system of exposed organisms, causing neuronal hyperactivity, consequent muscle paralysis and death (TINGLE et al., 2003).

Negative effects of fipronil on the collembolan’s survival, reproduction and bioaccumulation are reported in the literature (SAN MIGUEL et al., 2008; ALVES et al., 2014; ZORTÉA et al., 2018a; ZORTÉA et al., 2018b). Collembolans are considered a representative group of soil fauna and are essential to the maintenance of soil quality (CULIK and ZEPPELINI, 2003). This organisms are suitable bioindicators due to their high sensitivity to contamination and environmental stress (CULIK and ZEPPELINI, 2003). The exposure of these organisms at drough scenarios may affect negatively their metabolism, may compromise the growth, survival and reproduction (BURSELL, 1970; EDNEY, 1977; VAN GESTEL and VAN DIEPEN, 1997; JÄNSCH et al., 2004). However, even that effects of drought on collembolans are known, no studies were found describing the relationship between fipronil used in the treatment of agricultural seeds and reducing soil moisture under these organisms.

In this study, we hypothesize that a) the toxic effects and risk of fipronil via seed dressing formulations vary in contrasting soil types, being higher in natural sandy soil; b) the reduction of soil moisture content increase the fipronil toxicity to collembolans, depending on the tropical soil type. In this way, this study aimed to assess the influence of soil type and soil moisture content on the toxicity and risk of fipronil to collembolans *Folsomia candida*. For this, the effects of fipronil on the reproduction and growth of *F. candida* were assessed via chronic toxicity tests in three tropical soils (Tropical Artificial Soil – TAS, Entisol and Oxisol) under a standard (60% WHC) and a water restriction (30% or 45% WHC) conditions.

3.2 MATERIAL AND METHODS

3.2.1 Test organism

The collembolans *F. candida* were cultured in the laboratory according to ISO 11267 (ISO, 2014). The organisms were kept in plastic containers containing a substrate composed of plaster of Paris, water, and activated charcoal (10:7:1, respectively). Twice a week, the culture medium received food (dry *Saccharomyces cerevisiae*) and had the moisture adjusted with a few drops of distilled water. The culture was kept in a room with controlled temperature (20 ± 2 ºC) and photoperiod (12h). The species sensitivity was confirmed by using a positive control assay with boric acid in TAS (EC_{50} = 171.55 mg kg^{-1}), according to Niemeyer et al. (2018).

3.2.2 Test soils

The ecotoxicological tests were performed in an artificial tropical soil (TAS) and two contrasting natural tropical soils.

The TAS (GARCIA, 2004) consisted of a mixture of fine sand, kaolin and powdered coconut husk (75:20:5, respectively). Calcium carbonate ($CaCO_3$) was used to adjust the pH to 6.0 ± 0.5 (ISO, 2014). A clayey soil (Oxisol) was sampled in Palmitos (SC; 27º 04'S 53º 09'W) and a sandy soil (Entisol) was sampled in Araranguá (SC; 29º 00′S 49° 31′W). The soils were taken from the top layer (0-20 cm) of the soil profile in areas with no history of contamination by pesticides. The soil samples were sieved (# 2 mm), defaunated through three freezing and thawing cycles and air-dried (ALVES et al., 2019).

For all test soils, the water holding capacity (WHC) and pH (1M-KCl) were determined according to ISO 11267 (2014), and the cation exchange capacity (CEC), soil organic matter (SOM), sand, clay, and silt contents (Table 3.1) were measured following Tedesco et al. (1995).

Table 3.1 - Mean values (± standard deviation; n = 2) of physical and chemical characteristics of Tropical Artificial Soil (TAS), Entisol and Oxisol, used in the ecotoxicological tests.

Parameter	TAS	Entisol	Oxisol
pH (1M-KCl)	5.9 ± 0.1	4.2 ± 0.2	4.8 ± 0.1
SOM (%)	1.4 ± 0.0	2.2 ± 0.1	3.2 ± 0.8
CEC ($cmol_c$ dm^{-3})	3.3 ± 0.2	1.4 ± 0.1	10.8 ± 0.4
WHC (%)	46.3 ± 1.7	31.6 ± 1.1	53.0 ± 1.4
Sand (%)	67.2 ± 0.0	93.8 ± 0.4	31.5 ± 0.1
Clay (%)	14.3 ± 0.0	4.1 ± 0.2	35.5 ± 1.4
Silt (%)	18.5 ± 0.0	2.1 ± 0.1	33.0 ± 1.6
Soil texture	Sandy Loam	Sandy	Clay Loam

SOM: Soil Organic Matter;
CEC: Cation Exchange Capacity;
WHC: Water Holding Capacity.

3.2.3 Test substance

Soils were spiked with a commercial formulation of an insecticide for seed dressing (Shelter®), which contains 250 g of fipronil L^{-1} as active ingredient (a.i.).

Soil samples were spiked with increasing concentrations (Table 3.2), which were based on literature (ALVES et al., 2014; ZORTÉA et al., 2018a) and on preliminary tests (data not shown). A control treatment without contaminant (only with distilled water) was prepared for each soil.

Table 3.2 - Nominal and real concentrations of the a.i. fipronil used in the chronic toxicity tests carried out in Tropical Artificial Soil (TAS), Entisol and Oxisol with *Folsomia candida*

	Fipronil concentrations (mg kg^{-1})			
Soil type	Treatment	Nominal	Actual	% Nominal
TAS	Control	0	0	-
	C1	0.25	0.21	84
	C2	0.5	0.44	88
	C3	1	0.9	90
	C4	2	1.62	81
	C5	4	2.89	72.25
Entisol	Control	0	0	-
	C1	0.125	0.17	136
	C2	0.25	0.25	100
	C3	0.5	0.57	114
	C4	1	1.03	103
	C5	2	1.71	85.5
Oxisol	Control	0	0	-
	C1	0.25	0.27	108
	C2	0.5	0.46	92
	C3	1	0.72	72
	C4	2	1.28	64
	C5	4	2.53	63.25

The spiking occurred through an aqueous solution, with volume sufficient to reach 30% and 60% of the WHC in TAS and Entisol, and 45% and 60% of the WHC in Oxisol, since the species did not reproduce in Oxisol at 30% WHC. The soil moisture was checked at the beginning and the end of each assay (Table 3.3).

Table 3.3 - Means (± standard deviation) of soil moisture (% WHC) and pH at the start and the end of the chronic toxicity assays with *Folsomia candida* in Tropical Artificial Soil (TAS), Entisol and Oxisol under different soil moisture treatments (30% or 45%, and 60% of the soil water holding capacity - WHC). Means were calculated by using the results of one replicate of each tested concentration plus the control (n=6).

Soil	30% WHC				45% WHC				60% WHC			
	Soil moisture (%)		pH		Soil moisture (%)		pH		Soil moisture (%)		pH	
	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End
TAS	27.0 ± 0.31	31. 9 ± 2.29	6.55 ± 0.09	5.99 ± 0.11	(-)[a]	(-)[a]	(-)[a]	(-)[a]	56.6 ± 0.32	56.3 ± 1.49	6.36 ± 0.06	6.14 ± 0.08
Entisol	24.8 ± 2.71	27.8 ± 4.40	4.45 ± 0.02	4.51 ± 0.03	(-)[a]	(-)[a]	(-)[a]	(-)[a]	54.1 ± 7.13	55.2 ± 2.16	4.42 ± 0.04	4.40 ± 0.05
Oxisol	(-)[a]	(-)[a]	(-)[a]	(-)[a]	43.6 ± 1.14	42.2 ± 1.47	4.94 ± 0.03	5.16 ± 0.02	57.5 ± 1.21	58.5 ± 2.15	5.03 ± 0.03	4.99 ± 0.06

[a]- Assay not performed

The time-weighted average Predicted Environmental Concentrations at 28d (PEC) for fipronil were estimated based on the methodology proposed by the European and Mediterranean Plant Protection Organization (EPPO, 2003). The software ESCAPE (2013) was used to calculate the PEC based on a soybean crop scenario. It was considered a sowing density of 60 kg of seeds per hectare (EMBRAPA, 1988) at a depth of 0-5 cm (ALVES et al., 2013) and soil densities of 1.0 g cm^{-3} for TAS and Oxisol, and 1.5 g cm^{-3} for Entisol (SOUZA et al., 2005; BANDEIRA et al., 2020). A single Shelter® application dose of 12 g a.i. per 60 kg of seeds (lowest manufacturer's recommendation), over a single planting cycle, with an interception rate of 5% by the plants (JACKSON et al., 2009) and a dissipation half-life of 31 days (EFSA, 2006) were assumed.

3.2.4 Chemical analysis

For all tests, soil samples from each treatment were taken at the start of the test for the quantification of the real concentration in soils. The extraction of fipronil was based on the modified QuEChERS acetate method (PRESTES et al., 2011), without the cleaning step. The chemical analysis was performed in triplicates. For all treatments, wet soil was extracted with 50/50 (v/v) ultrapure water/acetonitrile. The flasks with the mixture were shaken manually and afterward, samples were centrifugated at 9000 rpm for 5 min. 1 mL of supernatant was taken out and diluted in 10 mL methanol. The final diluted extract was filtered through a 0.2 µm polyvinylidene fluoride Millex syringe filter directly into vials. The fipronil content was measured in an HPLC (LC-MS 2020, Shimadzu) with electrospray ionization source, quadrupole mass analyzer and LabSolution data acquisition system, using the Varian C_{18} column.

3.2.5 Test procedures

The chronic toxicity tests were performed according to ISO 11267 (ISO, 2014) at the temperature of 25 ºC and 12h-photoperiod.

The experimental units were constituted by glass containers with airtight closure caps that received 30 g of spiked soil or control soil. Ten organisms with synchronized age (10-12 d) were inserted into each container, which was fed (≈2 mg of dry *Saccharomyces cerevisiae*) on the 1st and 14th day of the test. Weekly, the containers were opened to allow gas exchange and for soil moisture adjustment with distilled water (by weight difference). At the end of the test (28 d), the content of each replicate was transferred to another vessel that received water and a few drops of black ink to allow the flotation and to facilitate the visual contrast of organisms with the water. The surviving adults were visually counted. The tests in each soil occurred individually, however, both moisture treatments were tested simultaneously with the same batch of animals. All treatments were performed in five replicates plus an extra replica, which was used to assess the soil moisture and pH at the end of the bioassays.

To evaluate the reproduction and growth of collembolans, each vessel was photographed from a superior view in high resolution. The photos were analyzed through ImageJ® software (2013), where all juveniles from each experimental unit were counted through the manual counting tool. In addition, the length (from the front head to the end of the abdomen) of generated collembolans was mensurated on the images by using the same software. For this, at least 30 juveniles of each replicate were randomly selected. For the replicates with less than 30, all juveniles were measured. In each replicate, the collembolans were classified in three distinct body sizes: small (≤ 3 mm), medium (> 3 and ≤ 4.5 mm) and large (> 4.5 mm). The results were presented in percentage of each size class.

3.2.6 Data analysis

The normality and homoscedasticity of the data from chronic toxicity tests were checked through the *Kolmogorov-Smirnov* and *Bartlett* tests, respectively. When necessary, data logarithmic transformations were used to meet the analysis of variance (ANOVA) assumptions. For reproduction and growth data, the differences between treatments were tested by the one-way ANOVA ($p < 0.05$). When statistical differences were detected in reproduction data, the treatments containing fipronil were compared with the control by using the post-hoc *Dunnett's* test ($p < 0.05$), allowing to determine the non-observed effect concentration (NOEC) and lowest observed effect concentration (LOEC). Differences between the average percentages of small, medium and large juveniles at 30% (or 45% in Oxisol) and 60% WHC, in the same soil type and concentration, were checked via *Tukey's* test ($p < 0.05$).

The effect concentrations causing a reduction in the collembolan's reproduction or growth in 10 or 50% (only reproduction), in relation to the control (EC_{10} or EC_{50}, respectively), were estimated by using non-linear regression models (ENVIRONMENT CANADA, 2007). The EC values were estimated based on real concentrations (Table 3.2). Significant differences between EC_{50} values (reproduction data) from different soil types (at 60% WHC) or moisture contents (within the same soil), were assumed when their 95% confidence intervals did not overlap (JEGEDE et al., 2017).

A two-way ANOVA was used to evaluate the interaction between the soil moisture and fipronil concentrations, for each soil, under reproduction of collembolans and growth of juveniles generated. All statistical analyzes were performed using the Statistica® software, version 13.5.0.17 (TIBCO DATA SCIENCE, 2013).

The risk estimation was performed through the toxicity-exposure ratio (TER) approach, by dividing the EC_{10} (EC, 2002) of each soil moisture scenario by the respective PEC value ($TER = EC_{10} / PEC$). A significant risk for *F. candida* was considered when TER < 5.

3.3 RESULTS

The validity criteria for chronic toxicity tests with *F. candida* (ISO, 2014) were met for all performed tests (Table 3.4).

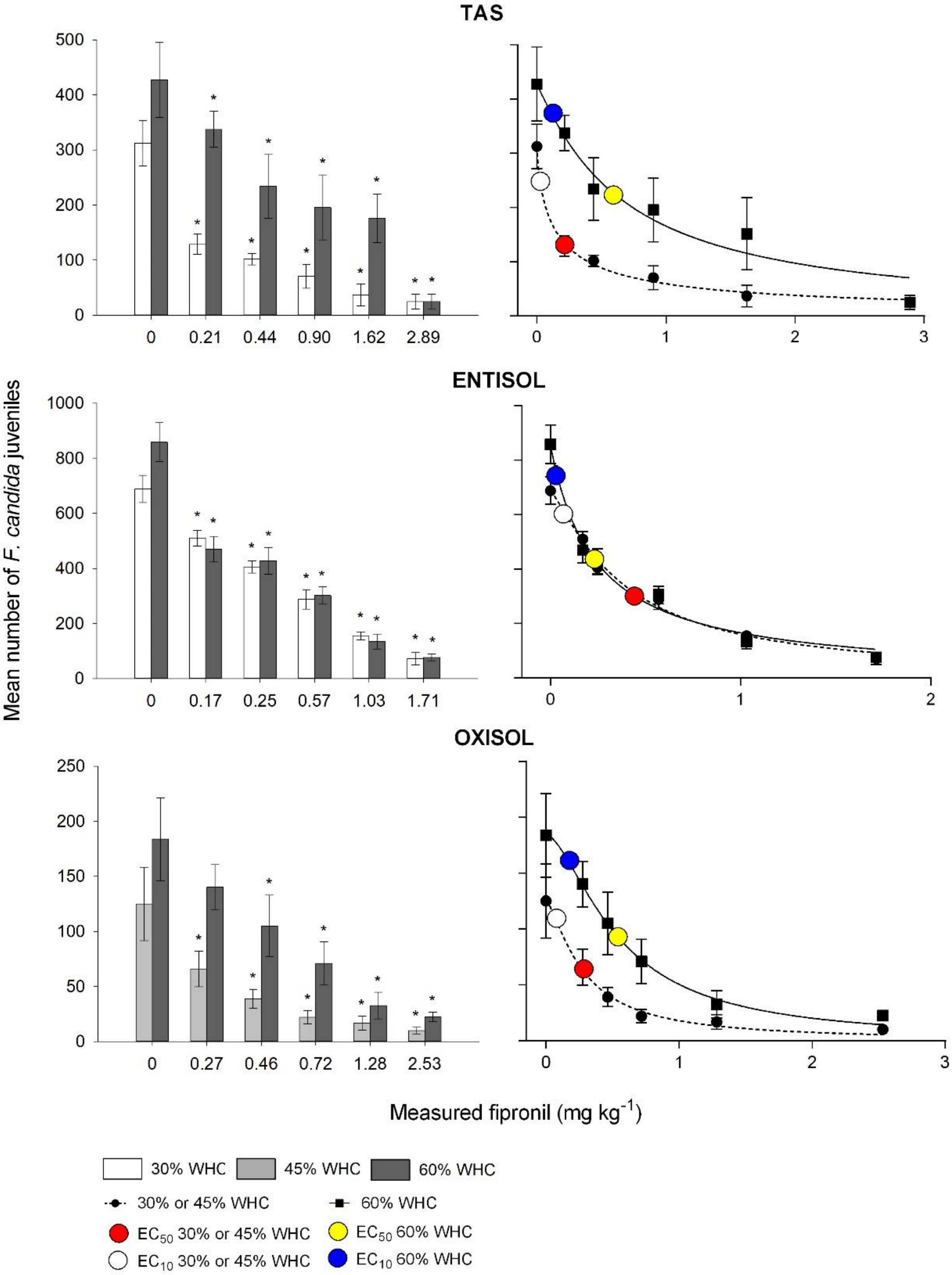
Table 3.4 - Validity criteria based on the mean number (± standard deviation) of juveniles and/or on adult survivors of *Folsomia candida* (n=5), as well as on the coefficients of variation (%) found in controls of the chronic tests performed in Tropical Artificial Soil (TAS), Entisol and Oxisol, under different soil moisture treatments (30% or 45%, and 60% of the soil water holding capacity - WHC).

Validity criteria	TAS		Entisol		Oxisol	
	30% WHC	60% WHC	30% WHC	60% WHC	45% WHC	60% WHC
Survivors[a]	10 ± 0.0	9.8 ± 0.4	10 ± 0.0	10 ± 0.0	8.8 ± 1.0	9.2 ± 0.8
Juveniles[a]	313 ± 41	428 ± 68	688 ± 50	858 ± 71	125 ± 33	184 ± 38
Coeficient of variation[a]	13%	16%	7%	8%	27%	20%

[a] - ISO 11267:2014 criteria for control treatments: adult mortality ≤ 20%; juveniles per vessel ≥ 100; coefficient of variation ≤ 30%.

Fipronil caused a reduction in the number of *F. candida* juveniles in all exposure scenarios, with effects varying according to the soil type and soil moisture (Figure 3.1).

Figure 3.1 - Mean number (bars ± standard deviation, n=5) and dose-effect curves (with effect concentrations of 50% - EC_{50}) of juveniles *Folsomia candida* generated after 28 d of exposure to different concentrations of fipronil in Tropical Artificial Soil (TAS), Entisol and Oxisol under regimes of 30 or 45% and 60% of soil water holding capacity (WHC). Asterisk (*) represent significant reduction in the number of juveniles compared to the respective control (*Dunnett's* test, $p \leq 0.05$). In dose-effect curves, the EC_{50} values were plotted as red and yellow circles for the exposures at 30 or 45% (for Oxisol) and 60%, respectively.



Source: prepared by the author (2021).

At 60% WHC, the fipronil toxicity (EC_{50}-based) did not differ between TAS and Oxisol. On the other hand, higher toxicity was found in Entisol, when compared with the other tested soils. In TAS and Oxisol, the dry condition (30 and 45% WHC, respectively) increased the toxicity of fipronil to collembolans, in comparison to 60% WHC (Table 3.5). For TAS, EC_{50} values indicated toxicity more than three times higher in the dry soil (30% WHC). For Oxisol, the toxicity at 45% WHC was twice higher than at 60% WHC. On the other hand, in Entisol, the toxicity at 60% WHC was higher when compared with 30% WHC (Table 3.5). The two-way ANOVA (Table 3.6) showed a significant interaction between the soil moisture content and concentrations of fipronil on the collembolan's reproduction and growth for all tested soils.

Table 3.5 - Ecotoxicological parameters for reproduction (NOEC, LOEC, EC_{50} and EC_{10}) and juvenile's growth (NOEC, LOEC and EC_{10}) from chronic toxicity assays with *Folsomia candida* exposed to increasing concentrations of fipronil in Tropical Artificial Soil (TAS), Entisol and Oxisol under two soil moisture contents (30% or 45%, and 60% of the soil water holding capacity - WHC). Asterisk (*) indicates significant differences between EC values at 30% or 45%, compared to 60%, within the same soil.

Measured endpoint	Parameter	Fipronil concentration (mg kg^{-1})					
		TAS		Entisol		Oxisol	
		30% WHC	60% WHC	30% WHC	60% WHC	45% WHC	60% WHC
Reproduction	NOEC	<0.21	<0.21	<0.17	<0.17	<0.27	0.27
	LOEC	0.21	0.21	0.17	0.17	0.27	0.46
	EC_{50}	0.20*	0.70	0.41*	0.24	0.27*	0.54
	Limits (95%)	(0.15 - 0.25)	(0.42 - 0.94)	(0.36 - 0.46)	(0.20 - 0.29)	(0.20 - 0.34)	(0.42 - 0.70)
	EC_{10}	0.01	0.08	0.04*	0.01	0.07	0.12
	Limits (95%)	(0.00 - 0.02)	(0.00 - 0.16)	(0.03 - 0.06)	(0.00 - 0.02)	(0.04 - 0.10)	(0.05 - 0.20)
Growth	NOEC	<0.21	<0.21	<0.17	<0.17	<0.27	<0.27
	LOEC	0.21	0.21	0.17	0.17	0.27	0.27
	EC_{10}	0.27	0.65	0.08	0.14	0.10	0.22
	Limits (95%)	(0.07 - 0.47)	(0.22-1.09)	(0.04 - 0.12)	(0.03 - 0.26)	(-)[a]	(0.10 - 0.33)

[a] - Confidence intervals could not be estimated

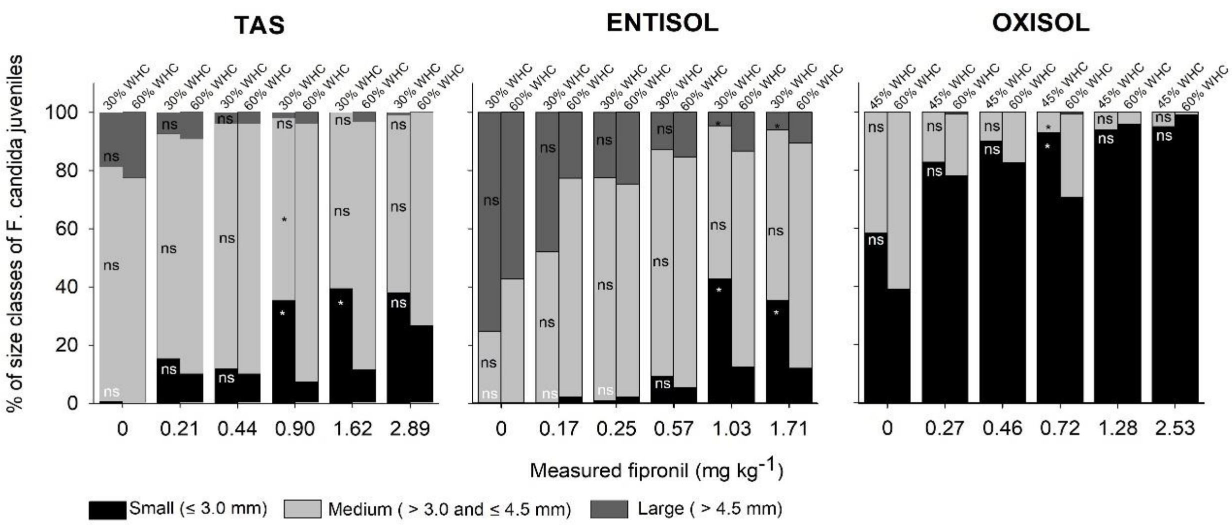
Table 3.6 - Summary of the two-way ANOVA on the effects of fipronil concentration in Tropical Artificial Soil (TAS), Entisol and Oxisol, under different soil moisture treatments (30% or 45%, and 60% of the soil water holding capacity - WHC), and their interaction on the reproduction and growth of *Folsomia candida*.

Measured endpoint	Soil type	Factor	SS	df	Mean squares	F	p
Reproduction	TAS	Intercept	200035..98	1	20035.98	72012..73	0
		Concentration	95.54	5	19.11	68.68	0
		Moisture	18.02	1	18.02	64.78	0
		Concentration x Moisture	8.56	5	1.71	6.16	0.000012
		Error	454.9	1635	0.28		
	Entisol	Intercept	27667.92	1	27667.92	70405.32	0
		Concentration	361.1	5	72.22	183.78	0
		Moisture	3.55	1	3.55	9.03	0.002689
		Concentration x Moisture	64.7	5	12.94	32.93	0
		Error	679.07	1728	0.39		
	Oxisol	Intercept	7621.049	1	7621.049	32924.02	0
		Concentration	119.605	5	23.921	103.34	0
		Moisture	4.028	1	4.028	17.4	0.00032
		Concentration x Moisture	14.554	5	2.911	12.58	0
		Error	328.693	1420	0.231		
Growth	TAS	Intercept	1750967	1	1750967	1185.677	0
		Concentration	699495	5	139899	94.733	0
		Moisture	211827	1	211827	143.44	0
		Concentration x Moisture	56832	5	11366	7.697	0.000024
		Error	69408	47	1477		
	Entisol	Intercept	7562852	1	7562852	5156.842	0
		Concentration	3148284	5	629657	429.341	0
		Moisture	9037	1	9037	6.162	0.016852
		Concentration x Moisture	67534	5	13507	9.21	0.000004
		Error	65995	45	1467		
	Oxisol	Intercept	278055.6	1	278055.6	787.6566	0
		Concentration	122247.4	5	24449.5	69.2588	0
		Moisture	30503.5	1	30503.5	86.4083	0
		Concentration x Moisture	8435.7	5	1687.1	4.7792	0.001337
		Error	16238.7	46	353		

All analyses were based on the real concentrations.
SS: sum of squares
df: degrees of freedom
F: Significance factor
p: p <0.05 the difference is significant

In general, while the percentage of small juveniles increased in all soils with increasing fipronil concentrations, the number of juveniles with large size decreased (in TAS and Entisol). When comparing the different soil moisture contents (Figure 3.2) for the same contamination scenario (Figure 3.2), the percentage of small collembolans was significantly higher at 30% WHC than at 60% WHC in TAS (i.e. at 0.90 and 1.62 mg kg^{-1}) and Entisol (i.e. at 1.03 and 1.71 mg kg^{-1}). On the other hand, no significant influence of soil moisture content on the fipronil effects on juvenile's growth was observed in Oxisol, except for the concentration of 1 mg kg^{-1}, in which the percentage of small organisms was significantly higher at 45% WHC compared to 60% WHC. For TAS, Entisol and Oxisol, the toxicity under the growth of juveniles (based on EC_{10}) was, respectively, about 2.2, 2.4 and 2.7-fold lower at 60% WHC when compared to the respective dry condition (Table 3.5).

Figure 3.2 - Number of small (≤ 3 mm), medium (> 3 and ≤ 4.5 mm) and large (> 4.5 mm) *Folsomia candida* juveniles (expressed as the % of total sample) found after 28 days of exposure to different concentrations of fipronil in Tropical Artificial Soil (TAS), Entisol and Oxisol, under of 30% or 45%, and 60% of soil WHC. Asterisk (*) indicate a significant difference between treatments with the same fipronil concentration but distinct soil moisture contents, and 'ns' indicate no significant difference (*Tukey's* test - $p \leq 0.05$).



Source: prepared by the author (2021).

The PEC values revealed that concentrations very close to that which cause reductions on 10% on collembolans reproduction can be found in the soil. TER values indicate risk for collembolans in TAS and Oxisol only at the dry condition (TER < 5). In

Entisol, however, a significant risk was found at both soil moisture contents (TER < 5; Table 3.7), when considering the lowest dose of Shelter® application to soybean culture.

Table 3.7 - Predicted Environmental Concentration (PEC) and Toxicity Exposure Ratio (TER) for the exposure of *Folsomia candida* to fipronil in TAS, Entisol and Oxisol under different soil moisture contents (30% or 45%, and 60% of the soil water holding capacity - WHC).

Soil	WHC (%)	EC_{10} (mg kg^{-1})	PEC (mg kg^{-1})	TER
TAS	30	0.01	0.017	**0.59**
	60	0.08	0.017	5.30
Entisol	30	0.04	0.014	**2.90**
	60	0.01	0.014	**0.73**
Oxisol	45	0.07	0.017	**4.13**
	60	0.12	0.017	7.07

TER values lower than 5 (in bold) indicate significant/unacceptable risk.

3.4 DISCUSSION

Fipronil was toxic to *F. candida* even at lower concentrations, in all soils tested (Figure 3.1). A decrease of 50% in the number of generate collembolans occurred at very low fipronil concentrations (Table 3.5), varying with the soil type. The reduction in collembolans reproduction caused by the exposure to fipronil are also reported in other studies (SAN MIGUEL et al., 2008; ZORTÉA et al., 2018a; ALVES et al., 2014), corroborating our findings. These effects are probably due to the mode of action of the insecticide on organisms that, in addition to causing inhibition of GABA neurotransmitter, which results in excessive stimulation of the nerves and consequent muscle paralysis and death of organisms (TINGLE et al., 2003), can also deregulate the endocrine system linked to sexual maturation and ecdysis in arthropods, causing reduced egg-laying (GAERTNER et al., 2011; CARY et al., 2004). The fipronil effects on the size of *F. candida* (Figure 3.2) was probably due to changes in physiological or biochemical processes into organisms (RIBEIRO et al., 2001), which are expected because they expend more energy to detoxification, thus reducing their resources for growth (SOUSA et al., 2000; RIBEIRO et al., 2001, HOFFMANN et al., 2003).

Based on the toxicity results, our first work hypothesis could be confirmed, since the effects of fipronil on the reproduction of *F. candida* (at 60% WHC) in Entisol (natural sandy soil) were greater than those found in TAS and Oxisol (Table 3.5). These findings can be related to the adsorptive characteristics of tropical soils on the environmental fate of fipronil (MASUTTI and MERMUT, 2007; SCORZA JUNIOR and FRANCO, 2013). Among the main factors that influence the bioavailability and consequently the ecotoxicity of pesticides in the soil, are the clay and OM contents, the reactivity of the mineral particles, and the sorption equilibrium of pesticide between pore water and solid phases of the soil (SMIT and VAN GESTEL, 1998; DOMENE et al., 2010; DOMENE et al., 2012; MANDAL and SINGH, 2013).

TAS contains 1.4% OM (Table 3.1) based on coconut fiber, which has very rich adsorptive properties such as porous morphology, many carboxylic, hydroxylic, and carbonyl sites (DA SILVA et al., 2012), with high WHC and ability to adsorb lipophilic pesticides, such as fipronil (CARY et al., 2004). Oxisol has undergone intense weathering processes, having significant clay contents, high WHC (Table 3.1), and mineral surfaces with high amounts of iron oxy-hydroxides (MASSUTI and MERMUT, 2007), that favor chemical bonds with electronegative atoms of fipronil (F^-, Cl^-, O^{2-} and N^{3-}, SINGH et al., 2014; SINGH et al., 2016). In contrast, Entisol have low WHC, low clay and silt contents (Table 3.1) and, consequently, fewer binding sites and probably low pesticide adsorption capacity, favoring greater bioavailability of fipronil in soil pores for collembolans uptaking (VAN GESTEL, 2012; PEIJNENBURG et al. 2012).

Similarly to our findings, Bandeira et al. (2019) observed lower toxicity of an imidacloprid-based seed dressing formulation to *F. candida* in TAS and Oxisol than in Entisol. Zortéa et al. (2018a; 2018b) assessed the effects of fipronil in a veterinary medicine to collembolans and found high toxicity in Entisol when compared to TAS and Oxisol. Other studies evaluating the toxicity of swine manure to soil invertebrates also found that the deleterious effects are greater in Entisol than in Oxisol (SEGAT et al., 2015; MACCARI et al., 2016). Despite testing different contaminants, these studies corroborate our first hypothesis, that insecticides and other contaminants have greater effects on soils with higher sandy contents to collembolans.

The second work hypothesis proved to be true only in TAS and Oxisol where the dry condition increased fipronil toxicity to *F, candida*. Instead, in Entisol the higher toxicity was observed at 60% WHC (Table 3.5). The reason for these behavior can alsorelated to the adsorptive capacities of the soils, above mentioned, intrinsic to WHC, that regulate the bioavailability of water and solutes present in the pore soil water (DOMENE et al., 2012).

The adsorption of fipronil in the solid matrix depends not only on the number of adsorptive sites but also on the water content of the soil. According to Bobé et al. (1997), the adsorption of the fipronil increases as the ratio soil:water decreases. This is because the substantial increase in water should promote the breakdown of aggregates in the soil, increasing the specific surface area and, consequently, the adsorption of the pesticide (Bobé et al., 1997). Thus, the increase in soil moisture content may have provided an increase in the adsorption of fipronil in the soil particles (especially in clay and organic material), reducing the bioavailability and consequently the toxicity , while the decrease in soil moisture content may have provided a decrease in the adsorption of fipronil in the soil, becoming more bioavailable and toxic for organisms in the soil pores. This could partially explain the increase in the fipronil toxicity on collembolan’s reproduction in TAS and Oxisol with the decrease of soil moisture.

Besides the availability of contaminant, It’s possible that dry condition had contributed to the sensibility of organisms, since that under drought conditions collembolans may have their metabolic activities reduced, which may affect their growth, compromising the survival and reproductive performance (BURSELL, 1970; EDNEY, 1977; VAN GESTEL and VAN DIEPEN, 1997; JÄNSCH et al., 2004). Some studies have indicated that the optimum soil moisture for metabolic maintenance and guarantee of the existence of the species is about 60% WHC (VAN GESTEL and VAN DIEPEN, 1997; CROUAU et al., 1999; BANDOW et al., 2014a; BANDOW et al., 2014b). This may explain the poor reproductive performance in dry condition in all tested soils (Figure 3.1; Table 3.4).

On the other hand, sandy soils with lower OM content, such as Entisol, normally present lower sorption capacities compared to soils with greater clay and OM contents (SPOMER and KAMBLE, 2010). According to Harris (1964a), in the presence of high water content, the toxicity of pesticides in sandy soil depends not only of their ability to bind to adsorption sites, but also on the molecule's ability to compete with water for binding sites. In their study, Harris (1964a) concluded that in a sandy soil the high water content available in the soil pores may compete better for the binding sites than pesticides heptachlor, DDT, diazinon, V-C13 and parathion, increasing their bioavailability and, consequently, their toxicity for insects. Sandy soils as Entisol, have poor adsorptive properties with a lower number of binding sites and lower WHC (SHARMA et al., 2013; Table 3.1), therefore, at higher moisture content, probably water has occupied most of the few adsorption sites that exist on soils like this, making fipronil more bioavailable in soil porewater, increasing the toxicity to organisms by uptaken, while in a dry situation the adsorption of the pesticide in the soil may have been favored, reducing the toxicity of fipronil to collembolans.

Collembolans's size has been considered a promissor assessment endpoint in understanding the effect of contaminants at long-term exposure (GUIMARÃES et al., 2019; SZABÓ et al., 2020). Our datas showed a higher proportion of small juveniles at 30% WHC, while in 60% WHC for some concentrations in TAS (0.90 and 1.62 mg kg^{-1}) and Entisol (1.03 and 1.71 mg kg^{-1};Figure 3.2).

In addition to the factors previously mentioned, the reduction in soil moisture may have caused a reduction in the thickness of the water layer that surrounds the soil particles, negatively affecting the habitat of collembolans and the access to food and water, compromising their development (COLEMAN et al., 2004).

In Oxisol, it appears that the texture of the soil was the main factor t influenced the growth of juveniles, given the predominance of small juveniles in relation to the occurrence of medium and large size juveniles in the control treatment in this soil, regardless of the moisture regime. This occurred probably because, in clayey soils, such as Oxisol (Table 3.1), the porous space available to be occupied by collembolans inside the soil are reduced, which can difficult the collembolans colonization and impair body development by limiting the access to water and resources (DOMENE et al., 2011). On the other hand, TAS and Entisol, which have higher sand content (Table 3.1), probably offer a better environmental condition to collembolans due to the greater volume of large pores (AMORIM et al., 2005; DOMENE et al., 2011; BANDEIRA et al., 2019). This assumption can also be demonstrated through the number of juveniles generated and through the proportion of medium and large size juveniles in the control treatments of each soil (Table 3.4).

In TAS and Entisol, in general, at the same chemical stress condition (i.e., at the same fipronil concentration), the percentage of small juveniles was higher in the drier condition. The results on the growth of the collembolans under both soil moisture contents were especially interesting for Entisol, because fipronil effects on the reproduction was twice higher at 60% WHC, while the effects on juveniles' growth were greater at 30% WHC. A possible explanation for this may be related to the collembolans' needs and mode of life. In the dry condition, although the bioavailability of fipronil is likely to be lower due to the greater retention of these molecules in the solid phase, the availability of resources essential to metabolic activity and the consequent development of the collembolans (especially water) is reduced compared to the standard moisture condition. This can contribute to explain the higher proportion of small juveniles generated in the drought scenario (30% WHC) compared to 60% WHC in the same chemical stress condition (e.g., 1.03 and 1.71 mg kg^{-1}). From these inferences, the problem of reduction on soil moisture content become evident on ecological scale for collembolans, whereas smaller collembolans can be more susceptible to stress and predation, resulting in an additional risk for these organisms (GUIMARÃES et al., 2019).

Other studies also evaluated the influence of soil moisture content on the toxicity of pesticides to collembolans. Hennig et al. (2020) observed that the toxicity of imidacloprid for *F. candida* in an Oxisol was greater at 45% WHC when compared to 60% WHC, while in Entisol there was no clear influence of soil moisture content (30 and 60% WHC) on the toxicity. Like to this study, the authors relate their findings to the adsorptive properties of natural soils and consequent bioavailability of contaminant to collembolans. Others studies conducted in standard soils also agree our finds to TAS. Bandow et al. (2014b) observed that the toxicity of pyrimethanil on the reproduction of *F. candida* and *Sinella curviseta* on OECD soil (sandy loam soil - 5% OM) was greater at 30% WHC compared to 50% and 70% WHC. Højer et al. (2001) observed that the toxicity of phenols to collembolans can also increase with the reduction of moisture content in a LUFA 2.2 soil (Loamy Soil - 6% OM). For both studies, the observed effects were related to a synergistic or additive effect of the drought with the effect of the contaminant, as well as to the adsorptive properties of the soil and the consequent bioavailability of the contaminant for collembolans, as reported in the present study.

Our estimated PECs (0.014 to 0.017 mg kg^{-1}) fitted in the range of fipronil concentrations found in agricultural soils by some authors (LI et al., 2015) in peanut fields. Ying and Kookana (2006) found fipronil concentrations between 0.01 to 2.06 mg kg^{-1} in field soils after three years of application. Mandal and Singh (2013) reported fipronil concentrations of 1.90 and 5.50 mg kg^{-1} in two different soils 120 d after the application. Similarly, Silva et al. (2016) found about 3.5 mg kg^{-1} of fipronil in two soils 40 d after pesticide application. These values led us to suppose that, fipronil can be persistent in soils and, in some cases, soil invertebrates may be exposed to environmental levels higher than those estimated in our study.

Calculations based on TER approach indicated no risk for *F. candida* exposure to fipronil at the lowest recommended dose for soybean of the commercial formulation in TAS and Oxisol under standard soil moisture conditions. In contrasting, a significant risk of fipronil was observed in Entisol, revealing that *F. candida* populations can be affected when exposed to agricultural fields in sandy soils containing soybean seeds treated with fipronil (Table 3.7). Our results infer that soil properties, like clay and OM contents, may influence the risk of fipronil present in commercial formulation Shelter® to collembolans, as observed to mancozeb (CARNIEL et al., 2019) and imidacloprid (BANDEIRA et al., 2019) in Brazilian soils, where demonstrate more toxicity in soils with poor adsorptive characteristics. This seems to be a relevant finding for Brazil, since the environmental risk assessment of pesticides in the soil, as a requirement for registration and commercialization of a.i., is rarely conducted and not consider the type of soil in the risk (IBAMA, 2012).

In addition, the reduction in soil moisture as a consequence of climate change also revealed risk of fipronil for collembolans in TAS and Oxisol, while in Entisol risk occurred regardless of soil moisture regime. Similar results were reported for imidacloprid (HENNIG et al., 2020), where dry Oxisol increased the risk of this insecticide for collembolans, and for Entisol risk was observed regardless soil moisture regime, corroborating our findings. The authors attributed their results to WHC of soils and adsorption properties of soils. and reveal a problem of fundamental importance for tropical countries like Brazil, where severe drought scenarios are predicted for the coming years (PBMC, 2014).

Based on the risk assessment observed in this study, considering the influence of abiotic factors such as soil type and soil moisture regime in prospective risk assessments of pesticides in soil seems to be essential to avoid underestimation the effects of these chemicals on collembolans in future scenarios.

3.5 CONCLUSIONS

Our results showed that the toxic effects of fipronil on *F. candida* reproduction are dependent on the tropical soil type, and are enhanced in the sandy soil when compared to a sandy loam artificial soil and natural clay soil. There was a relationship between adsorption properties (by clay, silt, sandy and OM contents) of soil in driving the toxicity of fipronil for collembolans. The reduction of soil moisture may increase or decrease the toxic effects of fipronil on *F. candida* reproduction, depending on the soil type. Apparently, soils with higher WHC and good adsorptive properties (by clay and OM content) tend to increase fipronil toxicity when dry, while those with less WHC and less favorable adsorptive properties can reduce a.i. toxicity. In all tested soils, the percetage of small juveniles increased with fipronil concentration regardless of soil moisture, but more small juveniles (than medium or large) were generated at dry condition compared to the standard soil moisture in TAS and Entisol. Disregarding the climatic variables, it was found that the risk of fipronil depends on the texture of the soil, presenting risk for collembolans in Sandy soil. On the other hand, when considering the reduction of precipitation regimes, the reduction of soil moisture resulted in an increased risk of a.i. in soils with higher WHC.

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4 TOXICITY AND RISK ESTIMATION OF FIPRONIL FOR COLLEMBOLANS UNDER INCREASING TEMPERATURES IN CONSTRASTING NATURAL SUBTROPICAL SOILS

ABSTRACT

We evaluated the toxicity and the risk, via toxicity exposure ratio (TER) approach, of the insecticide fipronil to collembolans in three tropical soils, under increasing atmospheric temperatures. Chronic toxicity tests were performed with *Folsomia candida* in Tropical Artificial Soil (TAS), Entisol and Oxisol spiked with increasing concentrations of fipronil, at a standard (20 ± 2 ºC), a tropical condition (25 ± 2 ºC) and at global warming scenario (27 ± 2 ºC). Fipronil toxicity varied with the type of soil by clay and silt content, showing higher toxicity in TAS and Oxisol at 27 °C, compared to 20 and 25 °C ($EC_{50\ TAS}$ = 0.81, 0.70, 0.31 mg kg^{-1}; $EC_{50\ OXISOL}$ = 0.52, 0.54, 0.40 mg kg^{-1}; to 20, 25 and 27 ºC, respectively). In Entisol the toxicity at 27 ºC was reduced compared to the lower ones ($EC_{50\ ENTISOL}$ = 0.12, 0.24, 0.33 mg kg^{-1}, to 20, 25 and 27ºC, respectively), probably due to the effect of pesticide degradation influenced by the high temperature in this soil. TER approach revelead risk of fipronil in Entisol regardless temperature regime, while in TAS risk occured at 20 and 27 ºC and in Oxisol only at 27 ºC, showing that, regardless soil type and the way of temperature affecting the fipronil toxicity, at highest temperature for all soil types, there is risk of pesticide for collembolans when exposed at this temperature.

Keywords: Global warming; *Folsomia candida*; Pesticides; Terrestrial ecotoxicology; Risk assessment.

4.1 INTRODUCTION

Fipronil (5 - amino - 1 - [2,6 - dichloro - 4 - (trifluoromethyl) phenyl] - 4 - (trifluoromethylsulfinyl) -1H –pyrazole - 3 - carbonitrile) is a broad spectrum phenylpyrazole pesticide, capable of disrupter the central nervous system of the target organisms, being considerate effective against a wide range of pests (HAINZL et al., 1998; MOHAPATRA et al., 2010). To protect or minimizing damage by pests and pathogens that attack seeds and seedlings early in the crop cycle, one of the main applications of this active ingredient (a.i.) is in the chemical treatment of seeds (BONMATIN et al., 2015). However, despite the advantages of its for crops, this a.i.

residues in soil can cause deleterious effects in non-target invertebrates, which are often in direct contact with the pesticide in the soil and play important ecosystem services (ALVES et al., 2013, ALVES et al., 2014; SIMON-DELSON et al., 2015; DAAM et al., 2019).

Climatic factors can also play an important role, since they affect the fate and route of pesticides in the soil by desorption process and cosequently degradation and volatilization, as well as affect the physiology, composition and diversity of soil invertebrates (DAI et al., 2019). According to the Intergovernmental Panel on Climate Change (IPCC), Earth's climate is changing substantially due to anthropogenic increase of emission greenhouse gases (IPCC, 2019). Under the current global warming perspective, it is estimated that an increase in global temperature of about 1 ºC has already occurred since the beginning of the century, being predicted an increase of about to 1.5 ºC between the years 2030 and 2052 (IPCC, 2019). Ectothermic arthropods, such as collembolans, that have their corporal temperature dependent of the environment temperature (DAI et al., 2019), may suffer with increases on temperature due changes in their homeosthasis, altering the thermal tolerance (HUEY and KINGSOLVER, 1989) impacting the community composition due damage in survival, fitness and reproduction of this organisms (JÄNSCH et al., 2005; SINCLAIR et al., 2016). In addition, the increase in temperature may influence the dynamics and fate of pesticides in the soil, through the increase in degradation and volatilization rates (SHUNTHIRASINGHAM et al., 2010). The exposure of soil invertebrates to pesticides at increased temperatures can result in changes in rates of absorption, metabolism, bioaccumulation and the dynamics of excretion of pollutants, which may potentiate the toxic effects of the substances (SEELAND et al., 2012; DELCOUR et al., 2015; DAI et al., 2019)

The effects of atmospheric temperature at toxicity increasing of several pesticides like pyrethroids, organophosphates and neonicotinoids have are already known (BANDOW et al., 2014a; JEGEDE et al., 2017; BANDEIRA et al., 2020) and how these effects vary with the type of soil where the exposure occurs (BANDEIRA et al., 2020). Despite some ecotoxicological studies has reported negative effects of fipronil in survival, reproduction, and bioaccumulation in collembolans (SAN MIGUEL et al., 2008; ALVES et al., 2014; ZORTÉA et al., 2018a; ZORTÉA et al., 2018b) no studies has reported the relationship of temperature increasing and toxicity of fipronil to collembolans, when used in seed treatment formulations, especially in tropical soils.

Therefore, this study aimed to assess the influence of increasing atmospheric temperatures on the chronic toxicity of fipronil to collembolans *F. candida*. To achieve this goal, collembolans chronic toxicity tests were conducted in three different soil types, under atmospheric temperatures of 20, 25 and 27 ºC. We hypothesize that increase in atmospheric temperature increases the toxicity of fipronil to collembolans, but that this effect may vary with the type of soil used.

4.2 MATERIALS AND METHODS

4.2.1 Test species

The collembolans *F. candida* were cultured in laboratory according to ISO 11267 (ISO, 2014). The organisms were kept in a room with controlled temperature (20 ± 2 ºC) and photoperiod of 12 h, in plastic containers containing a mixture of plaster of Paris, water and activated charcoal (10:7:1, respectively). Twice a week the collembolans were fed with dried Baker's yeast (*Saccharomyces cerevisiae*) and the moisture of the breeding medium was adjusted with few drops of distilled water. The sensitivity of *F. candida* was checked though a positive control assay with boric acid in TAS (EC_{50} = 175 mg kg^{-1}), following Niemeyer et al. (2018).

4.2.2 Test soil

The ecotoxicological assays were performed in an Artificial Tropical Soil (TAS) and two contrasting natural soils from Brazil. The TAS (GARCIA, 2004) was composed of a mixture of fine sand (75%), kaolin (20%) and powered coconut husk (5%). The TAS pH was adjusted to 6.0 ± 0.5 with $CaCO_3$ (ISO, 2014). An Entisol was sampled in Araranguá-SC (29º00′S 49°31′W) and an Oxisol, was sampled in Palmitos-SC (27º04'S 53º 09'W). Both were collected in the top layer of the soil profile (0-20 cm) in

Brazilian areas with no history of contamination by pesticides. Soils samples were sieved (# 2 mm), dafaunated through three freezing and thawing cycles, air-dried (ALVES et al., 2019) and stored at local ventilated and protected from light.

The water holding capacity (WHC) and pH (KCl- 1 M) were measured according to ISO 11267 (ISO, 2014).Cation exchange capacity (CEC), soil organic matter (SOM), and the sand, clay and silt contents were measured according to Tedesco et al. (1995). All results for these parameters are shown in Table 4.1.

Table 4.1 - Mean values (± standard deviation; n = 2) of physical and chemical characteristics of Tropical Artificial Soil (TAS), Entisol and Oxisol, used in the ecotoxicological tests.

Parameter	TAS	Entisol	Oxisol
pH (KCl 1M)	5.9 ± 0.1	4.2 ± 0.2	4.8 ± 0.1
SOM (%)	1.4 ± 0.0	2.2 ± 0.1	3.2 ± 0.8
CEC ($cmol_c$ dm^{-3})	3.3 ± 0.2	1.4 ± 0.1	10.8 ± 0.4
WHC (%)	46.3 ± 1.7	31.6 ± 1.1	53.0 ± 1.4
Sand (%)	67.2 ± 0.0	93.8 ± 0.4	31.5 ± 0.1
Clay (%)	14.3 ± 0.0	4.1 ± 0.2	35.5 ± 1.4
Silt (%)	18.5 ± 0.0	2.1 ± 0.1	33.0 ± 1.6
Soil texture	Sandy loam	Sandy	Clay loam

SOM: Soil Organic Matter;
CEC: Cation Exchange Capacity;
WHC: Water Holding Capacity

4.2.3 Test substance

Acommercial formulation for seed treatment Shelter®, with 250 g of Fipronil, i.a. L^{-1} ingredient was used to teste the toxicity of fipronil to *Folsomia candida* at increasing temperature in subtropical soils. The soil samples were spiked with increased concentrations of Fipronil at Shelter. (Table 4.2), which were chosen based on literature (ALVES et al., 2014; ZORTÉA et al., 2018a) and preliminary tests (ISO, 2014). The soil spiking was performed through an aqueous solution, in required volumes to reach 60% of the WHC of each tested soil.

Table 4.2 - Nominal and real concentrations of the a.i. fipronil used in the chronic toxicity tests carried out in Tropical Artificial Soil (TAS), Entisol and Oxisol with *Folsomia candida*, at 20, 25 and 27 ºC.

Soil type	Fipronil concentrations (mg kg^{-1})									
	Treatment	20 ºC			25 ºC			27 ºC		
		Nominal	Actual	% Nominal	Nominal	Actual	% Nominal	Nominal	Actual	% Nominal
TAS	Control	0	0	-	0	0	-	0	0	-
	C1	0.25	0.21	84	0.25	0.21	84	0.064	0.06	93.75
	C2	0.5	0.44	88	0.5	0.44	88	0.16	0.18	112.5
	C3	1	0.9	90	1	0.9	90	0.4	0.36	90
	C4	2	1.62	81	2	1.62	81	1	0.9	90
	C5	4	3.6	90	4	3.6	90	2.5	2	80
Entisol	Control	0	0	-	0	0	-	0	0	-
	C1	0.25	0.25	100	0.125	0.17	136	0.064	0.04	62.5
	C2	0.5	0.57	114	0.25	0.25	100	0.16	0.1	62.5
	C3	1	1.031	103	0.5	0.57	114	0.4	0.37	92.5
	C4	2	1.71	85.5	1	1.031	103	1	1.03	103
	C5	4	2.94	73.5	2	1.71	85.5	2.5	2.15	86
Oxisol	Control	0	0	-	0	0	-	0	0	-
	C1	0.25	0.27	108	0.25	0.27	108	0.125	0.15	120
	C2	0.5	0.46	92	05	0.46	92	0.25	0.27	108
	C3	1	0.72	72	1	0.72	72	0.5	0.46	92
	C4	2	1.28	64	2	1.28	64	1	0.72	72
	C5	4	2.53	63.25	4	2.53	63.25	2	1.28	64

4.2.4 Chemical analyses

Soil samples with the concentrations or control were taken on the first day of each assay, to perform chemical analysis. The extraction o fipronil from soil was based on the modified QuEChERS acetate method (PRESTES et al., 2011) and the quantification were performed on a HPLC (LC-MS 2020, Shimadzu) with electrospray ionization source, quadruple mass analyzer and LabSolution data acquisition system (according described in section 3.2.4 of this work).

4.2.5 Risk assessment

The time-weighted average Predicted Environmental Concentrations at 28d ($PEC_{TWAC-28d}$) for fipronil in all tested soils were calculated by using the ESCAPE software (2013), based on the methodology of the European and Mediterranean Plant Protection Organization (EPPO, 2003). The considerations adopted to $PEC_{TWAC-28d}$ calculate were assuming a single Shelter® application dose of 12 g a.i. per 60 kg of seeds (lowest manufacturer's recommended dose to soybean crops), over a single planting cycle, an interception rate of 5% by the plants (JACKSON et al., 2009), sowing density of 60 kg of seeds per hectare (EMBRAPA, 1988) at a depth of 0-5 cm (ALVES et al., 2013). Soil densities of 1.0 g cm^{-3} for TAS and Oxisol, and 1.5 g cm^{-3} for Entisol (SOUZA et al., 2005). Also, a dissipation half-life (DT_{50}) of 68 (YING and KOOKANA, 2006), 31 (EFSA, 2006) and 28 days (SHUAI et al., 2012) were assumed for the temperatures of 20, 25 and 27 ºC, respectively.

The Toxicity-Exposure Ratio (TER) approach was used to estimate the risk for *F. candida*. For each soil and temperature scenario, TER value was the ratio between the estimated fipronil concentrations that reduced collembolans reproduction by 10% (EC_{10}) and the $PEC_{TWAC-28d}$ ($TER = EC_{10}/PEC_{TWAC-28d}$). When TER was lower than the trigger value of 5, a significant risk was assumed (EC, 2002).

4.2.6 Test procedures

The chronic toxicity tests with *F. candida* were performed according to ISO 11267 (ISO, 2014). The individuals were exposed to increased concentrations of a.i. (Table 2) in three tropical soils (TAS, Entisol and Oxisol), under 20 ± 2 ºC (a standard condition; ISO, 2014), 25 ± 2 ºC (typical tropical temperature; NIVA et al., 2016) and 27 ± 2 ºC, where it was assumed an increase of 2-degree in the atmospheric temperature, due global warming consequences (IPCC, 2014).

In glass bottles with airtight closing lids, containing 30 g of wet soil (control) or spiked soil, were insertedten individuals with synchronized age (10 - 12 d). On the 1st and 14th day of the test, about 2 mg of dried Baker's yeast was offered as food per replicate. For each treatment, including control, five replicates were performed. An extra replica was used to assess soil moisture and pH at the end of the tests.

Weekly, the experimental units were opened to adjust the soil moisture (by weight difference) and to allow gases exchange. At the end of the test (28 d), the content of each replicate was transferred to a plastic container, which received water and few drops of black ink, allowing the flotation and contrast of organisms in the suspension. The surviving adults were visually counted at the end of the test. For reproduction evaluation, the content of each container was photographed from a superior angle in high resolution, and the juveniles were counted on-screen viewing, through ImageJ® software (2013).

4.2.7 Data analysis

The homogeneity of variances and normality of residuals were checked through *Bartlett* and *Kolmogorov-Smirnov* tests, respectively. Logarithmic transformations were applied in the data when the assumptions were not met. Reproduction data were submitted to analyses of variance (ANOVA One-way) and when significant differences were found (p<0.05), the treatments containing fipronil were compared with control trough *Dunnett´*s test. From this analysis, the non-observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) were determined. The fipronil concentrations that reduce collembolans reproduction on 50 and 10% (EC_{50}

and EC_{10}, respectively) were estimated via non-linear regression models, following Environmental Canada (2007). All statistical analyses were performed using the Statistica® 13.5.0.17 (TIBCO DATA SCIENCE, 2013). To detect significant differences among the EC values obtained for the different temperatures regimes/soil types a generalized likelihood ratio test (p<0.05) was applied (NATAL-DA-LUZ et al., 2011).

4.3 RESULTS

For all tests performed, all the validity criteria established by ISO 11267 (ISO, 2014) for the chronic ecotoxicological test with *F. candida* were met. In all tests, the adult survival was > 80%, the mean number of juveniles generated was > 100 and the coefficient of variation in control replicates was < 30% (Table 4.3).

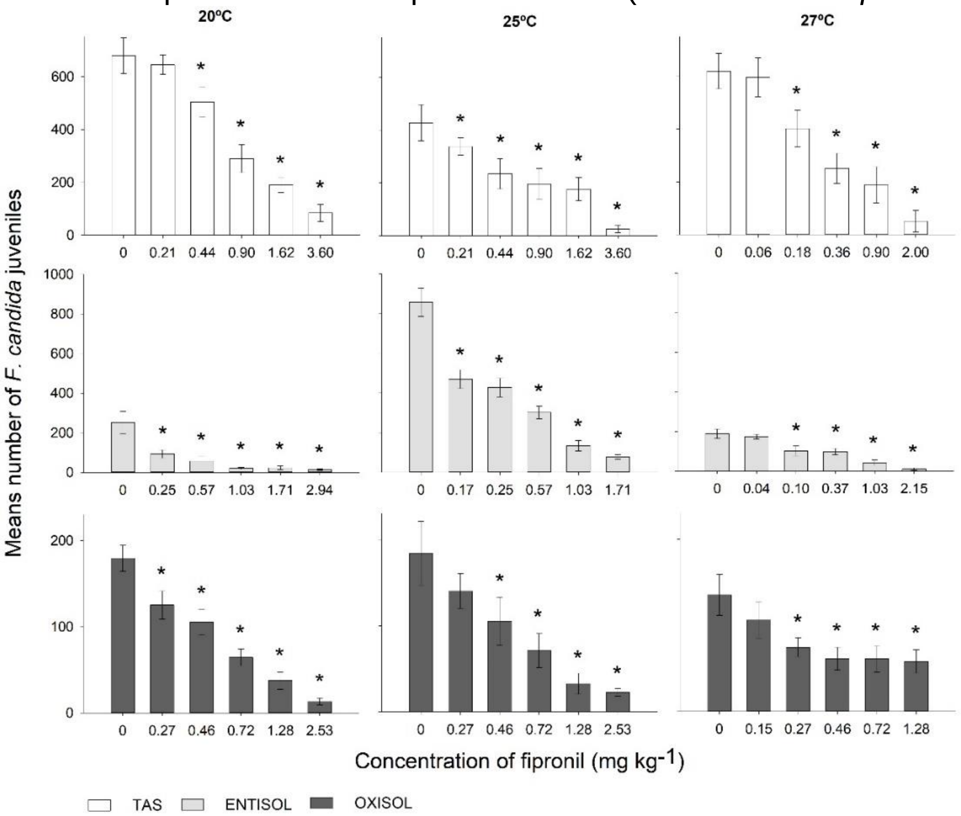
Table 4.3 - Validity criteria based on the mean number (± standard deviation) of juveniles and/or on adult survivors of *Folsomia candida* (n=5), as well as on the coefficients of variation (%) found in controls of the chronic tests performed in Tropical Artificial Soil (TAS), Entisol and Oxisol, under different temperature regimes (20, 25 and 27 ºC).

Validity criteria	TAS			Entisol			Oxisol		
	20 ºC	25 ºC	27 ºC	20 ºC	25 ºC	27 ºC	20 ºC	25 ºC	27 ºC
Survivors	9.6 ± 0.5	9.8 ± 0.4	10	10	9.8 ± 0.4	9.6 ± 0.5	9.8 ± 1.8	9.2 ± 0.8	9.2 ± 1.3
Juveniles	679 ± 61	428 ± 68	619 ± 67	251 ± 57	311 ± 66	188 ± 24	180 ± 15	184 ± 38	135 ± 24
Coeficient of variation	10%	16%	11%	23%	21%	13%	8%	20%	18%

ISO 11267:2014: adult mortality in the control ≤ 20%; juveniles per control vessel ≥ 100; coefficient of variation in the control ≤ 30%.

Increase fipronil concentrations caused a reduction in the number of *F. candida* juveniles in all tested scenarios (Figure 4.1), however, the intensity of the effect varied between the tested soils and/or temperatures.

Figure 4.1 - Mean number (± standard deviation, n = 5) of *Folsomia candida* juveniles generated after 28 days of exposure to increasing concentrations of fipronil in Tropical Artificial Soil (TAS), Entisol and Oxisol, under 20 ºC, 25 ºC and 27 ºC. Asterisk (*) indicates a significant reduction of juveniles in comparison to the respective control (*Dunnett's* test - $p \leq 0.05$).



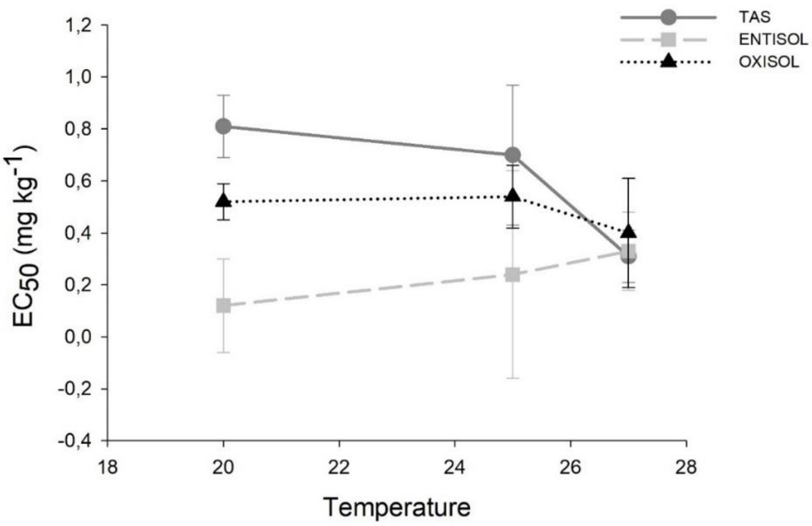
Source: prepared by the author (2021).

In TAS, the EC_{50} values (Table 4.4; Figure 4.2) didn't differ at 20 and 25 ºC, while a difference of almost 2.5 times were observed when comparing EC_{50} values at 25 and 27 ºC , which indicatethat the increase of temperature can enhance the toxicity of fipronil to collembolans in TAS. In Entisol, the toxicity (based on EC_{50} values) at 27 ºC was almost three times lower, when compared to 20 ºC, indicating that fipronil toxicity can decrease at a higher temperature. No differences between EC_{50} values at 25 and 27 ºC were found in Entisol. In Oxisol, the EC_{50} values estimated at 20 and 25 ºC didn't differ, while at 27 ºC the value was 1.3 times lower if compared to that obtained at the lowest temperatures. It reveals that in Oxisol andTAS, the fipronil toxicity was higher at the highest temperature.

Table 4.4 - Endpoint measurements (EC_{50} and EC_{10}, with respective 95% confidence intervals) of chronic toxicity assays with *Folsomia candida* exposed to increasing concentrations of fipronil in TAS, Entisol and Oxisol, under three temperature levels (20, 25 and 27 ºC). Different letters indicate significant differences between EC_{50} or EC_{10} from different temperatures within the same soil.

Soil type	Endpoints	Temperature		
		20 ºC	25 ºC	27 ºC
TAS	EC_{50}	0.81 (0.69 - 0.93)a	0.70 (0.43 – 0.96)a	0.31 (0.21 – 0.40)b
	EC_{10}	0.20 (0.12 - 0.27)a	0.08 (0.00 – 0.16)b	0.05 (0.01 – 0.08)b
Entisol	EC_{50}	0.12 (0.03 - 0.21)a	0.24 (0.20 - 0.29)b	0.33 (0.18 – 0.48)b
	EC_{10}	0.02 (0.00 - 0.04))a	0.01 (0.00 - 0.02)a	0.02 (0.00 – 0.04)a
Oxisol	EC_{50}	0.52 (0.45 – 0.59)a	0.54 (0.42 - 0.70)a	0.40 (0.19 - 0.61)b
	EC_{10}	0.12 (0.08 - 0.16)a	0.12 (0.05 - 0.20)ab	0.05 (0.00 – 0.10)b

Figure 4.2 - EC_{50} values (± 95% confidence intervals) estimated from chronic toxicity tests performed with *Folsomia candida* in Tropical Artificial Soil (TAS), Entisol and Oxisol, at 20, 25 and 27 ºC.



Source: prepared by the author (2021).

TER values (Table 4.5) indicated risk of fipronil for *F. candida* in TAS at 25 and 27 ºC. In Entisol, there was significant risk regardless of temperature. In Oxisol, risk was found only at 27 ºC.

Table 4.5 - Toxicity-Exposure Ratio (TER) values calculated for the exposure of *Folsomia candida* to the time-weight average predicted environmental concentrations of fipronil 28 days after seed planting ($PEC_{TWAC-28d}$) in Tropical Artificial Soil (TAS), Entisol and Oxisol at 20, 25 and 27 ºC.

Soil type	20º C		25 ºC		27 ºC	
	PECtwa 28d	TER	PECtwa 28d	TER	PECtwa 28d	TER
TAS	0.0198	10.08	0.0170	**4.715**	0.0165	**3.036**
Entisol	0.0132	**1.512**	0.0113	**0.884**	0.0110	**1.821**
Oxisol	0.0198	6.048	0.0170	7.073	0.0165	**3.036**

TER values lower than 5 (**in bold**) indicate significant/unacceptable risk.

4.4 DISCUSSION

Regardless of the temperature and soil tested, fipronil proved to be toxic to *F. candida* even at low concentrations, revealing a decrease in collembolans reproduction with increasing concentration (Figure 4.1). Our results showed that a decrease of 50% of the organisms' reproduction (EC_{50}) may occur at concentrations of fipronil between 0.12-0.81 mg kg^{-1}, depending on the soil type and temperature tested. Others studies also reported EC_{50} values for the fipronil toxicity to *F. candida* varying between 0.12-0.79 mg kg^{-1} (SAN MIGUEL et al., 2008; ALVES et al., 2014; ZORTÉA et al., 2018a), corroborating our results. According to the literature, the fipronil toxicity occurs due its directly action on the nervous system, which results in a loss of neuron signaling control and an interruption of system function by hyperexcitation (COLE et al., 1993; TINGLE, 2003). Besides that, fipronil can affect the reproduction of collembolans through deregulating the endocrine system associated with the sexual maturation of organisms (GAERTNER et al, 2011; CARY et al, 2004).

Our work hypothesis was partially confirmed in this study, since highest fipronil toxicity to collembolans was observed temperatures were increased in Oxisol and artificial soil, according EC_{50} values (Table 4.4; Figure 4.2). In TAS and Oxisol the fipronil toxicity increased with increased temperature, while in Entisol an opposite behavior to fipronil toxicity was observed, since EC_{50} values revealed a lower toxicity at higher temperature in comparison with others tested temperatures.

Although studies relating the fipronil toxicity to collembolans with an increase in temperature have not been found, some studies verified that collenbolans may be lower tolerance to the exposure to contaminated soils at high temperatures. According Snider and Butcher (1973), in a scenario of disturb in the environment (e.g. contamiantion of soil), collembolans can have their reproductive functions affected at temperatures from the 26 ºC. Bandow et al, (2014b) and Jegede et al. (2017) performed studies evaluating the toxicity of a fungicide and organophosphates to collembolans, respectively, at temperatures of 20 and 26 ºC, and concluded that increased on temperature enhanced its toxicity of chemicals to collembolans, corroborating our results obtained in TAS and Oxisol.

At high temperatures, like above 25 ºC, collembolans can suffer with several physiological alterations, such as damage to integral proteins, DNA and at the membranes permeability (HOCHACHKA and SOMERO, 2002; SULMON et al., 2015), and also with disturbance of da ATP-generation, that results in perturbation of homeostasis (WAAGNER et al., 2010). According Holmstrup et al. (2010), the effects by increased on temperature may be enhanced if soil invertebrates are exposed high temperature and pesticides simultaneously. Is possible that organisms allocated more resources to regulate their metabolism, trough detoxification mechanisms, to compensate the disturbance by high temperatures (WALKER et al., 2001), may compromise their growth and reproduction (HOFFMAN et al., 2003). This may be the main reason for the higher fipronil toxicity to *F. candida* in TAS and Oxisol at 27ºC, compared to 25 and 20ºC (Figure 4.2).

Among the factors influencing the toxicity and bioavailability of pesticides on soil, the soil adsorption properties are stands out (DOMENE et al., 2012). This may help to explain the lower fipronil toxicity to collembolans observed in Entisol at the highest temperature. In the soil, fipronil has a high affinity to OM and clay particles (MANDAL and SINGH, 2013). Unlike TAS and Oxisol, that have good adsorptive properties due to clay minerals and silt content, Entisol has lower bindings sites (SHARMA et al., 2013), in function mainly of it sandy texture (Table 4.1), showing poor properties adsorptives not favorable to retain fipronil, and allowing that a.i. become more bioavailable in soil pores.

Some studies have reported that sorption (by Freundlich coefficient - K_f) and persistence (by half-life values) of fipronil tend to be lower in sandy soils (e.g. Entisol), when compared to soils with higher levels of clay or silt, and with higher OM contents (MANDAL and SINGH, 2013; SPOMER et al., 2009; DORAN et al., 2006). Consequently, sandy soils provide conditions for greater bioavailability of pesticides in soil pores (SHARMA et al., 2013), where is more susceptible to losses by chemical degradation processes by high temperatures. The increase of temperature in addition to provide the desorption of pesticide of the soil matrix, increase the microbial activity and the action of catalytic substances, that works in degradation process of the chemicals (NAVARRO et al., 1992). In this way, due to a possible higher and rapid degradation at the highest tested temperature (RÖMBKE et al., 2007), it is expected lower availaible concentrations of fipronil had for collembolans uptake in Entisol, resulting in lower toxicity at a higher temperature (Table 4.4). Corroborating with our find, Jegede et al. (2017) verified that deltamethrin toxicity to *F. candida* in an artificial soil (Sandy Loam Soil) was twice less at 26 ºC than effects observed at 20ºC. Römbke et al. (2007) and Daam et al. (2020) also verified a similar behavior to toxicity of benomyl and carbendazim, respectively, for *Eisenia fetida* in standard soils (LUFA, TAS and OECD), showing a descrease in toxicity at higher temperatures (28 ºC – RÖMBKE et al., 2007; 26 ºC – DAAM et al., 2020) in comparison with observed at 20 ºC. All these studies attributed their results to degradation like factor of cause of decrease toxicity of molecules at high temperatures, however, in present study for fipronil, this effect seems to be dependent on the soil texture.

For all tested soils, differences between EC_{50} values in each temperature can be attributed to soil type, however, these differences were more expressive in tests conduced at 20 and 25 ºC, since at 27 ºC EC_{50} values for all tested soils were very similar (Table 4.4; Figure 4.3). Bandeira et al. (2020) evaluated the influence of increase in temperature on toxicity of imidacloprid for *F. candida* and *E. andrei* in TAS, Entisol and Oxisol and found that influence of the soil type is overlaid by the higher temperature (28 ºC). However, for fipronil this effect has not been fully proven, whereas for TAS and Oxisol, the effect of high temperature was added to that of the contaminant, increasing its toxicity in these soils, while for Entisol, the increase in temperature possibly influenced the degradation of the pesticide, reducing its toxicity. Then, it seems that higher temperature prevails over the soil properties factor only to

TAS and Oxisol, since the fipronil toxicity and degradation was dependent of soil texture for Entisol.

Concentrations similar to those estimated ($PEC_{TWAC-28d}$) in the present study (Table 4.5) have been found in clay soil with peanut fields by Li et al. (2015). Ying and Kookana (2006) found reduction in fipronil concentrations from 0.57 to 0.01 mg kg^{-1}, 0.53 to 0.02 mg kg^{-1} and 0.49 to 0.04 mg kg^{-1} in Sandy, Loamy and Clay soils, respectively, after three years of application. Mandal and Singh (2013) found concentrations of 1.90 (± 0.25) mg kg^{-1} and 5.50 (± 0.36) mg kg^{-1} in a Sandy loam and in a Clay loam soil, respectively, after 120 d of application of 100 mg kg^{-1} of fipronil dose at laboratory conditions. These studies indicate that our PECs may be found in the environment, even at temperatures different from those tested in this study, presenting risk to soil organisms, such as collembolans, when exposed in soils contaminated with fipronil.

In Entisol, the influence of the temperature on risk was not clear, while in TAS and Oxisol contrasting influences on the risk for collembolans were observed, which is probably related to the soil texture (BANDEIRA et al., 2019). Our results showed risk in Entisol regardless of tested temperature, while in TAS risk was pronunciated at 25 and 27 ºC, and in Oxisol risk occurred only at 27 ºC. Therefore, the organisms when exposed to fipronil to this temperature may be subject to a risk, which may compromise the population under these exposure conditions.

Some studies reported the influence of temperature and soil type on risk of pesticides for collembolans, corroborating with our results. Bandeira et al. (2020) evaluating the influence of temperature of toxicity and risk of imidacloprid for collembolans in TAS, Entisol and Oxisol, found that risk is greater at higher temperatures, beeing more expressive in Entisol and Oxisol in comparison with TAS. Jegede et al. (2017) observed that risk of chlorpyrifos for collembolans in OECD soil (Sandy Loam Soil) was higher at 28 ºC than 20 ºC. Other study conductect by Bandeira et al. (2019) evaluated the risk of imidacloprid in contrasting soils and found that soil type influenced significantly on risk of pesticide to collembolans, beeing particularly hazardous in sandy natural soils, such as Entisol. Carniel et al. (2019) verificated the soil type influenced the toxicity and risk of mancozeb to collembolans, where Oxisol demonstrate higher toxicity for molecule than Ultisol, due higher CEC and clay content in the last. Hennig et al. (2020), evaluated the influence of the soil moisture regime on imidacloprid toxicity and observed that soil texture can play important role on risk of pesticide for collembolans, where Entisol spiked with imidacloprid play risk for collembolans regardless soil moisture content, while in Oxisol the a.i. toxicity seems to be influencied by soil moisture.

Based in risk assessment (Table 4.5), is predicted that there may be a potential risk for collembolans from the application of a single dose of fipronil, may be vary with soil type and enhanced with increase in temperature regime, confirming our hypothesis. However this context can be even more severe, since in tropical contries, such as Brazil, that in addition to suffering from the increase in atmospheric temperature, the pesticide application occur in higher doses and applications (RERKASEM, 2005) may resulting in a greater pesticide exposure and hence risk, drawing attention to the importance of further studies.

4.5 CONCLUSIONS

Our results showed that increases in atmospheric temperatures can increase the toxicity of fipronil for collembolans, reducing their reproduction in soils with high clay and/or silt contents, while in sandy soil there may be a decrease in the toxicity of fipronil. The risk assesment for fipronil, based in a single application of commercial formulation (Shelter®), demonstred risk for collembolans at higher temperature, regardless the soil type, may pose a risk to collembolans in the field after lowest recommended dose for pasture cultivation.

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5 INFLUENCES OF CLIMATIC FACTORS ON THE TOXICITY OF FIPRONIL TO *Eisenia andrei* IN TWO SUBTROPICAL SOILS

ABSTRACT

This study aimed to assess how the increase in temperature may affect the fipronil toxicity and risk for earthworms *Eisenia andrei* in two Brazilian soils with contrasting textures (Oxisol and Entisol) and how the decrease in soil moisture content can influence fipronil toxicity in a sandy soil (Entisol). Earthworms were exposed for 56 d in soils spiked with increasing concentrations of fipronil under scenarios with different combinations of increased temperatures (20, 25 and 27 ºC) and soil moisture contents (60 and 30% of water holding capacity – WCH for Entisol; and only at 60% WHC for Oxisol). The number of generated juveniles was taken as the endpoint and a risk estimation was performed based on the Hazard Quotient (HQ) approach. In Entisol, at 60% WHC the influence of temperature on fipronil toxicity for earthworms was not clear. In Entisol at 30% WHC fipronil toxicity increased with increased of temperature (EC_{50} = 29.56, 7.84, 15.74 mg kg^{-1}, to 20, 25 and 27ºC, respectively). In Oxisol at 60% WHC, the fipronil toxicity increased at 27 ºC compared with 20 and 25 ºC (EC_{50} = 26.38, 239.17, 218.86 mg kg^{-1}, respectively). The fipronil toxicity increased in Entisol at 30% WHC compared with 60% WHC at 25 (EC_{50} = 7.84 and 30.07, respectively) and 27 ºC (EC_{50} = 15.74 and 57.53 mg kg^{-1}, respectively). Even that risk (TER) of fipronil was not significant in all exposure scenarios,this study showed that increase in temperature, as well as the reduction of soil moisture, can increase fipronil toxicity for earthworms, but this influence can vary according to the properties of the soil where the exposure occurs.

Keywords: Terrestrial ecotoxicology; *Eisenia andrei*; Soil type; Soil moisture regime; Temperature.

5.1 INTRODUCTION

According to the Intergovernmental Panel on Climate Change (IPCC), if greenhouse gas emissions are not controlled, increases in global temperatures, as well as the occurrence of extreme weather events, are expected for next years (IPCC, 2019). In Brazil, despite the predicted consequences of climate changes vary for different regions, in general, until the end of the century is expected a 5 ºC increase in atmospheric temperature and reductions by about 50% in rainfall patterns, which increases the probability of extreme and prolonged droughts events (PBMC, 2014).

Climate change consequences, especially those related to temperature and precipitation, are considered an imminent problem due to long-term impacts on species that inhabit the Earth (NOYES et al., 2009). These changes can affect the physical, chemical and biological properties of the soil ecosystem, and also can affect the structure and composition of the edaphic community through disorders on the metabolism of soil organisms, which can impair their growth and reproduction, making them susceptible to additional environmental disturbances, such as soil contamination by pesticides (NOYES et al., 2009).. In this sense, is expected that the increase of temperature and the reduction in rainfall patterns might enhance the risk of toxic substances present in the soil (GONZÁLEZ-ALCARAZ et al., 2015).

In soils, earthworms contribute to the decomposition of organic matter, nutrient cycling, soil genesis and, due to their high exposure and sensitivity, are suitable bioindicators to contaminants and environmental stresses (EDWARDS et al., 2004; HACKENBEGER et al., 2018). However, their exposure at high temperatures may cause a reduction in activities of detoxification enzymes (HACKENBERGER et al., 2018), as well changes in the permeability of cellular membranes (DAI et al., 2019). Earthworms are also vulnerable to reductions in soil moisture contents because they are soft-bodied organisms with highly permeable skin (PEIJNENBURG et al., 2012). However, to date, only a few studies have demonstrated how increases in temperatures and reductions in soil moisture content can affect earthworms performance in natural soils polluted by pesticides and how their toxicity may be influenced (e.g. LIMA et al., 2015; NUNES et al., 2016; HACKENBERGER et al., 2018; BANDEIRA et. al., 2020).

Fipronil is a phenylpyrazole pesticide widely used, with sales of 2 thousand tons in 2019 in Brazil (IBAMA, 2019). Although the application doses suggested by the manufacturer's for some commercial formulations are relatively low, the lack of training for pesticide application (WAICHMAN et al., 2007) and the accumulation, due to a continuous input, may result in greater a.i. residues in the soil, which may impact non-target organisms (DAAM et al., 2019). The ecotoxicological effects of fipronil on survival, reproduction and growth for different soil organisms are reported in the literature (e.g. SAN MIGUEL et al., 2008; ALVES et al., 2014; QIN et al., 2014; QU et al., 2014; ZORTÉA et al., 2018a; ZORTÉA et al., 2018b) and reveal that this molecule can cause population imbalances for different species. However, to date, the effects of fipronil as seeds treatment based associated with abiotic and climatic factors, especially for earthworms, were not found in the literature.

To help in this knowledge gap this study assessed how temperature increases and the reduction in soil moisture content influence toxicity and risk of fipronil-polluted tropical soils to the soil-dwelling species *Eisenia andrei*. For this, bioassays were conducted in two contrasting natural soils (Entisol and Oxisol) with different combinations of temperatures (20, 25 or 27 ºC) and soil moisture contents (60 or 30% of water holding capacity - WCH for Entisol; only 60% WHC for Oxisol), and the number of generated juveniles by the earthworms was used as endpoint. We hypothesized that increasing temperature and decreasing soil moisture content enhance the toxicity of the fipronil to *E. andrei* in tropical soils.

5.2 MATERIAL AND METHODS

5.2.1 Tests soils

Two Brazillian subtropical soils, an Entisol and an Oxisol, were sampled in the municipalities of Araranguá (29º00′S 49°31′W) and Palmitos (27º04'S 53º 09'W), respectively, in the soil top layer (0-20 cm), in areas without historic of contamination. The sampled soils were sieved (2 mm), defaunated according to Alves et al. (2019), air-dried and kept protected from light at room temperature. Soil characteristics were described in Table1, where WHC and pH (1M KCl) were measured following ISO 11267 (ISO, 2014), and the cation exchange capacity (CEC), soil organic matter (SOM), sand, clay and silt contents were measured following Tedesco et al. (1995).

Table 5.1 - Mean values (± standard deviation; n = 2) of physical and chemical characteristics of the tropical Entisol and Oxisol used in the ecotoxicological tests.

Parameter	Entisol	Oxisol
pH (1M-KCl)	4.2 ± 0.2	5.5 ± 0.1
SOM (%)	2.2 ± 0.1	4.7 ± 0.1
CEC ($cmol_c$ dm^{-3})	1.4 ± 0.1	18.3 ± 2.8
WHC (%)	31.6 ± 1.1	58.8 ± 2.2
Sand (%)	93.8 ± 0.4	28.6 ± 0.7
Clay (%)	4.1 ± 0.2	33.0 ± 0.0
Silt (%)	2.1 ± 0.1	38.4 ± 0.7
Soil texture	Sandy	Clay loam

SOM: Soil Organic Matter;
CEC: Cation Exchange Capacity;
WHC: Water Holding Capacity

5.2.2 Test species

Bioassays were performed using laboratory cultured individuals of the species *E. andrei* (Lumbricidae, Oligochaeta), obtained from Minhobox® Corporation, Minas Gerais State, Brazil. The organisms were maintained at a room with a temperature of 20 ± 2 ºC and 12h-photoperiod (ISO, 2012), in boxes with a moisturized substrate composed of dafauned horse manure (free of contaminants), coconut fiber and sand in the proportion of 70:20:10 (w:w:w), respectively. Once a week, the earthworms received cooked oatmeal as food and distilled water was used to adjust the substrate moisture.

5.2.3 Test substance

Ecotoxicological assays were performed using the commercial formulation Shelter®, an insecticide used for chemical treatment seeds, which contains 250 g of fipronil L^{-1} as an active ingredient (a.i.). Test soils were spiked with increasing concentrations of the a.i. (3; 9; 29; 72; 180 mg kg^{-1} for Entisol; 4.5; 13; 39; 117; 350 mg kg^{-1} for Oxisol), chosen from range-finding tests (data not shown). A control treatment was performed by using only distilled water.

The spiking was performed via an aqueous solution, with calculated volumes to reach 30 and 60% WHC in Entisol, and only 60% WHC in Oxisol, because the species did not reproduce at 30 and 45% WHC in this soil (data not shown). Soil moisture content and pH were checked at the beginning and the end of each bioassay (Table 5.2).

Table 5.2 - Means (± standard deviation) of soil moisture (% WHC) and pH at the start and end of the chronic toxicity assays with *Eisenia andrei* in Entisol and Oxisol at 20, 25 and 27 °C, under 30 and 60% WHC.

		30% WHC				60% WHC			
	Soil	Soil moisture (% WHC)		pH		Soil moisture (% WHC)		pH	
Temperature	type	Start	End	Start	End	Start	End	Start	End
20 ºC	Entisol	34.1 ± 13.82	31.8 ± 1.19	4.18 ± 0.02	4.41 ± 0.19	59.5 ± 0.99	61.3 ± 3.23	4.42 ± 0.07	4.43 ± 0.16
	Oxisol	(-)[a]	(-)[a]	(-)[a]	(-)[a]	64.6 ± 11.04	66.4 ± 1.50	5.67 ± 0.03	5.20 ± 0.01
25 ºC	Entisol	26.8 ± 0.62	32.8 ± 2.55	4.06 ± 0.03	4.08 ± 0.04	56.8 ± 0.62	65.9 ± 19.4	4.04 ± 0.01	4.04 ± 0.02
	Oxisol	(-)[a]	(-)[a]	(-)[a]	(-)[a]	58.2 ± 1.88	65.0 ± 1.40	5.52 ± 0.08	5.32 ± 0.09
27 ºC	Entisol	27.5 ± 0.63	29.14 ± 1.15	4.15 ± 0.04	4.22 ± 0.05	56.3 ± 5.85	57.32 ± 4.29	4.15 ± 0.02	4.25 ± 0.04
	Oxisol	(-)[a]	(-)[a]	(-)[a]	(-)[a]	57.3 ± 0.98	61.7 ± 1.60	5.60 ± 0.05	5.19 ± 0.04

[a] - Assay not performed

5.2.4 Estimation of PEC and Risk Assessment

The Predicted Environmental Concentrations after 28d (PEC) were calculated according to the European and Mediterranean Plant Protection Organization (EPPO, 2003 by using the software ESCAPE (2013). It was considered a scenario with soybean crop, and soil densities of 1.5 and 1.0 g cm^{-3} for Entisol and Oxisol, respectively. It was considered the worst-case scenario of Shelter® application, at a sowing density of 60 kg of seed per ha (EMBRAPA, 1988), with a higher recommended dose for soybean crop (37.5 g a.i. per 60 kg of seeds by manufacturer's recommendation), with 5% of interception by plants, during only one planting cycle. Values of dissipation half-life (DT_{50} values) of 68 d (YING & KOOKANA, 2002), 31 d (EFSA, 2006) and 28 d (SHUAI et al., 2012) were assumed to increase temperatures of 20, 25 and 27 ºC, respectively.

The Toxicity-Exposure Ratio (TER) approach was used to estimate the risk for *E. andrei*. For each soil and temperature scenario, TER value was the ratio between the estimated fipronil concentrations that reduced collembolans reproduction by 10% (EC_{10}) and the $PEC_{TWAC\text{-}28d}$ (TER = $EC_{10}/PEC_{TWAC\text{-}28d}$). When TER was lower than the trigger value of 5, a significant risk was assumed (EC, 2002).

5.2.5 Chronic toxicity assays

The toxicity assays were performed according to ISO 11268-2 (ISO, 2012), where organisms were exposed to the concentrations of fipronil in two soil types (Entisol and Oxisol), two different soil moisture contents in Entisol (30 and 60% WHC) simulating different scenarios of water availability. In Oxisol assays were performed only at 60% WHC. All the assays were performed at the temperatures of 20, 25 and 27 ºC, simulating different scenarios of global warming.

The experimental units consisted of plastic containers (15 cm diameter and 10 cm height) with perforated lids (to allow gas exchanges), where about 650 g of wet soil (control or spiked with concentrations) were added. Ten grams of horse manure moisturized with 20 mL of distilled water were also offered as food at the start of the tests. Ten earthworms with known individual weight (250 - 600 mg; Table 5.3) were inserted in each replicate. Four replicates were performed for each treatment. Once a week, the soil moisture was adjustedwith distilled water (weight-based), and horse manure was added as food (consumption-based). After 28 d from the beginning of the test, surviving adult earthworms were removed. During the next 28 d, only the soil, and generated cocoons and juveniles remained in the containers. At the end of the test (56 d), the experimental units were immersed in a water bath (60 ± 5 º C) for one hour, and the *E. andrei* juveniles were manually counted.

Table 5.3 - Means (n=480; ± standard deviation) of the initial weight (mg) of *Eisenia andrei* earthworms used in the chronic toxicity assays, mean number (± standard deviation) of *Eisenia andrei* adult and juveniles survivors (n=4) and coefficients of variation (%) found in controls of the chronic toxicity tests performed in Entisol and Oxisol at 20, 25 and 27 °C, under 30 and 60% WHC.

Temperature	parameter	Entisol		Oxisol
		30% WHC	60% WHC	60% WHC
20 ºC	Initial weight[a]	367 ± 28.9	366 ± 31.9	370 ± 28.7
	Survivors[b]	10.0 - 0.0	10.0 - 0.0	10.0 - 0.0
	Juveniles[b]	99.5 - 10.8	140 - 5	105 - 14.3
	Coeficient of variation[b]	11%	3%	14%
25 ºC	Initial weight[a]	383 ± 29.6	371 ± 23.1	384 ± 21.4
	Survivors[b]	10.0 - 0.0	10.0 - 0.0	9.75 - 0.5
	Juveniles[b]	65.5 - 9.3	132.5 - 9.2	107.2 - 12.3
	Coeficient of variation[b]	14%	7%	11%
27 ºC	Initial weight[a]	416 ± 21.5	412 ± 25.38	358 ± 33.5
	Survivors[b]	10.0 - 0.0	10.0 - 0.0	9.75 - 0.5
	Juveniles[b]	81.7 - 9.2	118.2 - 16.9	63.2 - 17.8
	Coeficient of variation[b]	11%	14%	28%

[a] - ISO 11268-2: The weight of earthworms must be between 250 - 600 mg.
[b] - ISO 11268-2 validity criteria: adult mortality in the control ≤ 20%; juveniles per control vessel ≥ 30; coefficient of variation in the control ≤ 30%.

5.2.6 Data analysis

The homoscedasticity and normality of the data were tested via the *Bartlett* and *Kolmogorov-Smirnov* tests, respectively. Logarithmic transformations was applied only to the data obtained for Oxisol at 20 ºC, to achieve the assumptions.. Significant differences ($p < 0.05$) between treatments were tested using ANOVA. Factorial

ANOVA was applied for evaluate the interaction of fipronil concentrations, temperature and soil moisture on fipronil toxicity for earthworms, and a generalized linear model (GLM) was applied to data to estimate the percentage of contribution of the same factors on toxicity effect observed. Effect concentrations causing a reduction of 10 or 50% in the species reproduction (EC_{10} or EC_{50}, respectively) were estimated by using non-linear regression models, according to Environment Canada (2007). All statistical analyzes were performed using Statistica® version 13.5.0.17.

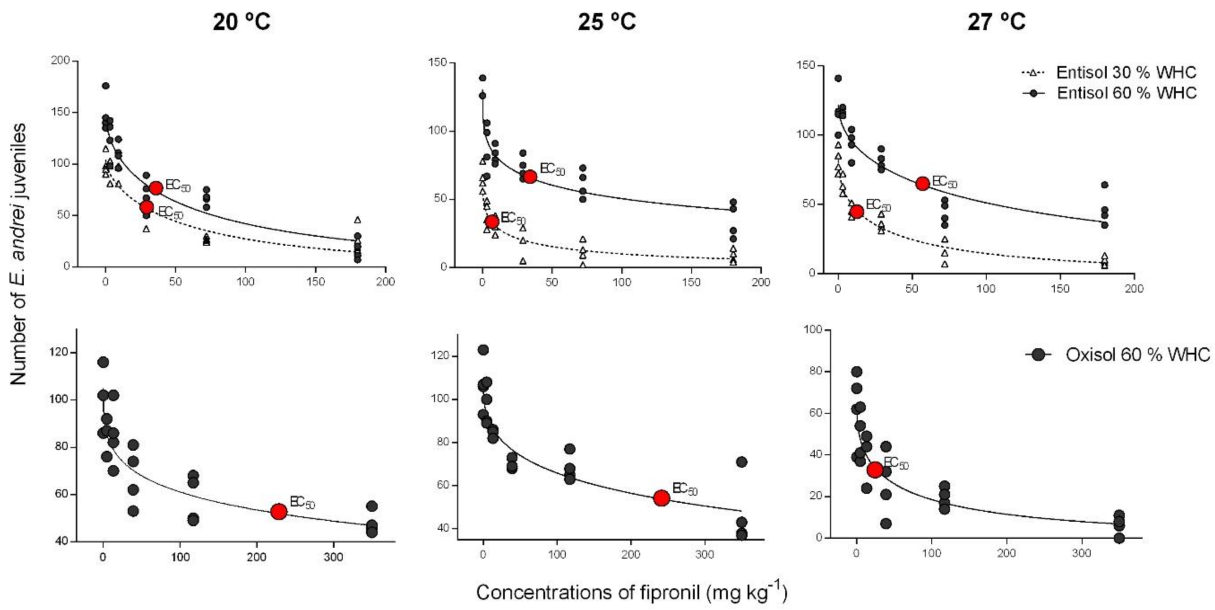
To assess the significant differences between EC values for soils (Entisol *vs* Oxisol) at 60% WHC, temperatures (20 *vs* 25 *vs* 27 ºC) or between soil moisture contents for Entisol (30 *vs* 60 % WHC), a generalized likelihood ratio test ($p <0.05$) as used following Natal-da-Luz et al. (2011).

5.3 RESULTS

All validity criteria for chronic ecotoxicological tests with earthworms (ISO, 2012) were met (Table 5.3).

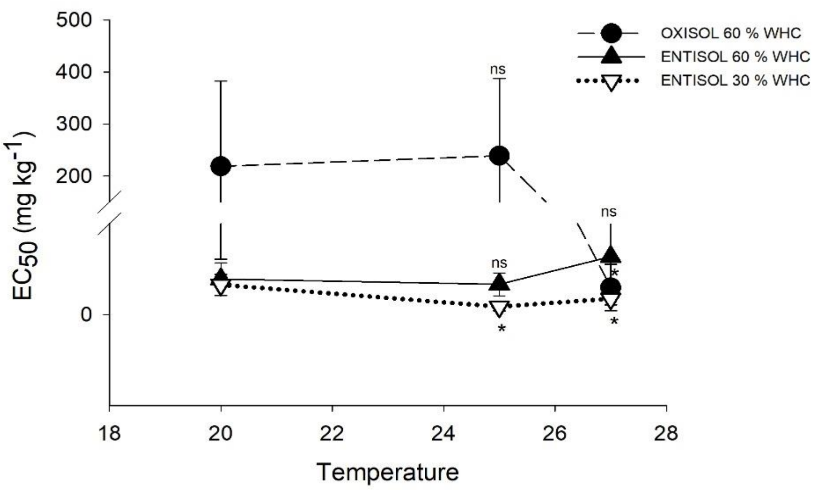
In all exposure scenarios, there was a decrease of *E. andrei* juveniles with increasing fipronil concentrations (Figure 5.1). The a.i. toxicity (EC_{50} based) for earthworms was higher in Entisol, compared to Oxisol , being this difference significant only at 20 and 25 °C (Figure 5.2; Table 5.4).

Figure 5.1 - Dose-effect response curves representing the effects of fipronil on the reproduction of *Eisenia andrei* exposed for 56 days to increased concentrations of fipronil in Entisol (30 and 60% WHC) and Oxisol (60% WHC) at 20, 25 or 27 ºC.



Source: prepared by the author (2021).

Figure 5.2 - EC_{50} values (± 95% confidence intervals) estimated from chronic toxicity tests performed with *Eisenia andrei* in Entisol (30 and 60% WHC) and Oxisol (60% WHC) at 20, 25 or 27 ºC. Asterisk (*) indicate a significant difference between EC_{50} within the same soil and moisture regime, and 'ns' indicate no significant difference, in relation to (likelihood ratio test - $p \leq 0.05$).



Source: prepared by the author (2021).

Table 5.4 – Ecotoxicological parameters (EC_{50} and EC_{10}, with 95% confidence intervals) from chronic toxicity assays with *Eisenia andrei* exposed to increasing concentrations of fipronil in Entisol (30 and 60% WHC) and Oxisol (60% WHC) at 20, 25 and 27 ºC. Different lowercase letters indicate significant differences between EC_{50} or EC_{10} from different temperatures within the same soil; Different capital letters indicate significant differences between EC_{50} or EC_{10} from different soil moisture in Entisol within the same temperature.

	Fipronil concentration (mg kg^{-1})			
Soil and moisture content	Ecotoxicological parameter	Temperature		
		20 ºC	25 ºC	27 ºC
Entisol 30% WHC	EC_{50}	29.56aA	7.84cB	15.74bB
	Limits (95%)	(19.02 - 40.10)	(3.79 - 11.90)	(9.87 – 21.62)
	EC_{10}	2.04aA	0.18aA	0.96aA
	Limits (95%)	(-)[a]	(-) [a]	(0.09 – 1.83)
Entisol 60% WHC	EC_{50}	35.26aA	30.07aA	57.53aA
	Limits (95%)	(18.96 - 51.55)	(18.76 - 41.37)	(24.12 – 90.94)
	EC_{10}	2.05aA	0.13aA	1.87aA
	Limits (95%)	(-)	(-)[a]	(-)[a]
Oxisol 60% WHC	EC_{50}	218.86a	239.17a	26.88b
	Limits (95%)	(54.71 - 383)	(90.35 - 387.10)	(3.85 - 49.90)
	EC_{10}	3.09a	1.91a[a]	1.24a
	Limits (95%)	(-)[a]	(-)[a]	(-)[a]

[a] Confidence limits (95%) could not be estimated.

Soil moisture content affected the reproduction of earthworms in Entisol, revealing a poor reproductive performance in controls replicates at 30% WHC in comparison with 60% WHC, regardless of temperature content (Figure 5.1; Table 5.3). Fipronil toxicity (EC_{50} based) was higher under the dry condition (30% WHC) at 25 and 27°C, while at 20 ºC the soil moisture did not significantly influence the effect (Table 5.4; Figure 5.2).

According to the factorial ANOVA results, the juveniles generation in Oxisol was influenced by concentrations of fipronil and temperature in isolation, while in Entisol the analysis indicated the interaction between a.i. concentrations, temperature and soil moisture on reproduction of earthworms (Table 5.5).

Table 5.5 - Results from a factorial ANOVA on the effects of temperature, soil moisture, fipronil concentration and their interaction on the toxic effects on *Eisenia andrei* in Entisol and Oxisol at 20, 25 or 27 °C.

Soil	Factor	SS	df	Mean squares	F	*p*
Oxisol	Intercept	270643.1	1	270643.1	2202.704	0
	Concentration	26028.9	5	5205.8	42.369	0
	Temperature	28768.2	1	14384.1	117.069	0
	Concentration x Temperature	391	10	39.1	0.318	0.972834
	Error	6389.2	52	122.9		
Entisol	Intercept	566975.5	1	566975.5	4925.717	0
	Concentration	110601.6	5	22120.3	192.175	0
	Temperature	12281.4	2	6140.7	53.348	0
	Moisture	51874.1	1	51874.1	450.667	0
	Concentration x Temperature	8284.7	10	828.5	7.197	0
	Concentration x Moisture	3641.2	5	728.2	6.327	0.00037
	Temperature x Moisture	3488.8	2	1744.4	15.155	0.000002
	Conc x Temp x Moisture	3048.1	10	304.8	2.648	0.006477
	Error	11855.8	103	115.1		

SS: sum of squares
df: degrees of freedom
F: Significance factor
p: p <0.05 the difference is significant

According to GLM model (Table 5.6), the fipronil concentrations, moisture and temperature can play different influences on the number of juveniles generated for both soils. Comparing the models obtained for both soils, the reproduction of earthworms in Entisol seems to be twice as affected by the concentrations of fipronil, in comparison to Oxisol. Regarding temperature, the reproduction of earthworms in Entisol seems to be twice as affected in Oxisol, compared to Entisol. About the influence of moisture regime in Entisol, the higher the moisture, the trend is a greater generation of juveniles.

Table 5.6 - Generalized linear model (GLM) for the effects of fipronil concentration, temperature and moisture on *Eisenia andrei* reproduction in Entisol (30 and 60% WHC) and Oxisol (60% WHC) at 20, 25 and 27 °C.

Soil	Model	R^2
Entisol	Juveniles = 86.7844700 - (0.37665615 × Fipronil concentrations) - (2.5578166 × Temperature) + (1.26135746 × Moisture)	0.68
Oxisol	Juveniles = 181.727511 - (0.12642862 × Fipronil concentrations) - (4.5245980 × Temperature)	0.49

Based on the TER approach, fipronil not represented a risk for earthworms in all exposed scenarios, revealing TER > 5. (Table 5.7).

Table 5.7 - Toxicity-Exposure Ratio (TER) values calculated for the exposure of to the time-weight average predicted environmental concentrations of fipronil 56 days after seed planting ($PEC_{TWAC-56d}$) in Entisol (30 and 60% WHC) and Oxisol (60% WHC) at 20, 25 and 27 ºC.

Soil and moisture content	20 ºC			25 ºC			27 ºC		
	$PEC_{TWA\ 56d}$	EC_{10}	TER	$PEC_{TWA\ 56d}$	EC_{10}	TER	$PEC_{TWA\ 56d}$	EC_{10}	TER
Entisol 30% WHC	0.0362	2.04	56.33	0.0272	0.18	6.63	0.0258	0.96	37.25
Entisol 60% WHC	0.0362	2.05	56.61	0.0272	0.13	5.16	0.0258	1.87	72.56
Oxisol 60% WHC	0.0543	3.09	56.89	0.0407	1.91	46.89	0.0387	1.24	32.08

TER: EC_{10}/ $PEC_{TWA\ 56d}$

5.4 DISCUSSION

The generation of juveniles was not affected by the increases in temperature in this study, based on control replicates (Figure 5.1). Moreover, regardless exposure scenario, fipronil affected *E. andrei* reproduction (Figure 5.1). This effect can be explained by the bioactivity of the molecule on soil invertebrates, which involves the loss of neuronal signaling control and disturbance of the normal central nervous system (COLE et al., 1993).

Effects of fipronil on earthworms are reported on literature, and revealed that fipronil may affect growth (QIN et al.,2014), reproduction (ALVES et al., 2013; ZORTÉA et al., 2018) and survival (QU et al., 2014) of earthworms. Therefore, despite these studies being conducted in standard conditions of soil, temperature and moisture, reinforce our findings, indicating that fipronil may pose harmful effect to earthworm population in the soil.

About the influence of temperature on fipronil toxicity to earthworms in tropical soils, our hypothesis was confirmed, since that increase in temperature only reduced earthworms' reproduction in Oxisol at 60% WHC and in Entisol at 30% WHC (Table 5.4). A possible explanation for these results can be related with an additive effect of toxic effects caused by fipronil with the metabolic stress to organisms caused by increased on temperature. Similar results were found to imidacloprid (BANDEIRA et al., 2020), carbaryl (LIMA et al., 2009), carbofuran and chlorpyrifos (DE SILVA et al., 2009) for earthworms, where increases in temperature also increased the toxicity of pesticides. According do these authors, higher temperatures may increase the activity of earthworms, inducing a higher contact and uptake of chemicals into soil. Associated with this, the increase in temperature can promote the desorption of chemicals of the solid matrix of soil, making it more bioavailable to contact and uptake by soil organisms in soil pores (NAVARRO et al., 1992; BELFROID et al. 1994). Supporting our findings, the results of factorial ANOVA indicate that there was the influence of factors on toxicity process (Table 5.5). Also, through GLM results (Table 5.6), was observed a great impact on juveniles number as the factors chemichal stress (concentrations), temperature and soil moisture are combined. For Entisol, the increases in concentrations and temperature added with the reduction of soil moisture, should generate a smaller number of juveniles. For Oxisol, the model also indicated that an increase in fipronil concentrations added to increase in temperature can enhance the inhibition on reproduction.

In Entisol, under 60% WHC there was no clear influence of temperature. The GLM analysis (Table 5.6) showed that, regardless fipronil concentration and temperature, when moisture contents is higher the reproduction tends to increase. However, the factorial ANOVA (Table 5.5) showed that temperature interact with fipronil concentrations in the toxic effects. These observations indicate that may be occurred a increase on earthworms reproduction in Entisol spiked with fipronil under 60% WHC at 27 ºC. A possible explanation for this effect can be related to loss of a.i. on soil by degradation caused by high temperature associated with high moisture content. According to Domene et al. (2012), the bioavailability of pesticides on soil depends on adsorptive soil properties. Studies reported that sorption and persistence of fipronil are lower in sandy soils compared to fine-textured soils (MANDAL and SINGH, 2013; SPOMER et al., 2009; DORAN et al., 2006). These factors can have contributed to a higer bioavailability of fipronil in soil pores of Entisol and, associate with higher moisture and temperatures, fipronil being more susceptible to degradation (SCORZA JUNIOR and FRANCO, 2013). According to Navarro et al. (1992), higher temperatures provide the enhanced activities of microorganisms and catalytic substances degradation promoters of chemical substances. In addition, higher water contents in soil with lower WHC, such as Entisol, can accelerate the fipronil degradation process due to breaking the molecule by hydrolysis processes (TINGLE, 2003). Although the present study did not present fipronil degradation data for the respective contamination scenarios and climatic factors,tis possible that this effect occurred, explaining the high EC_{50} value for Entisol at 27 ºC under 60% WHC.

The soil moisture content also influenced fipronil toxicity in Entisol, and also affected the reproduction performance of earthworms in this soil, regardless of the temperature (Figure 5.1). The reduction in soil moisture content can reduce the extent of water films around soil particles, namely hygroscopic water (COLEMAN et al., 2004). This aspect may be affected earthworms in the soil by make harder the search for food, favorable microhabitats and hatching of cocoons, affecting also survival and reducing growth and reproduction of organisms (SINGH et al., 2019). In this study, for Entisol at 27 ºC, dry condition revealed significantly higher a.i. toxicity in comparison with the standard soil moisture (EC_{50} based; Table 5.4). Effects of increased toxicity with reduced soil moisture were reported to fluoranthene (LONG et al., 2009), carbaryl (LIMA et al., 2011) and propiconazole (Hackenberger et al., 2018) for earthworms. According to Lima et al. (2011), dehydration due to drought conditions can enhance concentrations of a.i. into earthworms' bodies, increasing the toxicity of pollutants. Moreover, when combined high temperatures with drought conditions, the toxicity of chemicals on poikilothermic organisms, such as earthworms, may be enhanced due to the greater uptake rates of substance at higher temperatures (DONKER et al., 1998; ŠUSTR and PIŽL, 2010; GONZÁLEZ-ALCARAZ and VAN GESTEL, 2016), corroborating our findings.

In addition to temperature and moisture regime, characteristics of soil play important role on toxicity of pesticides for soil organisms, as above mentioned. In both test soils under 60% WHC, at 20 and 25 ºC fipronil was more toxic to earthworms in Entisol compared to Oxisol. This one, due to its high clay, amounts of iron oxy-hydroxides and SOM content, make fipronil less bioavailable for earthworms, compared to Entisol, which has poor adsorptive capacities, like above mentioned (Table 5.1; MASSUTI and MERMUT, 2007; MANDAL and SINGH, 2013; SINGH et al., 2014). However, at 27 ºC, EC_{50} for both soils at 60% WHC was not different (Table 2; Figure 5.4). This effect demonstrates that, at higher temperatures, the soil type not play a relevant role in the toxicity of fipronil to earthworms, as by Bandeira et al. (2020).

The PEC values (Table 5.7) calculated indicated that in a worst exposure scenario, residues of fipronil between 0.02 - 0.05 mg kg^{-1} may be present in the soil, even after 56 d of application. Some studies indicated that these concentrations may be realistic since were found concentrations of 0.003 to 0.15 mg kg^{-1} in peanut field (LI et al., 2015), 0.011 - 0.155 mg kg^{-1} in cotton field (CHOPRA et al., 2011) and 0.01 to 2.06 mg kg^{-1} in other agricultural fields (YING and KOOKANA, 2006).

However, risk, by TER approach, was not observed on evaluated scenarios. Probably because the EC_{10} values of fipronil to earthworms were high (0.13 - 2.05 mg kg^{-1}; Table 5.4), compared to the estimated PEC and with concentrations found in the environment reported in the literature, resulting in no potential environmental risk for earthworms, from the application of fipronil to natural tropical soils, regardless climatic scenario.

5.5 CONCLUSIONS

The present study showed that: 1) Higher temperatures increased toxicity of fipronil to earthworms in Oxisol and in dry Entisol, while in wet Entisol, the influence of temperature was not clear; 2) The reduction in soil moisture increased the toxicity of fipronil to earthworms only at highest temperature; 3) The risk of fipronil to earthworms occurred in all exposure scenarios, however, in Oxisol the risk increased with increase on temperature, while in Entisol the influence of temperature, and also moisture regime, was not clear. Our findings reinforce the need to include abiotic and climatic factors in risk and ecotoxicological assessments of pesticides, such as different soil types and different temperature and moisture scenarios.

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6 GENERAL DISCUSSION AND CONCLUSIONS

Based on results showed in all experiments of this study, it was possible to confirm that the presence of fipronil as a seed dressing formulation in tropical soils may negatively affect the non-target edaphic organisms. Fipronil has shown greater toxicity to collembolans than in earthworms. The influences of the decreases in soil moisture content and increases in atmospheric temperature on the fipronil toxicity also were more representative to collembolans than earthworms.

The first experiment (Section 3) showed that fipronil toxicity for collembolans depends on soil type, being higher in Sandy soil, in comparison with Sandy Loam Soil and Clay Loam Soil, and these effects are probably due to the adsorptive soil properties, that regulate the pesticide bioavailability for soil organisms. Based on this inference, it is considered that soil type is an important attribute in assessing pesticide toxicity and should not be neglected by Brazilian regulatory agencies. In addition, the experiment showed that the reduction moisture increased the fipronil toxicity for collembolans reproduction in Sandy Loam Soil and Clay Loam Soil, but decreased the toxicity in Sandy Soil, also due to the adsorptive soil properties relative to clay and SOM contents. It was also observed that the proportion of small juveniles increased with increasing concentrations, moreover, in Sandy and Sandy Loam soils, the reduction in soil moisture indicated an increase in the amount of small juveniles, in comparison with observed in standard moisture. These effects on growth/development of collembolans, mainly in Sandy soils, revealed a problem on an ecological scale, because even if fipronil toxicity is lower at dry condition, this environmental situation my impar the development of the individuals, making them more susceptible to stress or predation, with impact in future generations.

The second experiment (Section 4) indicated that increases in temperature can play different influences on fipronil toxicity for collembolans in different soils type. In soils with high clay and silt content, like Sandy Loam and Clay Loam soils, the fipronil toxicity increased at highest temperature, while in Sandy soil, that have poor adsorptive properties, occurred a decrease in fipronil toxicity with increase on temperature, probably due a effect of degradation by higher temperatures. Therefore, the effect observed in Sandy soil can implies an increase in the rate of application of pesticides in soils like this, which may cause more drastic effects on the population of collembolans living in exposure scenarios like this. From this, it is expected that the increase in temperature due to the effects of global warming will result in different toxicity responses from pesticides to different soils, bringing to light the importance of considering the type of soil and temperature in the ecotoxicological assessment of pesticides.

The fipronil toxicity for earthworms (Section 5) increased with increase on temperature in Clay Loam Soil and in dry Sandy Soil. The fipronil toxicity also was influenced by soil moisture in Sandy Soil, where there was a increase on fipronil toxicity for earthworms in dry situation with the increase of temperature. The influence of temperature on fipronil toxicity in wet Sandy soil was not clear, however, to seems demonstrate a degradation of molecule like consequence of its exposure to high moisture content associate with highest temperature. Like to above mentioned, this possible effect of degradation of molecule can imply on increase on rates of application of fipronil in Sandy soils under higher moisture and temperature regimes. From the climate changes predicted until the end of the century, knowing the effects of climate variables on the toxicity of pesticides in natural soils is important to prevent and preserve the terrestrial ecosystem from possible imbalances. Therefore, this experiment reinforce the importance of the use of natural soils and the simulation of real and predicted climatic conditions in predictive ecotoxicological assessments.

Although our results have been demonstrated in laboratory settings, the measurement in semi-field or field studies should help to clear the results obtained. The realization of more realistic studies considering contaminated agricultural soils, crops, relief, native edaphic species, successive applications of fipronil as well as real rain and temperature regimes, would help to further clarify the effects observed so far, leaving a gap open for further studies.